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The "University of Sindh Journal of Animal Sciences (USJAS)" is dedicated to addressing critical issues within the fields of animal sciences and zoology. Focused on the promotion of research and the dissemination of knowledge, this journal covers a wide spectrum of subjects, including biodiversity conservation, highlighting the significance of preserving the planet's diverse animal species amid growing global challenges. Additionally, USJAS delves into the ecological impact of human activities, advocating responsible practices and ecosystem preservation. It also emphasizes Sustainable Food Production, recognizing the pivotal role of animals in the global food supply chain. Furthermore, USJAS explores ecosystem services provided by animals, contributes to understanding Human-Animal Interaction, encourages interdisciplinary research spanning entomology, endocrinology, molecular biology, parasitology, wildlife management and Conservation, and Animal Diversity and Systematics. Serving as a platform for researchers, educators, and students, the journal facilitates the exchange of ideas and research findings while promoting access to advanced education programs. Lastly, USJAS underscores the urgency of global ecosystem preservation, aiming to equip stakeholders with knowledge and strategies to safeguard our planet's ecosystems. Through these endeavors, the journal seeks to advance animal sciences, promote responsible practices, and address the intricate challenges faced by both animal species and human societies.

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DETECTION OF AFRICAN SWINE FEVER VIRUS IN PIGS SLAUGHTERED AT ABATTOIR, JOS, PLATEAU STATE NIGERIA

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ABSTRACT

African swine fever (ASFV) is a reportable disease of swine, with serious economic limitations associated with losses in terms of production and international trade. About 100 spleen tissue samples were collected from abattoir Jos, Plateau State over a period of five weeks. The samples were prepared and analyzed using blocking enzymatic immunoassay (Blocking ELISA) test kit to test for antigen against ASFV. The result revealed an overall prevalence of 17% (95 CI: 10.6-25.3). The prevalence based on sex indicate a higher prevalence in male pigs 21.73 (95% CI 11.6 - 35.34) than in female pigs 12.96 (5.85 - 23.97); however, the difference in prevalence is not statistically significant $p > 0.05$. The finding confirms that ASF is endemic in Plateau and the ASFV is prevalent in the study area. The circulation of the virus is a great risk and has a negative impact on the means of livelihood and food security in the state and the country at large. It is therefore recommended that further studies should be carried out to investigate the possible source of the infection on ASFV positive pigs and also characterized the ASFV antibodies in pigs and pig's farmers should ensure strict adherence for biosecurity measures on their farms.

1. INTRODUCTION

African swine fever (ASF) is a major reportable and transboundary disease affecting pigs worldwide, causing heavy financial losses in epidemic and endemic countries, impacting negatively on food supply, and threatening famers livelihoods (Beltran-Alcrudo et al., 2019; Uwishema et al., 2022; Onoja et al., 2023). Detection of ASFV in pigs can lead to international trade restrictions, Exacerbating national economic losses (Taylor et al., 2020). First outbreak of ASF in Nigeria was documented in 1973, with subsequent outbreaks in the years 1997, 1998 and 2001 (Babalobi et al., 2003). Since the 1997 outbreaks, sporadic cases of ASF have been persisting, indicating that ASF is enzootic in the country (Awosanya et al., 2015).

The disease has been reported in various states, including Taraba (Abwage et al., 2015), Benue (Asambe et al., 2018; 2019), Ogun, Oyo, and other southwestern states (Awosanya et al., 2015; Oluwole et al., 2016; Omowon et al., 2019). ASF poses a serious threat to pig production due to economic losses and impacting the national economy (Saka et al., 2010; Omowon et al., 2019; Adekola et al., 2022) and the loss of nutrients in the form of protein and fats from the food supply, further exacerbating food security issues (Okoth et al., 2013; Abwage et al., 2015). About 50 outbreaks of ASF was reported in 12 state of Nigeria in the year 2020. As of May 30, 2021, there were outbreaks in six states with nine confirmed cases (Chieloka and Mogaji, 2022; WAHIS, 2023; Ogundijo et al., 2023).

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African swine fever etiology is a tick-borne DNA virus belonging to the Asfivirus genus and Asfarviridae family, which replicates in the cytoplasm (Sánchez-Cordón et al., 2018). The *Ornithodoros* genus of soft ticks, is a member of the family Argasidae, serves as the biological vector and reservoir host for ASFV. Approximately eight species of *Ornithodoros* have been recognized as vectors for the transmission of ASF (Golnar et al., 2019; Gaudreault et al., 2020). Three epidemiological cycles of infection are recognized: Sylvatic, Domestic and Sylvatic-Domestic cycles. Most African countries have reported the three cycles, but in Nigeria the most recognized is the domestic cycle, which harbor the virus among domestic pigs, although some reported the ASF among riverine hogs (Awosanya et al., 2015). The clinical manifestation of ASF varies, presenting in per acute, acute form, sub-acute form and chronic form. In the per acute form, infected pigs are found dead within a period of four days following infection with the absence of gross pathological lesion. In the acute form there is usually high number of dead pigs, with 90-100% mortality in 4-21 days' post infection. Common postmortem changes are vasculitis which may include pulmonary edema, skin erythema, hyperemic splenomegaly, hemorrhagic lymphnodes and lungs, kidneys and urinary bladder with petechial hemorrhages. The sub-acute form is characterized by mortality of about 30-70% due to moderately virulent strains, with pigs dying around 20 days' post-infection. The clinical signs are less severe than those in the acute form, with more pronounced vascular changes, including hemorrhage and edema. The chronic form, caused by low virulent isolates, is characterized by lack of vascular gross lesions and few mortality rates. Clinical signs include poor growth, emaciation, swelling of joints, skin ulcers, and secondary bacterial infections (Sánchez-Vizcaíno et al., 2015; Sánchez-Cordón et al., 2018).

Numerous laboratory diagnostic techniques have been developing for rapid control of ASF since inception. At The moment priority is to develop affordable, accurate, rapid and field based diagnostic tests that are highly sensitive and specific (Gaudreault et al., 2020). Currently, there is no available drug for proper treatment and effective vaccines for control of the disease (WOAH, 2019; Onoja et al., 2023; Weka et al., 2023; Ogundijo et al., 2023). ASF control require early disease detection in domesticated and wild reservoir pigs through active surveillance and control programs which will serve as effective means of outbreak prevention (Danzetta et al., 2020). Due to the impact of ASF on the pork production value chain and food security it is important to provide an update on ASF situation in Nigeria. The objective of this study is

to detect the prevalence of African Swine Fever virus in pigs slaughtered at abattoirs in Jos, Plateau State.

2. MATERIALS AND METHODS

Study Area

This research study was conducted in Jos, Abattoir. Jos is the capital city of Plateau state with about 1,238 meters above sea level. The city has a dense population of about 900,000 inhabitants according to the National census of Nigeria 2006 (2006 Population Census).

Sampling Method

Convenient sampling technique was used to collect the sample, during which 100 spleen tissue samples were collected from the pigs slaughtered at the abattoir, Jos, Plateau State (20 samples every week for the period of five weeks). The samples were put into sample bottle labeled, and transported in ice pack to the Viral Research Laboratory, NVRI and stored at -20°C until use.

Equipment

Distilled water, ELISA Kits, Pipette tips, Sample bottles, Refrigerator, Centrifuge, pistol and mortar, incubator, scissor.

Laboratory Analysis

Sample Preparation

10% tissue homogenate was prepared; thus, about 1g tissue sample taken, pistil and mortar were used to macerate the sample using sterile sand. The samples were Centrifuge at 1500rpm (revolution per minute) for 10 minutes. The supernatant was used for the analysis.

Test Procedure

The kit is a blocking ELISA based on the procedure of blocking enzyme immune assay and steps were carried out in accordance with the manufacturer's instructions. The steps taken include the Following:

1. All reagents (except conjugate) where allowed to come to room temperature before used.
2. To the wells A1 and B1 100 μl of the negative control was added, and to A2 and B2 100 μl of the negative control was added. 100 μl of samples test was added to each well. The plate was then sealed and incubated for a period of 1 hour at 37°C .
3. After the incubation period, the wells where emptied and washed four times using wash solution.

4. 100µl of specific conjugate was added to each well. The plate was sealed and incubated for 1hour at 37°C. It was washed 4 times following the described procedure.
5. To the plate 100µl of the substrate solution was added, in to every well. The plate was kept for a period of 15 min at 37°C.
6. Lastly to each of the well 100µl of stop solution was added to every well. The Optical density OD values were read on a MultiSkán® spectrophotometer ELISA plate reader (Thermo Scientific, USA) at 450nm wavelength.

Data Analysis

The data obtained were saved in Microsoft Excel® spreadsheet. The Microsoft Excel® spreadsheet was used to perform Descriptive statistics and open Epi Version 2.3.1 statistical tool (Dean et al., 2013).

3. RESULTS AND DISCUSSION

In this study 100 spleen tissue sample were obtained from pigs slaughtered from Jos, abattoir Plateau State, and analyzed for antigen against ASFV. The result revealed an overall prevalence of 17% (95% CI: 10.6 - 25.3). The prevalence based on sex indicate a higher prevalence in males 21.73 (95% CI 11.6 - 35.34) than in females 12.96 (5.85 - 23.97); however, the difference in prevalence is not statistically significant $p > 0.05$. In table 1 and 2 below:

Table 1: Prevalence of ASFV from slaughtered pigs

Total no of Animals	Number Positive	Number Negative
100	17	83
Total	17%	

Table 2: Prevalence of ASFV based on Sex

Sex	Number of animals	Number Positive	Number Negative	Proportion (%)	95% CI
Female	54	7	47	12.96	5.85 - 23.97
Male	46	10	36	21.73	11.6 - 35.34
Total	100	17			

P-value = 0.1304; $P > 0.05$

Numerous serological studies have been conducted in Nigeria to measure the antibodies levels of African swine fever (ASFV) in pig populations, with examples including studies by Mailafiya and Iliya (2009) in kumo, Gombe State and Kperebeyi and Amostsuka (2010) in Delta State. The prevalence of ASFV in this study is significantly higher than previously reported in other studies in Nigeria. Compared to Owolodun et al. (2010) who found a 14% prevalence in Plateau State, the current study's findings are higher. Similarly, the prevalence is higher than the 13.2% reported by Abwage et al. (2015) in Taraba State and the 1.7% reported by Asambe et al. (2018) in Benue State, indicating a more widespread presence of ASFV in Nigeria.

However, the seroprevalence of ASFV obtained in this study is lower than the rates reported by several other studies in Nigeria. Compared to the high prevalence rates reported by Olugasa et al., (2007) (65.2%), Saka et al. (2010) (88%), and Awosanya et al (2015) (28%) in Commercial piggry South western Nigeria. Additionally, the prevalence is also lower than rates reported by Fasina et al., (2010) in different state, which are Ogun, Delta, Cross River, Plateau, Benue and Oyo states (60%), (60%), (53%), (47%), (44%) and (36%) respectively. The higher prevalence rates recorded in the study by Fasina et al. (2010) can be attributed to the fact that samples were collected during outbreaks of ASF, which is a significant factor that can impact the prevalence rate. In contrast, the current study's samples were collected during a period without an outbreak, which could explain the lower prevalence rate. The detections of ASFV in the pig population may also be due to the circulation of low pathogenic strains, development of resistance and carrier state in the local pig population, or poor reporting practices by farmers, who may not report outbreaks to authorities and instead dispose of affected pigs, as reported by Fasina et al. (2010).

The study found a higher prevalence of ASFV in male pigs (21.73%) compared to female pigs (12.96%), but the difference was not significant statically. This result is similar to that of Abwage et al. (2015), but contrasts with the report of Asambe et al. (2018) which found a higher prevalence in female pigs. The study's findings indicate the endemicity of ASFV in Plateau State, and highlight the importance of addressing biosecurity issues, such as poor compliance, inadequate protective clothing and insecure, due to loss in revenue, which can contribute to the resurgence of outbreaks (Janse van Rensburg et al., 2022). Additionally, the study suggests that inadequate knowledge of biosecurity procedures among small-scale pig farmers may be a

factor in the occurrence of ASF outbreaks (Jongeneel et al., 2021; Ogundijo et al., 2023).

4. CONCLUSION

This study detected the present of ASF antigen in pigs slaughtered in Jos main Abattoir Plateau State; this of course has a negative impact on the means of livelihood and food security in the state and the country at large.

5. RECOMMENDATIONS

Pig's farmers should ensure strict adherence to biosecurity measures on their farms and Further studies should be carried out to investigate the possible source of the infection on ASFV positive pigs and also characterized the ASFV in circulation in Jos Plateau State.

6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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PRODUCTION PERFORMANCE AND ECONOMIC BENEFITS OF SHEEP FARMING IN MANATUTO MUNICIPALITY, TIMOR-LESTE

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ABSTRACT

Sheep are one type of small ruminant livestock that are very important for rural communities to meet the customary and economic needs of families. This study aims to obtain information about the livestock system, productivity, and benefits of sheep products for the cultural and economic needs of sheep farming families. This study used a survey method with a 95% confidence interval. The determination of the survey location was carried out using the purposive sampling technique and the determination of the sample size using the Slovin method. The selection of respondents used the random sampling method. The variables observed were herd structure, reproductive performance, utility of sheep products to meet the customary and economic needs of farming families, and product sales systems. The survey results showed that around 95% of respondents still use the traditional extensive system, without technical control and attention to the quality and frequency of animal feed. This system is less appropriate in efforts to overcome reproductive problems, the age of the first pregnancy of sheep, resulting in low productivity, mortality rates reaching 1%, the age of first lambing reaching 12 months, and the age of weaning of sheep usually 3 to 6 months. All informants stated that culturally, sheep are the most important animals in their lives, therefore, sheep farming must be maintained at all times. Sometimes they sell the sheep production, but only to finance some of the family's sudden needs. It is concluded that it is difficult to influence farmers to increase sheep productivity, especially in the research location because the main purpose of sheep farming is only to maintain cultural life, not market oriented.

1. INTRODUCTION

The development of animal production is often used as one of the other main targets in an effort to provide highly nutritious food to consumers, in the context of reducing hunger and malnutrition through the provision of animal protein-based foods, and to improve the producer's economics. Sheep is a type of small ruminant that has great potential for developing production and this type of livestock has adapted well to the environment and local food availability.

Sheep in Timor-Leste are kept by 2983 households, and in the Manatuto municipality only around 103 households, whose main objective is to firstly satisfy the family's cultural needs and secondly the economic needs (Timor-Leste Agricultural Census, 2019). Farmers generally still do not care about the rearing system, as well as livestock structure and productivity, which has a negative impact on the development process and efforts to increase the quality of production and selling value. Thus, in efforts to increase the sheep production, it is very necessary to improve the rearing system, reproductive management, feed quality and technical assistance, especially on productivity issues in order to minimize calving intervals and growth as far as possible (Dagnew et al., 2017). The factors that

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influence the productivity of sheep and goats are the raising system, age, production frequency, nutritional needs and period of gestation (Susilawati, 2008). Implementing good management in sheep production activities is very helpful in determining the right weaning age and calving interval to enable ewes to be lambing twice in eighteen months with a high-quality litter size of lambs. Basically, sheep breeders in rural areas have two important goals, namely maintaining social and cultural status as respected people and secondly as saving in the family. This research aims to obtain information regarding the rearing system used by breeders, productivity performance, and the cultural and economic benefits of sheep raised using a traditional system.

2. MATERIALS AND METHODS

2.1 Description of the research site

Manatuto is one of the municipalities of Timor-Leste, located in the center of the country. It has a population of 45,541 inhabitants and an area of 1,783.3 km². The municipality of Manatuto borders the municipalities of Baucau and Viqueque to the east and Manufahi, Aileu and Díli to the west. It reaches both the south and north coasts of the island, and is only one of two municipalities to do so (the other is Lautém in the far east), and has the most geographical diversity. To the north is the Strait of Wetar, to the south is the Timor Sea (Timor-Leste population Census, 2015). The research data collection period starts on September 20 to December 20, 2023 or is carried out for three months.

2.2 Sample size estimation

This study used the survey method with 95% interval confidence, including interviews with sheep owners as respondents and carried out direct observation of the object under study, including the rearing system, feeding method, frequency and the quality of food provided. In the process of determining the research site used the intentional sampling method based on secondary data from the household Census of Manatuto municipality. In determining the sample size, Slovin method was used according to the recommendation of Sugiyono (2014) with the following formula:

$$n = \frac{N}{N \cdot d^2 + 1} \quad \text{where:}$$

n = Total sample (head household as respondee)
N = Total population (sheep farmer) d² = Desired percentage.

A total number of households as sheep breeders who were concentrated in the municipality of Manatuto out of a total of 103. Therefore, the formula is as following:

$$n = \frac{103}{1 + 103 \times (0.1)^2} = 50,74 \sim 51$$

According to the Slovin formula, the minimum research sample that must be interviewed is 51 sheep breeders. To determine the respondents, it used the simple random sampling method with the intention that all producers registered in the municipality of Manatuto had the same opportunity to be selected as respondents.

2.3 Observed variables

The variables observed in this research were livestock structure, pregnancy period, number of lambs, age at weaning, age at which ewes first gave birth, production system, mortality rate, and method of sale, as well as the economic benefits (value) of sheep products.

2.4 Interview method

The survey method in this research uses direct techniques, namely conducting interviews with respondents based on a semi-structured questionnaire designed to obtain primary data. Questionnaires are a very important material in survey research to obtain the primary information, to be processed and analyzed according to each variable that wants to measure and observe. The interview is a technique used to ask questions carefully so that the interviewee, possibly, provides accurate information according to the researcher's objectives. The conception is the implementation of a survey is a process whose objective is the collection of thematic, valid, and reliable information, obtained from the individual answers given to a set of questions by a representative group of respondents, which produced conclusions that can be generalized to the universe of the study population (Sugiyono, 2014).

2.5. Data analysis

The data obtained in this study were coded, tabulated, and subjected to descriptive statistical analysis according to [Sampurna & Nindhya \(2008\)](#), using the SPSS program, Version 25, to determine the mean, mode, standard deviation, standard of mean error and relative percentage of each variable observe

3. RESULTS AND DISCUSSION

3.1 Production system

Based on the results of observations and interviews, it shows that about 70% of the total respondents still use the extensive or subsistence system. Approximately 25% of total respondents use a semi-intensive system and 5% are starting to try using an intensive maintenance system. The factors that encourage farmers to continue using the system are because it is considered easy and does not require high production costs, as well as technical assistance in the process of raising sheep. This system greatly affects the productivity and quality of sheep production and reproduction because the maintenance system is still simple and primitive. Farmers are unfamiliar with modern maintenance systems that use technology to improve feed quality and good management to improve sheep productivity. The other factors that influence the breeders main to change the production system from extensive to intensive is culture and economic viability. According to [Murdjito et al. \(2011\)](#) and [Welday et al. \(2019\)](#), breeders who have small capital only keep sheep and goats on a small scale, which is around 2-7 heads so that production is low. [Supriyanto et al. \(2019\)](#) states that the more sheep and goats owned by the breeder will affect the way of thinking to accept and adopt new innovations in their livestock production. In addition, basically, sheep farming activities are still classified as secondary activities from other agricultural activities. Detailed research data about the sheep-rearing system, is shown in Figure 1.

3.2 Sheep feed resource

The feed resource and availability in research area is shown in Figure 2. In general, sheep breeders state that during the day the sheep are taken to the pasture to look for food, and in the afternoon, they can be given additional food in the form of leaves available at the breeding site, such as leucaena and hibiscus leaves.

Local feed production, especially legumes such as leucaena and hibiscus, has great potential and is developing well, but because it has not developed well and in large quantities, production is still minimal and not enough to meet the needs of sheep feed. One of the important factors that need to be considered in sheep fattening is a guarantee of feed continuity ([Mayulu and Suhardi, 2016](#)).

However, due to the limited knowledge of farmers and feed utilization was not maximized to increase sheep production. About 90% of respondents stated that according to their knowledge, good feed for sheep is classified into three types, namely field grass, young leaves, and legumes, as well as separate feeding methods to maximize feed efficiency. The respondents stated that, there is no need to pay attention to the nutritional problems of the feed because the purpose of keeping sheep is for a long period and can sell or slaughter when needed. Whereas sheep really need energy, protein, vitamins, minerals, fiber, and water to live and produce properly. Nutritional needs for sheep in generally not the same or different between nations but the implementation always refers to a list of tables that have been available for livestock conditions in the Asian region, even though the agro-climate is both tropical ([Mayulu and Suhardi, 2016](#)). Therefore, the results of the observations in the research indicate that there is a need for technical assistants, especially with regard to advising and transferring new technologies in intensive livestock farming, but with a reduction in production costs so that can be achieved by small rural producers.

3.3 Herd structure

According to the results of the study, it was found that the total sheep per household (breeder) was 2 to 16 heads with an average of 7.98 ± 2.84 heads per establishment. Total sheep calculation is performed during the dry season, namely in May or at the end of the rainy season, as ruminant productivity is highly dependent on feed quantity and quality. In May, the availability of food (local grass) is high enough to increase the production, which is one of the factors that determine the productivity of sheep. The results of the observations show that basically the producers do not know a good way to maintain the size of sheep, mainly in the summer period. Sheep are small ruminant animals that reproduce quickly and have great potential to be used by the community as a source of

protein, besides being able to be sold to get money quickly and as an important material in customary matters (Lusi et al., 2022). About the sheep population structure, the results showed that there were differences in the number of sheep population structures in each breeder. Basically, it can be classified according to age, namely lamb aged 1-3 months, young lamb aged 4 to 7 months, and adult sheep aged 7 months and over. The results of the analysis showed that, the total number of sheep owned by farmers, the herd structure was as follows: ram lamb is 14,98%, ewe lamb is 11,61%, and adult ram is 16,85% and 56,55% is the adult ewe of the total sample observed. The proportions of physiological status and sex were classified into two groups as shown in Table 1.

In accordance with the results of the classification in Table 1, it can be seen that the highest percentage is the adult sheep and adult ram, meaning that from March to May the sheep structure is still stable, without additional any stock, for reasons of no disturbance such as illness, loss, and theft. The sex ratio is still in accordance with the needs, because several adult ram can be sold to meet the economic needs of the family. The number of sheep that have been observed as many as 5579 heads with details as shown in Table 1 with a sex ratio of 1:3 (1 adult ram versus 4 female sheep). This result is not the same as the recommendation from Mulyono and Sarwono (2012) that to get a good sheep production, the reproduction ratio should be 5:16 (5 adult ram compared to 16 adult ewe) or one adult ram can serve 3-4 adult ewe.

3.4 Sheep reproduction performance

The results of the descriptive statistical analysis of sheep reproduction performance for each variable observed in this study, consisting of mean, standard deviation, mode and percentage are shown in Table 2. These results indicate that sheep kept in subsistence production systems showed low reproductive performance.

3.5 Gestation period, litter size and mortality rate

3.5.1 Gestation period

Based on the results of the descriptive statistical analysis presented in Table 2, it appears that the

gestation period of adult ewe is 5.78 ± 0.07 months, so it shows that the fastest is 5.71 months and the longest is 5.86 months, and about 43.40% of the total respondents stated that the adult ewe gestation period was normally around 5 months, and it is considered normal gestation period. According to Welday et al. (2019) the normal gestation period for adult ewe is 144.90 - 150.94 days or about 4.83-5.09 months.

3.5.2 Litter size

The result of litter per ewe lambing in this study was at least 1.11 and the largest was 2.79 with a mean of 1.95 ± 0.08 . This means that the number of lambs born from each ewe ranges from one to two and can reach three, although this rarely happens. Therefore, this is still considered a low ewe production and is most likely caused by poor farm management. According to Mulyono and Sarwono (2012) that normally a ewe can produce 2 heads per lambing, although there are also ewe that can produce 4 to 5 heads per ewe lambing, which rarely happens. The results of this study do not differ from the findings of Sarwono (2011) that a ewe can produce 1.31-2.69 offspring, with an average of 2.0 ± 0.88 per ewe lambing. The respondent state that peak fertility is from late September through November. Ewes have an average cycle length of 17 days, with most being between 14 and 20 days, while does have an estrus cycle of 18 to 22 days, and they display estrus for 24 to 48 hours, and the gestation period ranges between 144 and 152 days.

3.5.3 Mortality rate

The results of the descriptive statistical analysis showed that the lamb mortality percentage from birth to weaning was 1.24 - 1.5%, and about 39.60% of those interviewed stated that the number of lamb mortality in general was 1 per lambing. Death of the Lambs occur due to lack of technical control, poor feed quality, lack of pens for safe shelter for sheep and lack of attention from producers. Therefore, mortality is one of the most dangerous and economically detrimental factors in the industry of animal production. According to Sudrajat et al (2021), the mortality rate in sheep production can reach 3.95 - 5.77% per ewe lambing. The high mortality rate is likely caused by the old pregnant mother in intensive maintenance not getting enough nutrition. With the grazing system, the feed obtained is likely to be only grass, so it is not sufficient for good production, when

compared to the semi-intensive and intensive maintenance systems the feed given in terms of quantity and quality can meet the needs of life and production.

3.6 Ewe first lambing and weaning age

One of the parameters is the age at first lambing. The results of the descriptive analysis showed that ewes kept extensively at the study site generally the ewe first lambing at 10.65 - 13.23 months or with an average age of 11.94 ± 1.29 months. The statistical analysis results showed that it was not much different from the answers from 61.30% of respondents that generally the ewe first lambing at the age of 12 months and can give lambing faster or less than 12 months if given high-quality feed. The results of this study are considered better when compared to the research results of [Perwitasari & Bastoni \(2019\)](#) which states that, ewes first lambing at 15-18 months of age with an average litter size of 1.57 per lambing. In accordance with the results of interviews, the weaning age of young ewes occurred in the age range of 5-6 months with an average of 5.61 ± 0.07 months. Approximately 43.10% of the total respondents stated that in general lambs can be weaned at the age of 7 months. [Mayulu and Suhardi \(2016\)](#) recommends that weaning can occur naturally at the age of 3 - 6 months, because at that age the young sheep and goat are able to find their own food. According to [Sulastri \(2001\)](#), when weaning young sheeps, it is also necessary to be aware of live weight, as there is a very close relationship with its development and survival in the future. As related by [Sarwono \(2011\)](#) that sheeps will grow healthy if their body temperature is around 39.5-40.5°C, heart rate is 70-80 per minute, and breathing rate is 12-13 times per minute.

3.7 Utility of sheep farming products in the culture and economy

Almost 95% of all respondents stated that they must maintain and develop sheep production because it is essential to meet traditional needs, especially as an irreplaceable material in offerings to the souls of people who have passed away. The respondents stated that the sheep's liver is very important for cultural purposes and this organ cannot be replaced by other organs from any animal. The type of cultural habits in the research location is one of the factors that encourage the producers to continue

breeding and developing sheep production, however, sheep farming always requires constant attention from the farmers, because the sheep cannot return to the pen alone without being herded. To ensure the safety and comfort of the sheep, the shepherd must lead them to pasture to find their food and bring them back to their pen in the afternoon.

Livestock farming (especially sheep breeding) has been the foundation supporting the livelihoods of local people from nomadic times until the present-day. [Welday et al. \(2019\)](#); [Utomo and Rasminati \(2009\)](#) state that the contribution of the agricultural production to the economy is crucial (about 45% of gross income). In Manatuto municipality, sometimes farmers sell their sheep when they need money to meet the family's needs, specially to fund their children's school and healthcare cost. Sales system normally based on the external condition (fat or thin), age and sex of the sheeps. Between young and adult there are always price differences and ewe and ram also have price differences. The price range is between \$25 to \$50 for lamb and \$75 to \$200 for adults, depending on the body condition and sex of the sheeps. About 87% of respondents stated that they always sell sheeps in the summer season because at this time there is a lack of quantity and quality of animal feed, especially grass and legumes such as grasses and young leaves that as potential type of animal feed in the research local. The annual income from sheep farming in the Manatuto municipality is shown in Figure 3 below.

The analysis results obtained presented in Figure 3 show that around 28% of sheep farmers only obtain income between \$100 and \$150 per year, 34% earned an income of US\$200 to US\$500 and 38% of respondents earned \$550 to \$2,000 income per year. Thus, although the main purpose of raising sheep is to fulfill traditional needs, but on the other hand, it can generate income to improve the family economy, even though its contribution is not much compared to other income of agricultural activities. In general, sheep farmers in the research location still consider sheep farming as a side activity compared to other agricultural activities in the family because the main goal is to maintain social status, especially in customary matters. Thus,

farmers are not business-oriented and do not prioritize the economic value of sheep farming.

4. CONCLUSION

The results of the research on the production system, herd structure, and productivity showed that all interviewees in general still use the traditional extensive system, not paying attention to the problem of herd structure, the quality and frequency of feeding, and productivity management, including production, reproduction and feed quality due to negatively affecting the productive performance of animals. Farmers sell their animal products when they need money to finance some urgent family needs. The price of animals is always based on the external condition, age, and sex of the animals intended for sale. In this way, breeders begin to understand that sheep farming is not only used to meet cultural needs but can contribute to improving the family's economic needs.

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6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1. Classification based on the phase and sex of sheep

Classification	Total	Percentage (%)
- Ewe lamb	648	11,61
- Male lamb	836	14,98
- Adult Ram	940	16,85
- Adult ewe	3155	56,55
Total	5579	100.00

Source: results of study (2023)

Table 2. Productivity Performance of Sheep kept in subsistence production system

Characteristics	$\bar{X} \pm sd$	Mode	Percentage (%)
Gestation period (month)	5.78±0.07	5	42.20
Number of lambs per birth	1.25±0.08	1	54.70
Mortality rate (%)	1.35±0.13	1	40.50
Mating interval (month)	12.24±0.12	12	65.50
Interval calving (month)	14.05±0.50	13	72.25
Weaning age (month)	5.61±0.07	7	43.10
Weaning weight (kg)	16.36±4.34	14	51.19

Source: Primary data

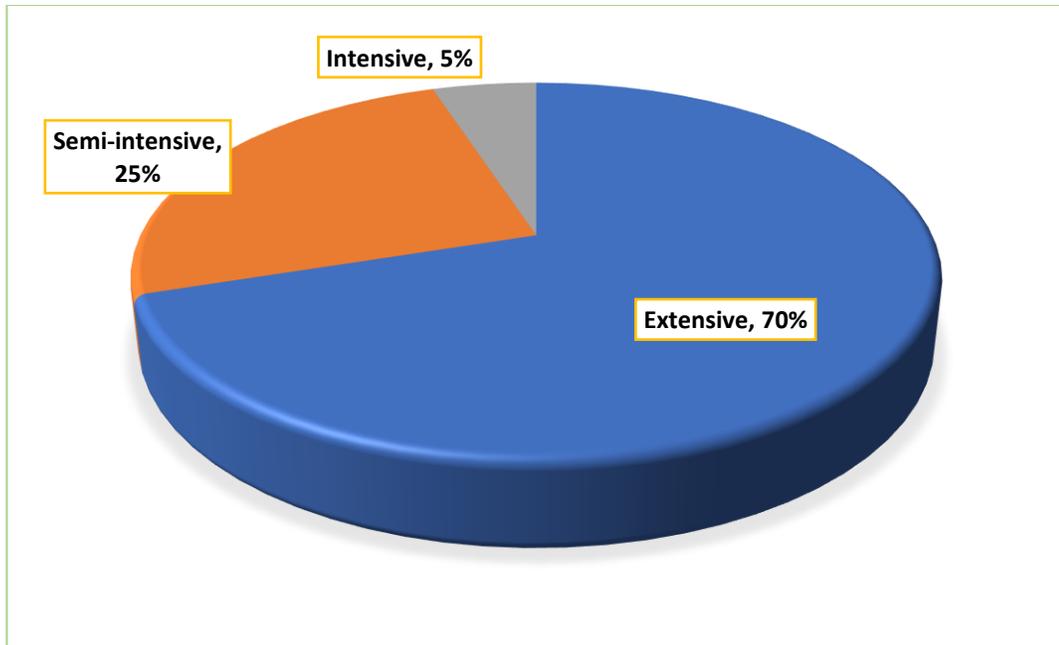


Figure 1. Sheep Production System in Manatuto Municipality (n=53)

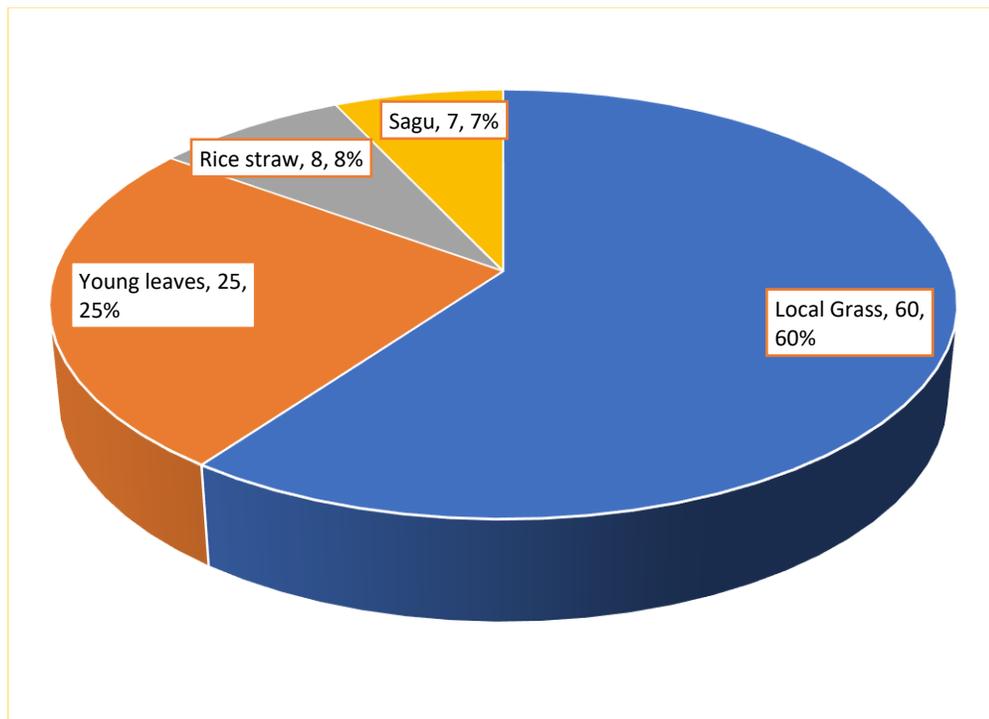


Figure 2. The type of feed most used by farmers in the research location (n=53)

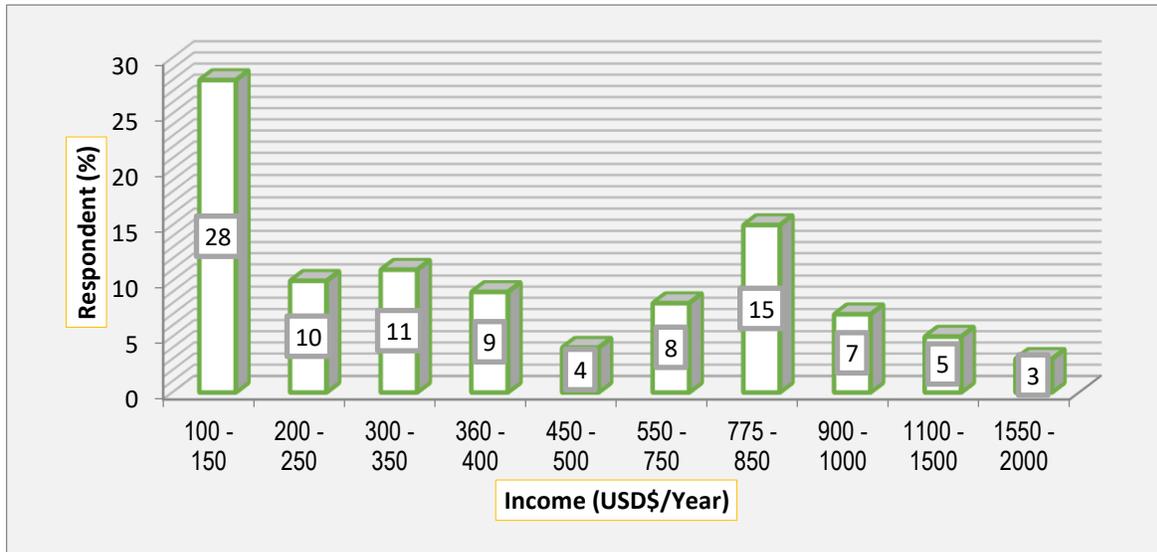


Figure 3. The annual income from sheep farming in Manatuto Municipality, Timor-Leste (n=53)

ANALYSIS OF ANTIBIOTIC RESISTANCE IN RAW MILK OF ORGANIC AND CONVENTIONAL COWS IN DISTRICT SIALKOT PAKISTAN

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ABSTRACT

Over the last few decades, antimicrobial resistance (AMR) has been recognized as one of the principal threats to public health. Even though the emerging crisis of AMR was formerly considered a problem to human health, it entails a “One Health” approach, because of the animal, human, and environmental reservoirs. Here of the all-encompassing antimicrobial use (AMU) in the livestock production systems can result in the occurrence of AMR genes in bacteria that can be transmitted in milk, leading them into the food chain. This study aimed to estimate the AMR prevalence in dairy farm, using susceptibility testing of cow’s raw milk bacteria as an indicator to evaluate whether AMR patterns differ between conventional and organic dairy herds. For this purpose, an AMR comparison was made between the milk obtained from organic (No AMU) and conventional (AMU) cows for which disk diffusion method was used where AMU data were based on the injections given to cows. The findings revealed that 44% of AMR was found among all the bacterial isolates against five antibiotics i.e., amoxicillin (AMOX), ampicillin + cloxacillin (AMC), cephradine (CED), ciprofloxacin (CFX), and oxytetracycline (OT) at five different concentrations (0.1, 1, 10, 100, and 1000 µg/ml) where organic samples have shown 52% of AMR and in conventional milk samples 39% of AMR was observed, most likely because of the good health of cows and consequently less AMU in both types of dairy farms illustrating the problems in determining associations with AMU. This was a surveillance study of antimicrobial susceptibility in district Sialkot which has provided us with a rundown on AMR in raw milk as well as on the role of AMU in the occurrence of AMR in raw milk. Further research of the complete dairy farm environment is required to unravel the complex web of AMR and its matrices on dairy farms.

1. INTRODUCTION

The growing prevalence of antimicrobial resistance (AMR) in bacteria is posing serious threats to human health (Rubiola, Chiesa, Dalmaso, Di Ciccio, & Civera, 2020).

Globally, approximately 700,000 people die each year due to antibiotic-resistant infections (Oved, 2021). In the O’Neill report composed by the well-known economist Jim O’Neill commissioned by the UK government it is stated that, at present rates, the number of human deaths could rise to approximately 10 million human deaths per year by 2050 (O’Neill, 2021). Like the rest of the globe, an increase in drug-resistant infections has been reported in Pakistan (Kumarasamy et al., 2010; Shah, Wasim, & Abdullah,

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2015). Currently, Pakistan is the third highest antibiotic consumer after India and China among all low to middle-income countries. In Pakistan, the rate of antibiotics consumption has been increased by 65% between 2000 and 2015 (Klein et al., 2018). This upsurge in antibiotic consumption is becoming a foremost healthcare challenge from an AMR perspective. According to a WHO report, Pakistan has been ranked among the top 5 countries in which the highest rate of neonatal deaths due to drug-resistant bacteria have been reported (Laxminarayan et al., 2016). In the past decade, resistance to *Enterobacteriaceae* against quinolones has increased in Pakistan (Yasmin, Akhtar, & Hameed, 2013). One of its examples was the outbreak of XDR *Salmonella* in 2016 that showed 100% resistance against fluoroquinolones (Qamar et al., 2018).

Besides the potentially pathogenic bacteria, antibiotic-resistant microbes are also present in raw milk (Alexa et al., 2020; Burakoff et al., 2018; Caudell et al., 2018; Godziszewska, Pogorzelska-Nowicka, Brodowska, Jagura-Burdzy, & Wierzbicka, 2018; Munsch-Alatossava & Alatossava, 2007), and thus the consumption of raw milk may facilitate the dissemination of ARGs to the human gastrointestinal tract. Currently, there is a lack of comprehensive understanding of the presence of antibiotic resistance in raw milk (Liu, Zhu, Jay-Russell, Lemay, & Mills, 2020). Observational research on cattle production systems has revealed that a higher prevalence of AMR enteric bacteria was found in conventional dairies as compared to organic dairies (Call, Davis, & Sawant, 2008).

Food animals are treated by AMU that affects human health via 2 mechanisms: 1) growing risk of antimicrobial residues, and 2) influencing the selection or generation of antimicrobial-resistant foodborne pathogens (Yan & Gilbert, 2004). The risk of increasing antimicrobial residues in the environment is widely recognized and has been addressed via the use of appropriate regulatory mechanisms however there is growing concern regarding the AMU impact on the development of AMR in food animals (Ruegg, 2013) (Fig. 1).

An upsurge in antibiotic-resistant infections has been reported in Pakistan which can be a result of over and misuse of antibiotics in livestock production systems therefore this surveillance study of antimicrobial susceptibility was performed in district Sialkot which would provide us with a rundown on antimicrobial resistance in raw milk as well as on the role of antimicrobial use (AMU) in the occurrence of AMR in

raw milk. Several international studies had compared AMR patterns in organic and conventional dairy farm (Sjöström et al., 2020). But to the best of our knowledge, there is no published study comparing AMR prevalence in the milk of organic and conventional cows in district Sialkot Pakistan. Because of the association among AMU and AMR, it was assumed that the farm with lesser AMU would also have a lesser AMR prevalence.

2. MATERIALS AND METHODS

This was a comparative and correlational study focusing on the prevalence of antibiotic resistance in the environment. The study involved a quantitative assessment of antibiotic susceptibility by determining the MIC. The sample size was 6 samples.

Sample Collection

Raw milk samples were collected near Power House, Shahabpura, and Abbot Road Sialkot from six lactating cows three of those were organic cows with no antimicrobial treatments and three were conventional cows with antimicrobial treatments in the recent past. For this purpose, the teats of each cow were disinfected with the help of a swab before taking the sample (Wattenburger, Schmidt, Placheta, Middleton, & Adkins, 2020). Samples were taken in a sterilized glass container. Immediately after taking the sample, the glass containers were placed in a bottle containing ice cubes to travel to prevent bacterial growth. Samples were transferred to the lab and refrigerated at -4 degrees Celsius within 2 hours until downstream processing.

Culturing

Serial dilutions of raw milk were prepared in distilled water. Samples were cultured by first applying the milk to the MacConkey agar media (Blood agar was used for reference and nutrient agar for CFU). The samples were plated using the spread plate culture technique so that bacteria spread out evenly on the plate (Kuehn et al., 2013). When the milk samples were plated, the left-over milk was stored in a freezer at -4°C for further analysis. The plates, after being spread with milk, were placed in the incubator upside down. The incubator was set at 37° C. Incubation was done for 24 hours so that bacteria would be given plenty of time for growth.

Colony-forming Unit (CFU)

For measuring the CFU, samples were grown on nutrient agar. Further, dilutions of these samples were prepared (by keeping the track of dilution factor) and cultured (Pious Thomas, Sekhar, Upreti, Mujawar, &

Pasha, 2015). For serial dilution, 1 ml of sample was aseptically weighed into a sterile glass bottle and 9 ml of sterile diluent was added to provide the initial 1:10 dilution from which serial dilutions were prepared as required (Harrigan & McCance, 2014).

The plates were incubated at 37 °C for 48 hours before counting colonies. The colonies obtained were counted on each per plate of each dilution by hand. The plate consisting of the countable number of colonies was selected along with the dilution factor assigned to that culture plate for measuring CFU. The colony-forming units (CFUs) were counted after 24 hours of incubation and calculated back to CFU/ml in the original sample (Sjöström et al., 2020). Colony counts estimated the number of colony-forming units per 1 ml of the sample (Sarao, 2017). So the number of bacteria (CFU) per milliliter of the sample was obtained by dividing the number of colonies by the dilution factor (P Thomas, Mujawar, Sekhar, & Upreti, 2014). CFU was calculated by applying a specific formula (Terzich, Pope, Cherry, & Hollinger, 2000) which is as follows:

$$\text{Bacteria (per mL)} \\ = \frac{\text{number of colonies} \times \text{dilution factor}}{\text{volume of the plate}}$$

Isolation and Purification of Bacterial Isolates

The bacterial colonies obtained through spreading were isolated by sub-culturing using the same protocol. Each bacterial colony was sub-cultured to identify each bacterial variant. The sub-cultures were labeled to avoid any confusion (Sasidharan, Prema, & Latha, 2011).

Morphological Characterization

Bacterial colony morphology was observed in terms of form (size and surface), elevation, color, texture and margins. Isolates were grown on MacConkey agar and checked for the occurrence of Lactose fermentation that changes the color of MacConkey agar due to pH change (Fig. 3A-D).

Gram Staining

On a grease-free slide, a thin spread was prepared by blending a small quantity of organisms selected from the stocked colony of 18–24 hours old pure culture into a drop of sterile distilled water in the first step. By moving it slowly over the flame, the spread was air-dried and heat-fixed. For 30–60 seconds, the slide was cautiously located on the staining rack and was swamped with primary stain (crystal violet). The addition of a gram's iodine was done (mordant) for 30 seconds. Later, the smear was moderately rinsed with tap water in the second step. The 70% ethanol that was

used as a decolorizer for 10–30 seconds was essential for the third step. The smear was stained with the secondary stain (safranin) for 30 seconds before getting rinsed with tap water and later it was given the due time to get dry in the final step. Through the help of an oil immersion objective (x100) the spread was observed under the microscope. Gram-positive organisms exhibited the purple color and gram-negative exhibited the red color (Dawodu & Akanbi, 2021). Microscopic appearance of bacteria was also observed.

Biochemical Testing for Identification

Microbial identification was performed by applying several biochemical tests. The biochemical test applied for each subculture of colonies obtained were oxidase test (OX) (Cheesbrough, 2005; Hussain et al., 2013), catalase test (CT) (Bennion et al., 1990; Dawodu & Akanbi, 2021), indole test (IND) (Hussain et al., 2013; Kuffner et al., 2010), motility test (Mot) (Bello, 2002; Islam et al., 2017), citrate utilization test (CIT) (Bello, 2002; Dawodu & Akanbi, 2021), gas, and urease test (URE) (Cheesbrough, 2005; Hussain et al., 2013).

By culturing the microorganisms in peptone water medium having tryptophan in a screw-capped tube, incubated for 24 h at 37°C, IND test was carried out and later on the addition of Kovac's reagent (0.5 ml) was done through which the positive results were identified not later than seconds of adding Kovac's reagent through the development of a pink-red layer on the broth (Hussain et al., 2013). Commonly, the fact that the bacteria has moved away from the stab mark (were motile) was identified if the entire tube was turbid. However, the organism was tending to be non-motile in the case, if the stab mark was evidently visible and the rest of the tube was not disorganized (Islam et al., 2017).

Simmon's citrate agar medium was formulated in a bijou bottle and was given the time to set in a slanting position to perform citrate test. To inject the test organism onto the slant medium and incubated at 37°C for 48 hours after which it was observed for color change, a sterile wire loop was utilized. A positive citrate test was determined by a bright blue color in the medium (Dawodu & Akanbi, 2021). Urea broth was immunized and incubated at 37°C for 24 to 48 h, for the URE test. The transformation of color of the broth from yellow-orange to bright pink was measured to be positive (Hussain et al., 2013).

To evaluate the bacteria which produce the enzyme cytochrome Oxidase, OXI test was carried out. Just a few drops of 1% tetramethyl-p-phenylenediamine

dihydrochloride were used to moisten the filter paper. The growth from the plate was spread over the paper by a wooden applicator. The development of the purple color proposed an encouraging result. No change in color specified a negative result (Hussain et al., 2013). To classify the bacterial strains that produce the enzyme catalase, catalase test was utilized. The addition of the drop of 3 percent hydrogen peroxide solution was done to the sterile slide containing a loopful of the organism. Foaming or bubble indicated a positive result (Dawodu & Akanbi, 2021).

To make a distinction among organisms on the basis of peculiarities in carbohydrate fermentation that was stipulated by the production of gas, the triple sugar-iron agar test utilizing Triple Sugar Iron Agar was structured. The production of gas (formation of CO₂ and H₂) was shown by bubbles or cracks in the agar (formation of CO₂ and H₂).

Minimal Inhibitory Concentration (MIC)

Serial dilutions of each antibiotic were prepared in distilled water. To determine the MIC value of antibiotics, bacterial isolates were grown on nutrient agar. A total of 5 antibiotics were used on each bacterial variant obtained from organic and conventional cows and each antibiotic is used in 5 different concentrations as shown in Table 3. The antibiotics of different classes were selected on the basis of their frequent use in cows. The antibiotics used were of commercial grade. First of all stock solution of each antibiotic was prepared from which further dilutions were made.

Following the disk diffusion method (Kowalska-Krochmal & Dudek-Wicher, 2021), filter paper disks were prepared for each concentration of each antibiotic by dipping in the particular dilution (Table 1). The filter paper disk was then placed onto the media on which particular bacterial isolate has been inoculated. The plates were then incubated for 24 hours at 36°C. After incubation, the plates were examined and the zone of inhibition (if formed) was measured. The particular concentration of a given antibiotic at which the bacterial growth was completely inhibited was considered the MIC value of that antibiotic. Following the CLSI guidelines, the bacterial isolates obtained from raw milk samples were reported as sensitive, intermediate, or resistant to a particular antibiotic (Weinstein, 2021).

Statistical Analysis

Statistical software SPSS 16.0 (Statistical Package for the Social Sciences) was used for the analysis of results. For graphical analysis, a scatter plot was used. Correlation analysis between antibiotics was also

performed. A comparison between the MIC value of different antibiotics tested against bacteria obtained from organic and conventional samples was made through a t-test. Correlation between MIC values of antibiotics against each sample was analyzed using Pearson correlation that investigated the degree of association between the antibiotics (Table 8). All calculations were performed at a level of significance of 0.05 or 0.01 (Table 9).

3. RESULTS AND DISCUSSION

Colony-forming Unit (CFU) Measurement

As the optimal count while evaluating CFU was 30-300 (i.e. a best single plate) we selected the 3rd dilution that falls in this range (Fig. 2).

For each sample, the original volume (initial volume) of the culture plate was 1ml and the final volume was 10ml through which the dilution factor was calculated. For each of the samples, the dilution factor was 10³. The number of colonies, the volume of the culture plate, and the dilution factor were required to measure the CFU (CFU/ml) which was the number of bacteria (CFU) per milliliter of the sample (Table 2).

$$F = \frac{V_f}{V_i} = \frac{10}{1} = 10$$

$$\text{Total dilutions} = \text{Current dilution} \times \text{Previous dilution}$$

$$2^{\text{nd}} \text{ dilution} = 10^1 \times 10^1 = 10^2$$

$$3^{\text{rd}} \text{ dilution} = 10^1 \times 10^2 = 10^3$$

In all raw cow milk samples, the SPC (Standard plate count) was below 250,000 CFU/ml. In conventional raw milk samples (210,000-230,000 CFU/ml) bacterial consortium was higher than organic raw milk samples (195,000-206,000 CFU/ml). Similarly in Serbia, Nada Smigic et al. reported that the bacterial consortium for organic raw milk samples was lower by approx. 1 log CFU/ml compared to conventional raw milk samples (Smigic et al., 2017). In contrast, Juraj Čuboň et al. reported in a similar study performed in the south part of Slovakia that the bacterial consortium was higher in organic milk (86.103 CFU/ml) than conventional (51.103 CFU/ml) (Čuboň et al., 2008). In a similar study in Wisconsin, M. Pol and P.L. Ruegg reported no association between the bacterial consortium and herd type as the SPC of raw cow's milk above 5,000 CFU/ml for 7 conventional and 10 organic herds and below 5,000 for 13 conventional and 10 organic herds (Pol & Ruegg, 2007).

As the minimum SPC of raw milk observed in this study was 195,000 CFU/ml which emphasizes the need to pasteurize the raw milk before consumption in

Pakistan. The regulatory requirements have been contributory in ensuring the raw milk's quality; however, most sectors of the dairy industry perceived that more strictly standards should be established. It was reported in some US states by Burke et al. that for direct consumption of unpasteurized milk there is a requirement of SPC value below 10,000 CFU/ml, which is regulated by state law. In England and Wales, for direct consumption of unpasteurized milk, the SPC must be below 20,000 CFU/ml, whereas 50,000 CFU/ml is the limit for certified raw milk in Germany (Brodziak et al., 2021).

Morphological Characterization

The colony morphological characteristics exhibited by the bacterial colonies were determined after 48 hours of incubation, which showed several variations regarding color, elevation, form, margin, texture, and lactose fermentation (Table 3).

Margins which are the edges of the bacterial colonies were soft i.e. entire except for two isolates that have irregular edges i.e. undulate. Bacterial colonies have also shown elevation as most of them were convex and remaining isolates were simply raised. All the colonies of bacterial isolates were circular while there was an exception for two that have shown slight variation in their shape and were irregular. Bacterial colonies had a smooth texture except for two isolates that were mucoid and one moist. Except for lactose fermentation, all the isolates have shown distinguishable morphological characteristics as all the isolates were lactose positive. As grown on MacConkey agar, most of the bacterial colonies have shown red color but some were dark pink in color (Fig. 3A-D).

Gram Staining

The microscopic appearance of bacterial isolates was studied with the help of the gram staining technique. The prepared slides of bacterial isolates were observed under a binocular light microscope (Fig. 3F-G).

All bacterial isolates were gram-positive. False-negative gram stain also occurred may be because of inadequate specimen or smear preparation Fig. 3F-G (Samuel, Balada-Llasat, Harrington, & Cavagnolo, 2016). The microscopic shape of bacteria was also observed as rod-shaped bacteria were detected in O1M, O2M, A2M-K, and A3M-K while in A1M, O3M, A2M, and A3M cocci in pairs and short chains were observed.

Identification of Bacterial Isolates

All the bacterial isolates were identified using several biochemical tests (Table 4) (Fig. 3H). Our present

study reported eight isolates out of which four microorganisms were detected after screening. The bacteria identified were *Streptococcus*, *Enterococcus*, *Lactobacillus*, and *Bifidobacteria*. All these bacteria were gram-positive, catalase, indole, and oxidase negative with no gas production. Among all isolates, *Lactobacillus* was the only motile bacteria present. Except for *Bifidobacterium*, all isolates were urease negative. On the other hand, *Streptococcus* and *Bifidobacterium* were the only citrate-positive bacteria among all isolates.

Our results were in complete agreement with a similar study performed in the Bahir Dar district by Yeshambel Taye et al. where they isolated and identified *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* from raw milk, yogurt, and cheese by morphological characteristics and biochemical testing (Taye, Degu, Fesseha, & Mathewos, 2021). In Qena city of Egypt, Margret Y. Shafeek et al. isolated *Enterococcus* from raw milk of cow and some dairy products including Kareish cheese, ice cream, and yogurt that were manufactured locally and then identified through biochemical testing and multiplex PCR and concluded that milk and dairy products can play a major role in the spread of *Enterococci* virulent strains to the humans through the food chain (Shafeek, El-Malt, Abdel Hameed, & El-Zamkan, 2018).

Except for *Enterococcus*, all isolates were detected in both conventional and organic raw milk samples. Similar to our results, Kouřimská et al. also observed no differences in terms of the number of coliforms in milk from conventional and organic farming (Kouřimská, Legarová, Panovská, & Pánek, 2014). On the other hand, *Enterococcus* was the bacteria that existed in only conventional raw milk samples not in organic samples. In Italy, Tiziana Silveti et al. also isolated *E. faecalis* strains in period (1997–2009) when higher rates of antibiotics were used (Silveti, Morandi, & Brasca, 2019). The reason may belong to the fact that *E. faecalis* is one of the most important active players of AMR because of its acquired or intrinsic resistance along with dissemination of resistance determinants beyond and within the genus (Silveti et al., 2019). In Gram-positive microorganisms, *E. faecalis* is an ideal indicator of AMR (Authority, Beloeil, Guerra, & Stoicescu, 2018). AMR exhibited by foodborne *E. faecalis* displayed a public health hazard since *E. faecalis* exposure to humans through food could lead to acquisition of AMR bacteria or bacteria-borne AMR genes (Andreolletti et al., 2008). Jamet et al. (2012) also reported that AMR is prevalent in enterococci isolated from French cheese, with chloramphenicol, erythromycin, and tetracycline as the most common

AMRs detected, emphasizing the origin of erythromycin- and tetracycline -resistant genes from the farms and environment (Jamet et al., 2012).

Minimal Inhibitory Concentration (MIC)

For the quantitative study of antibiotic susceptibility, the minimal inhibitory concentration of bacterial isolates was measured (Table 4). In vitro susceptibility testing was performed by the disk diffusion method. Like the MIC value, the category of susceptibility or resistance of the microbe to a given antibiotic was assessed by the bacterial growth inhibition zone (Kowalska-Krochmal & Dudek-Wicher, 2021). Inhibition zones were created by the antibiotics on an agar medium against susceptible bacteria. The diameter of the inhibition zones was measured (in millimeters) around each antibiotic disc and the diameter of the disc was also calculated (Benkova, Soukup, & Marek, 2020). The size of inhibition zones predicted the MIC value of antibiotics. Cultivation of resistant bacteria showed visible growth against antibiotics (Fig. 3E).

The MIC value was calculated using different parameters for each sample against a particular antibiotic such as the size of the disc which was 5mm for all samples. The X-value of each sample at a particular concentration of a given antibiotic was calculated by subtracting the size of the disc from the inhibition zone created on that sample. X^2 was then calculated from the X-value. Log of concentrations of antibiotic was taken and used to determine MIC. Calculation of MIC of each sample against a particular antibiotic was performed on SPSS software 16.0. Inhibition zones created at each concentration of a particular antibiotic were used to obtain the MIC value through a scatter plot. Inhibition zones were the dependent variable while concentrations of antibiotics were independent. X^2 was plotted on the y-axis and Log of concentrations on the x-axis. A fit line was added to the scatterplot which originates the X-intercept.

In each scatterplot, the R^2 value was given which was the coefficient of determination and its value ranged from 0 and 1. R^2 value closer to 1 indicated a stronger linear association (Kiernan, 2014) (Fig. 9). Antilog of X-intercept gave the value of MIC (Nahar, Khatun, & Kabir, 2020). The determined MIC value was compared with MIC clinical breakpoints to evaluate whether the bacterial strain was resistant or susceptible to the antibiotic (Kowalska-Krochmal & Dudek-Wicher, 2021) (Table 5 and 6). Inhibition zones of all samples created by different Antibiotics at each concentration ($\mu\text{g/ml}$) were measured in mm. All inhibition zones created on a particular sample at each

concentration were used to calculate MIC for that sample. Higher R^2 values indicated stronger linear associations (Kiernan, 2014).

Inhibition zones obtained were used for the susceptibility interpretation of each antibiotic against each bacterial isolate (Table 7). The bacterium was considered susceptible (growth inhibited), intermediate (growth partially inhibited), or resistant (growth not inhibited) against a particular antibiotic depending upon the size of the inhibition zone created by that antibiotic (Kowalska-Krochmal & Dudek-Wicher, 2021; Reller, Weinstein, Jorgensen, & Ferraro, 2009). The interpretive standards used were established by the Clinical and Laboratory Standards Institute (CLSI) 2020 ((CLSI), January 2020). The findings revealed that all isolates had shown resistance towards oxytetracycline and cephadrine. Bacterial strains resistant to oxytetracycline such as *Streptococcus* (Jeljaszewicz & Hawiger, 1966), *Enterococcus* (Yu et al., 2009), *Lactobacillus*, (Klare et al., 2007) and *Bifidobacterium* (Gueimonde et al., 2010) and bacterial strains resistant to cephadrine such as *Streptococcus* (Barie, Eachempati, & Shapiro, 2008), *Enterococcus* (Yameen, Iram, Mannan, Khan, & Akhtar, 2013), *Lactobacillus* (Saleem et al., 2018), and *Bifidobacterium* (Charteris, Kelly, Morelli, & Collins, 1998) have previously been reported by several studies. Besides, *Lactobacillus* has shown resistance towards each antibiotic. Resistant *Lactobacillus* strains were also detected in several studies against oxytetracycline (Klare et al., 2007), cephadrine (Saleem et al., 2018), Amoxicillin (Wang et al., 2018), Ciprofloxacin (Saleem et al., 2018), Ampicillin + Cloxacillin (Reuben, Roy, Sarkar, Alam, & Jahid, 2020). *Streptococcus* was resistant to both ciprofloxacin and amoxicillin. Samir N. Patel et al. and Viveka Schaar et al. also reported *Streptococcus* resistant strains against ciprofloxacin (Patel et al., 2011) and amoxicillin (Schaar, Uddbäck, Nordström, & Riesbeck, 2014) respectively (Fig. 4).

Resistance towards amoxicillin and ampicillin + cloxacillin by *Enterococcus* was also detected. Resistant *Enterococcus* strains to amoxicillin (Sanlibaba & Senturk, 2018) and ampicillin + cloxacillin (Bulajić & Mijačević, 2004; Róžańska, Lewtak-Piłat, Kubajka, & Weiner, 2019) have been observed in different studies. *Bifidobacteria* was resistant against ciprofloxacin ampicillin + cloxacillin (Fig. 5). W.P. Charteris et al. in a similar study isolated *Bifidobacterium* from the human gastrointestinal tract and investigated its antibiotic susceptibility and reported ciprofloxacin resistant *Bifidobacteria* strains (Charteris et al., 1998). *Enterococcus* resistant strains to ampicillin + cloxacillin were also observed. Hanna

Róžańska et al. and Snežana BULAJIĆ et al. while investigating AMR in *Enterococcus*, reported it resistant to ampicillin + cloxacillin (Bulajić & Mijačević, 2004; Róžańska et al., 2019). However, variability in resistance among samples was observed by *Streptococcus*, *Enterococcus*, and *Bifidobacterium* towards ampicillin + cloxacillin, ciprofloxacin, and amoxicillin respectively. There are several studies that also reported variability in resistance by bacteria towards antibiotics such as variability in resistance was detected by Hsieh J. C. et al. in *Streptococcus* towards ampicillin + cloxacillin (Hsieh, Yen, & Chuang, 2019). Daojin Yu et al. in *Enterococcus* towards ciprofloxacin (Yu et al., 2009), and W.P. Charteris et al. in *Bifidobacterium* towards amoxicillin (Charteris et al., 1998).

At lower concentrations (0.1 and 1 µg/ml), all isolates were resistant to amoxicillin, cephadrine, and oxytetracycline while for ciprofloxacin and ampicillin + cloxacillin all isolates were resistant at only 0.1 (µg/ml). At 1 (µg/ml), resistance was observed for ciprofloxacin and ampicillin + cloxacillin by *Lactobacillus* and *Bifidobacteria* while *Streptococcus* has shown resistance towards only ciprofloxacin. At higher concentrations (1000, 100, 10 µg/ml), *Enterococcus* and *Streptococcus* were resistant towards cephadrine, *Bifidobacteria*, and *Enterococcus* towards ampicillin + cloxacillin, while for amoxicillin only *Streptococcus* and *Bifidobacteria* were found resistant to oxytetracycline and ciprofloxacin. At 10 and 100 (µg/ml) of amoxicillin, resistant *Enterococcus* strains were detected. For ciprofloxacin, *Streptococcus* and *Lactobacillus* were resistant at 10 (µg/ml) only (Fig. 6).

The results revealed that 44% of AMR was found among all the bacterial isolates against five antibiotics at five different concentrations. Slight variation was reported in AMR among both conventional and organic raw milk bacteria. The AMR present in conventional samples was found to be 39% while organic samples have shown 52% of AMR. Conceivably, this unpredictability of AMR prevalence was because the association among AMR and AMU may be apparent on a larger scale, even though not easily revealed on an individual farm level (Ceccarelli et al., 2020). In a similar study in 2020, Karin Sjöström et al. investigated the difference in AMR patterns between Swedish conventional and organic dairy farms however observed no apparent difference in AMR prevalence among conventional and organic dairy farms (Sjöström et al., 2020). A possible AMR route for livestock may be from humans excreting antibiotic particles (and AMR strains) into the effluent (Hirsch, Ternes, Haberer, & Kratz, 1999) and then

further into surface water that is eventually used by grazing livestock (Atterby et al., 2017). This fact is supported by a study performed by Ivan Literak et al. in which AMR has been reported in bacteria isolated from rats, flies, and other animals that exist in farm environments (Literak et al., 2009). Considering the antibiotics, 47.5% of resistance was shown towards amoxicillin, 45% towards cephadrine, 42.5% towards ampicillin + cloxacillin, ciprofloxacin, and oxytetracycline (Fig. 7).

4. CONCLUSION

The issues regarding emerging antibiotic resistance in raw milk bacteria are complex and of high relevance to humans, animals, and the environment. The findings have shown 13% variation in AMR prevalence in both farm types i.e., 39% in conventional raw milk samples while 52% in organic raw milk samples. In this study, no obvious difference in AMR prevalence was detected among organic and conventional cows, most likely because of the good health of cows and consequently less AMU in both types of dairy farms. Although the number of farms was limited and minor variations may have been problematic to determine, the strict AMU regulation in conventional and organic production systems in district Sialkot highlights the question of whether there is a greater influence exerted by other farm level factors on AMR prevalence in this perspective.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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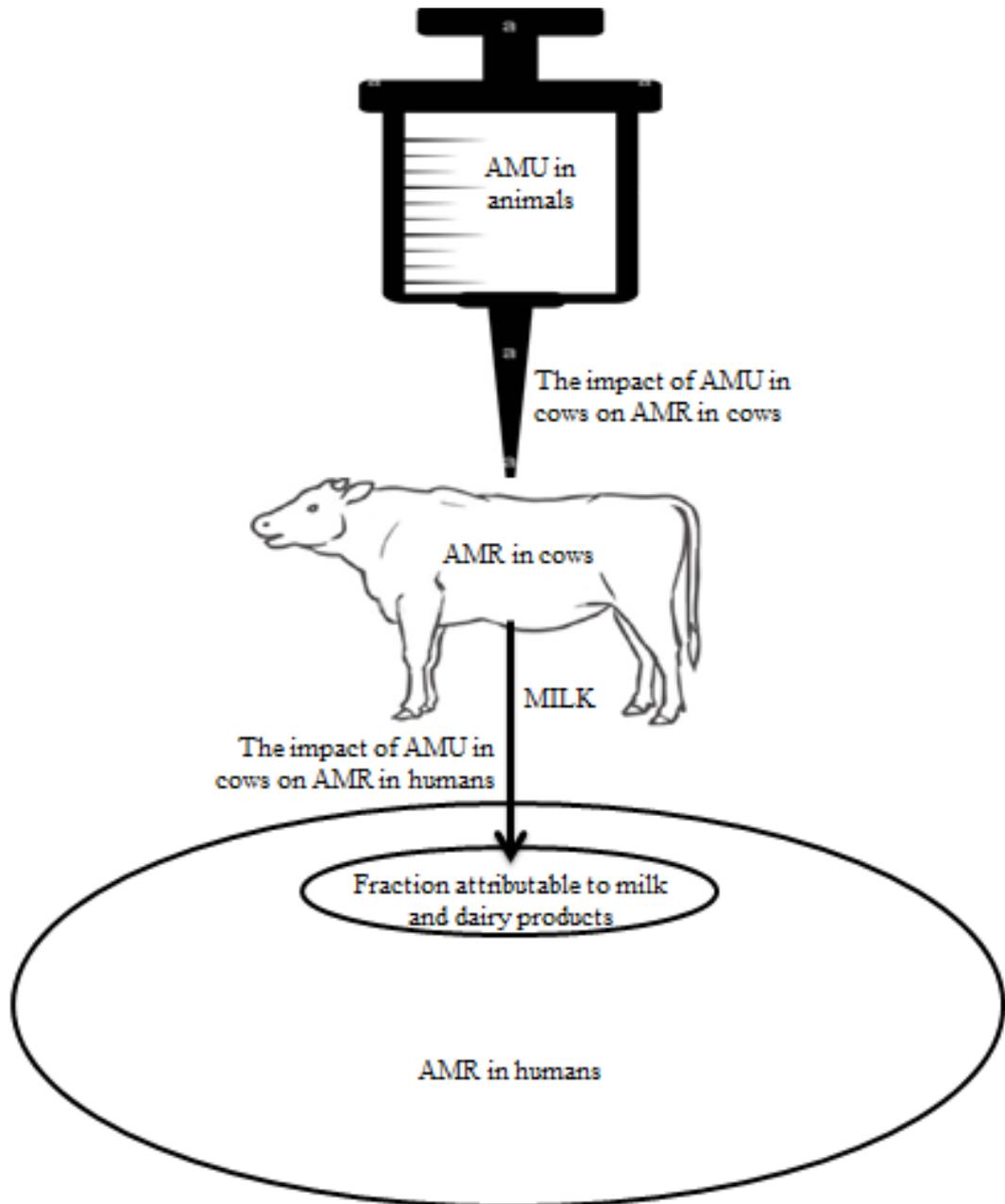


Figure 1. Framework for externality cost of AMR from AMU in cows. Cows treated with antimicrobials may develop AMR that can be transmitted to humans via milk and dairy products [17].

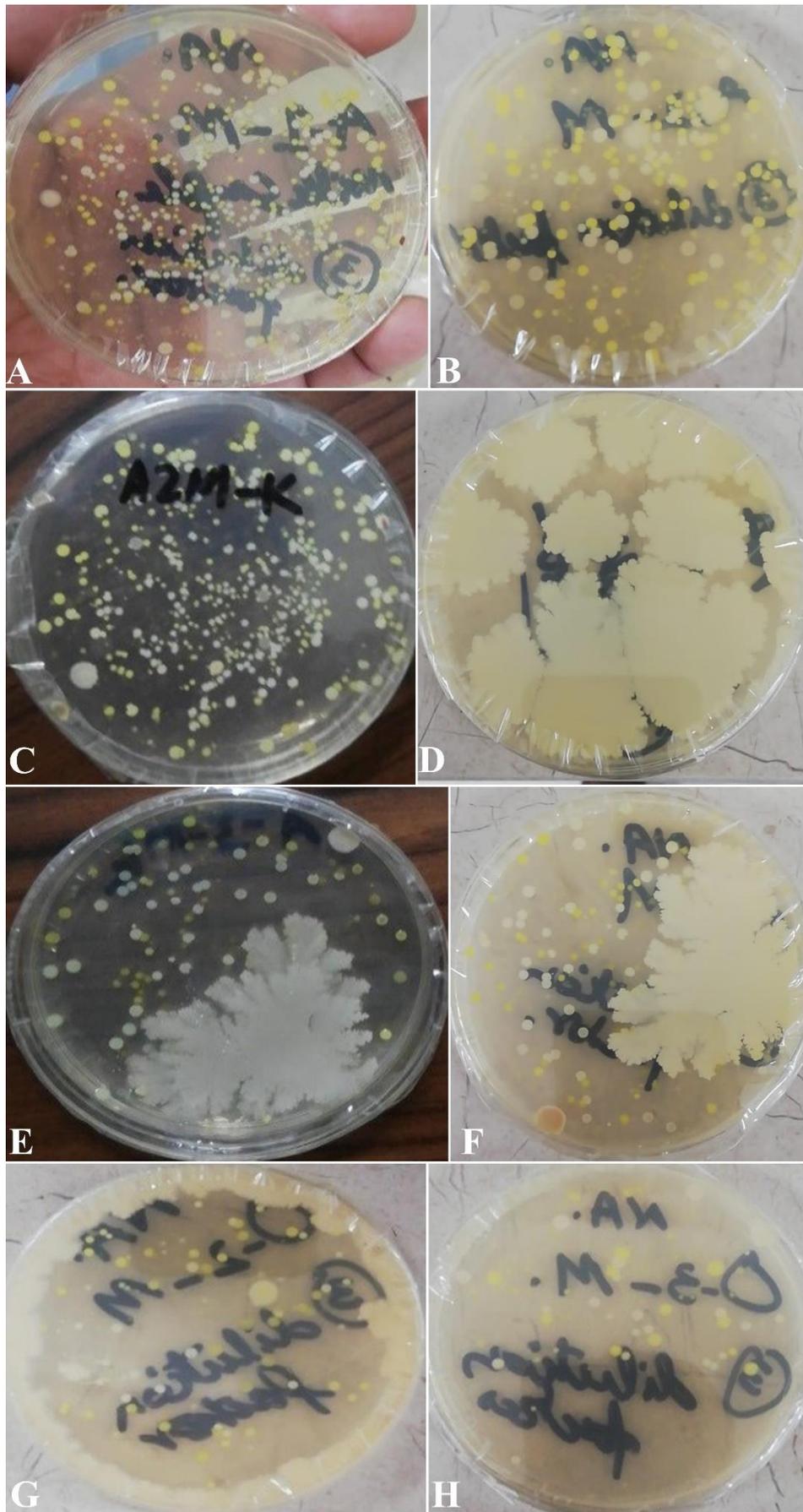
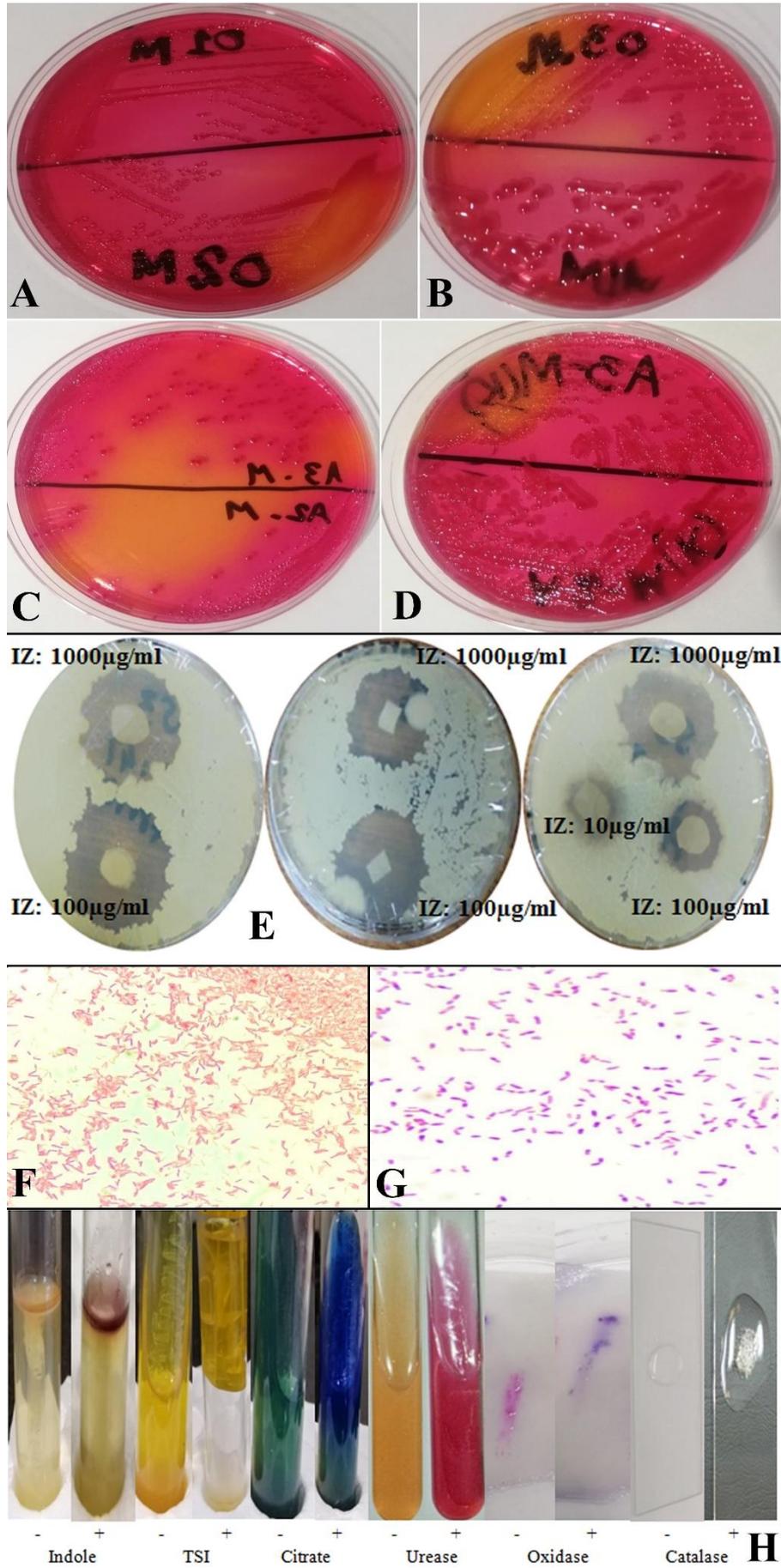


Figure 2. Culture plates showing the viable colonies.



Antibiotic Resistance in Raw Milk of Organic and Conventional Cows

Figure 3. Morphological characteristics exhibited by subcultures on MacConkey agar. **A)** Petri plate streaked with sample id O1M and O2M. **B)** Petri plate streaked with sample id O3M and A1M. **C)** Petri plate streaked with sample id A2M-E and A3M-E. **D)** Petri plate streaked with sample id A2M-K and A3M-K. **(E)** Inhibition zones created by antibiotics. The left and middle Petri plates suggested that bacteria were resistant at 3 concentrations i.e. 0.1, 1, and 10 ($\mu\text{g/ml}$) while inhibition zones (IZ) were created at 100 and 1000 ($\mu\text{g/ml}$). Whereas the right Petri plate indicated that bacteria were resistant at 2 concentrations of 0.1 and 1 ($\mu\text{g/ml}$) while IZs were created at 10, 100 and 1000 ($\mu\text{g/ml}$). All Petri plates depict the most trivial case where there was no overlapping of inhibition zones. **(F-G)** Gram stain testing showing gram-negative (left) in red and gram-positive (right) bacteria in purple under a microscope. **(H)** Different biochemical tests used in the identification of various bacterial isolates.

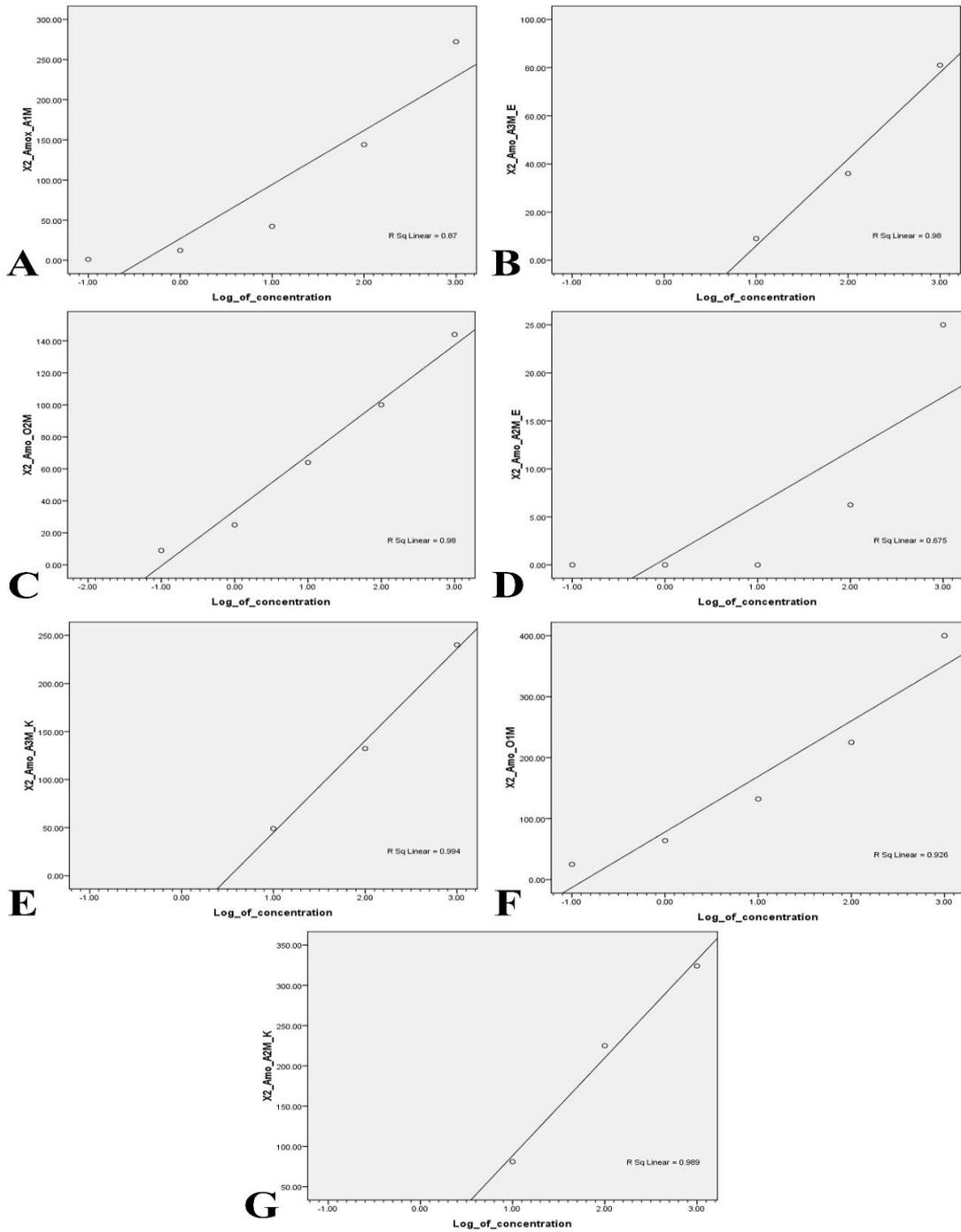


Figure 4. Scatter plot depicting X-intercept for the evaluation of MIC against Amoxicillin.

Antibiotic Resistance in Raw Milk of Organic and Conventional Cows

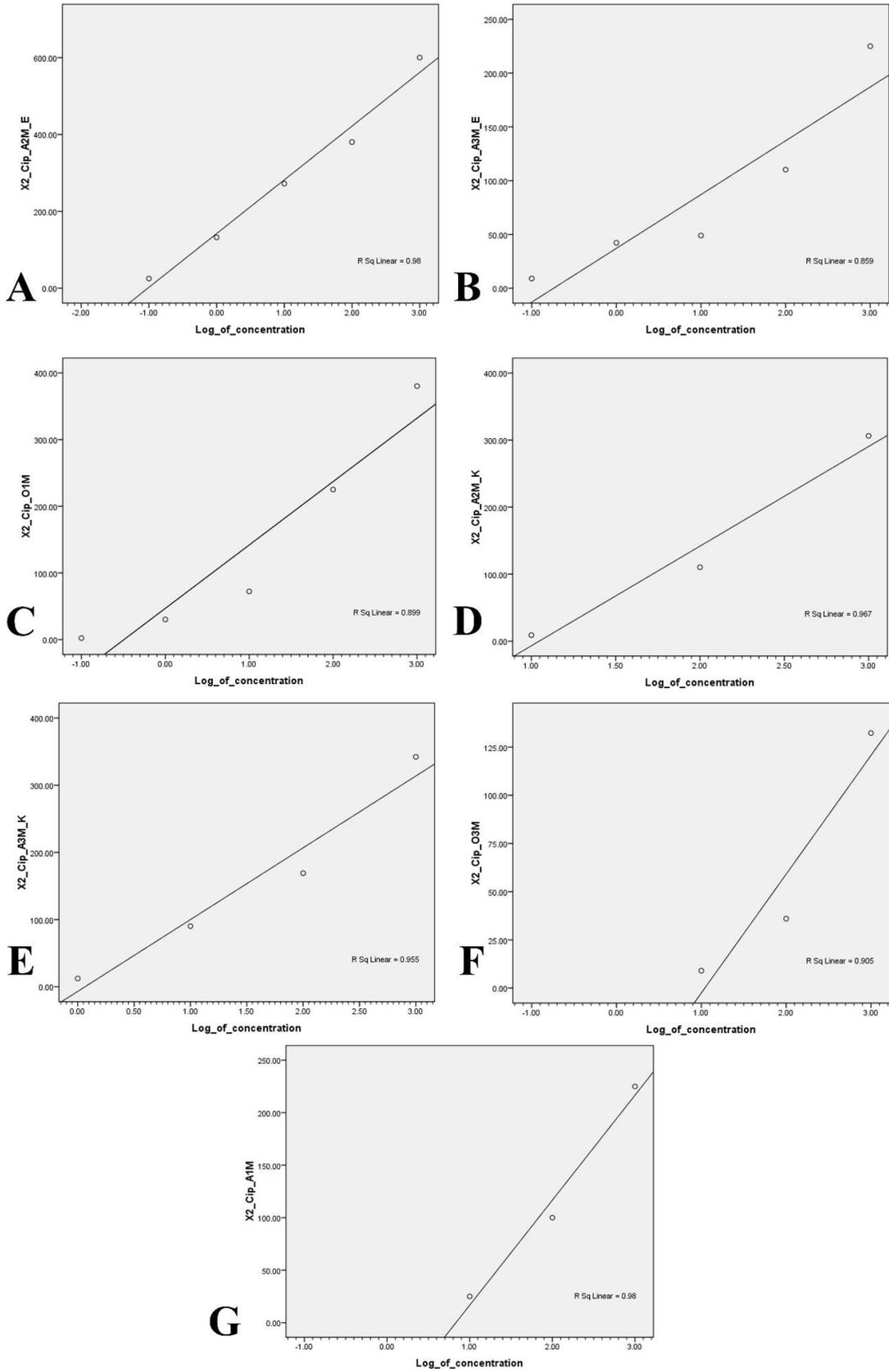


Figure 5. Scatter plot depicting X-intercept for the evaluation of MIC against Ciprofloxacin.

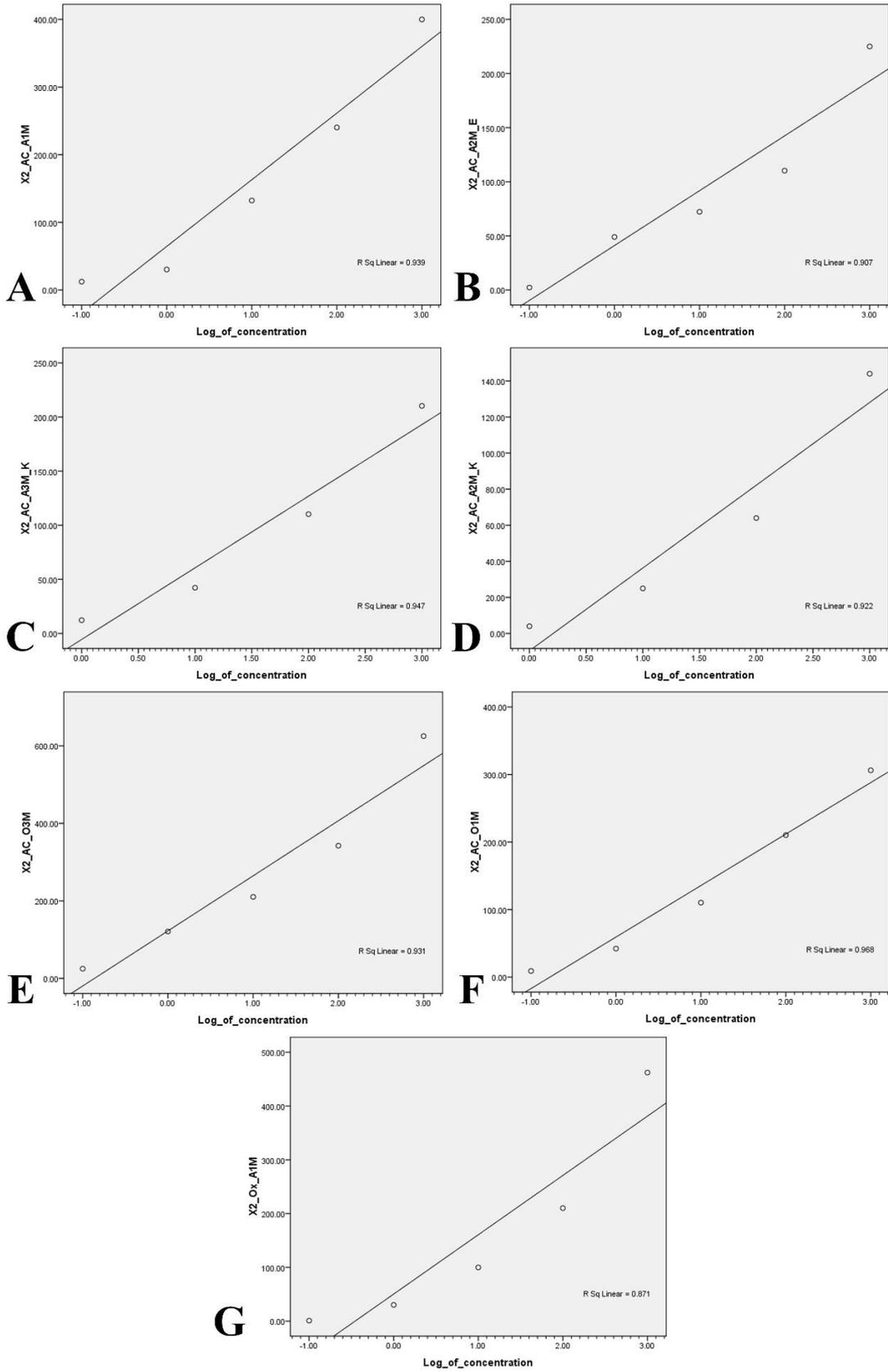


Figure 6. Scatter plot depicting X-intercept for the evaluation of MIC against ampicillin + Cloxacillin and Oxytetracycline.

Antibiotic Resistance in Raw Milk of Organic and Conventional Cows

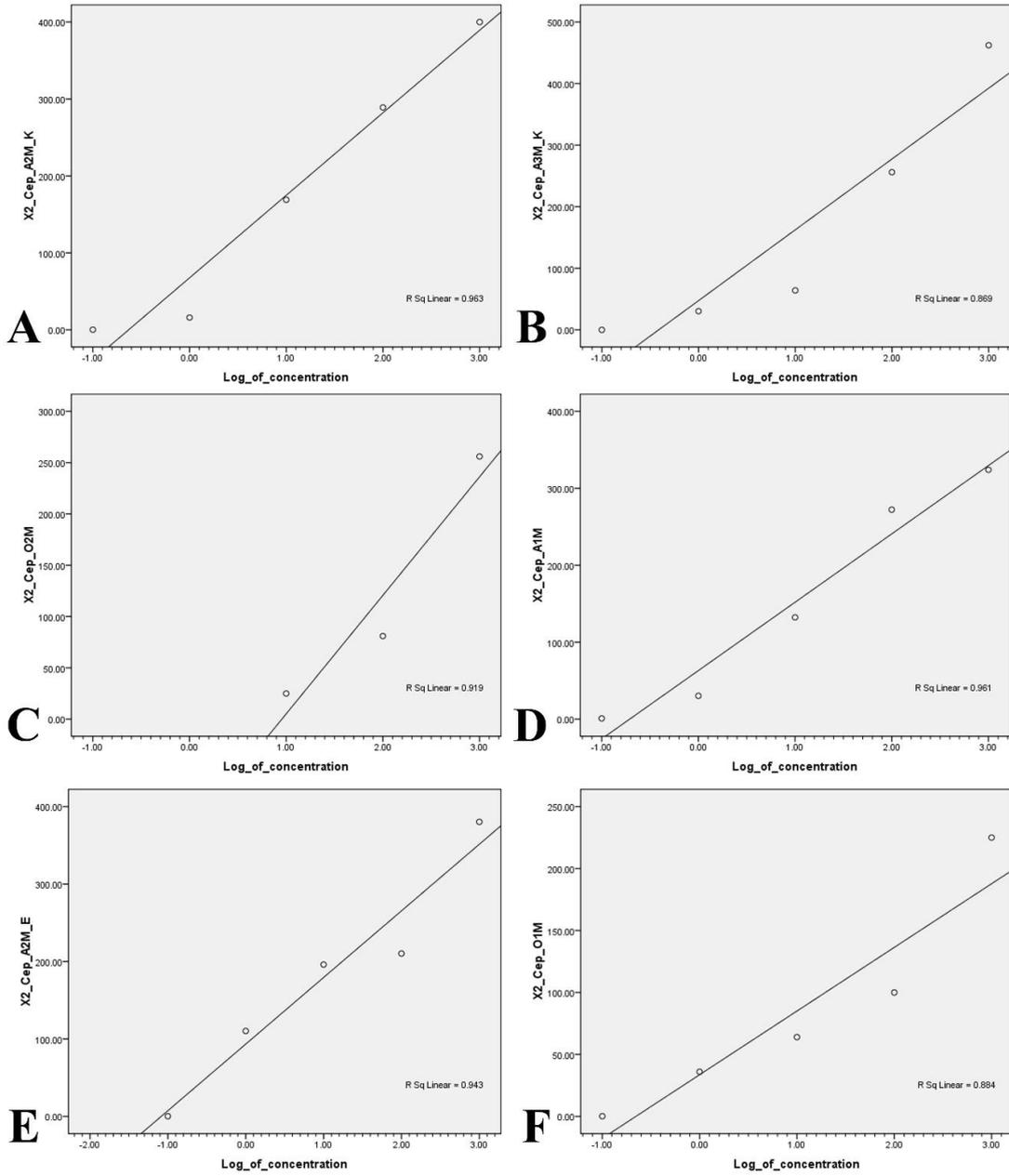


Figure 7. Scatter plot depicting X-intercept for the evaluation of MIC against Cephadrine.

Table 1. Antibiotics and their concentrations.

S/No.	Antibiotics	Concentration ($\mu\text{g/ml}$)				
		1	2	3	4	5
1	Amoxicillin	0.1	1	10	100	1000
2	Ampicillin + Cloxacillin	0.1	1	10	100	1000
3	Cephadrine	0.1	1	10	100	1000
4	Ciprofloxacin	0.1	1	10	100	1000
5	Oxytetracycline	0.1	1	10	100	1000

Table 2. Colony-forming unit of each bacterial isolate.

Sample ID	Dilution Factor	Number of Colonies	CFU (cu/ml)
A1M	10^3	230	230,000 or 2.3×10^6
A2M	10^3	215	215,000 or 2.15×10^5
A3M	10^3	210	210,000 or 2.1×10^5
O1M	10^3	195	195,000 or 1.95×10^5
O2M	10^3	201	201,000 or 2.01×10^5
O3M	10^3	206	206,000 or 2.06×10^5

Table 3. Morphological characteristics exhibited by bacterial isolates.

Sample ID	Color	Elevation	Form	Margins	Texture	Lactose Fermentation
A1M	Red	Convex	Circular	Entire	Moist	Positive
A2M-E	Dark pink	Convex	Circular	Entire	Smooth	Positive
A2M-K	Red	Raised	Circular	Entire	Mucoid	Positive
A3M-E	Dark pink	Convex	Circular	Entire	Smooth	Positive
A3M-K	Red	Raised	Circular	Entire	Mucoid	Positive
O1M	Red	Convex	Irregular	Undulate	Smooth	Positive
O2M	Red	Convex	Irregular	Undulate	Smooth	Positive
O3M	Red	Convex	Circular	Entire	Mucoid	Positive

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Table 4. Biochemical testing of bacterial isolates shows probable bacterial species.

Sample ID	O3M	O2M	O1M	A3M-K	A3M-E	A2M-K	A2M-E	A1M
Gram Stain	+	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-
Urease	-	+	+	-	-	-	-	-
Citrate	+	+	+	-	-	-	-	+
Gas	-	-	-	-	-	-	-	-
Motility	-	-	-	+	-	+	-	-
Probable Bacterial Species	<i>Streptococcus</i> <i>spp.</i>	<i>Bifidobacteria</i> <i>spp.</i>	<i>Bifidobacteria</i> <i>spp.</i>	<i>Lactobacillus</i> <i>spp.</i>	<i>Enterococcus</i> <i>spp.</i>	<i>Lactobacillus</i> <i>spp.</i>	<i>Enterococcus</i> <i>spp.</i>	<i>Streptococcus</i> <i>spp.</i>

Table 5. Calculation of X² value for the evaluation of MIC.

Antibiotic	Amoxicillin against A1M					Amoxicillin against A2M-E				
Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3
Inhibition zone (mm)	7	12	18	29	38	R	R	R	13	15
X	1	3.5	6.5	12	16.5	R	R	R	2.5	5
X2	1	12.25	42.25	144	272.25	R	R	R	6.25	25
Antibiotic	Ampicillin + Cloxacillin against A1M					Ampicillin + Cloxacillin against A2M-E				
Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3
Inhibition zone (mm)	12	16	28	36	45	8	19	22	26	35
X	3.5	5.5	11.5	15.5	20	1.5	7	8.5	10.5	15
X2	12.25	30.25	132.25	240.25	400	2.25	49	72.25	110.25	225
Antibiotic	Cephadrine against A1M					Cephadrine against A2M-E				
Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3
Inhibition zone (mm)	7	16	28	38	41	6	21	28	34	44
X	1	5.5	11.5	16.5	18	0.5	10.5	14	14.5	19.5
X2	1	30.25	132.25	272.25	324	0.25	110.25	196	210.25	380.25
Antibiotic	Ciprofloxacin against A1M					Ciprofloxacin against A2M-E				
Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3
Inhibition zone (mm)	0	0	15	25	35	15	28	38	44	54
X	R	R	5	10	15	5	11.5	16.5	19.5	24.5
X2	R	R	25	100	225	25	132.25	272.25	380.25	600.25
Antibiotic	Oxytetracycline against A1M					Oxytetracycline against A2M-E				

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Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3
Inhibition zone (mm)	7	16	25	34	48	9	14	25	37	48
X	1	5.5	10	14.5	21.5	2	4.5	10	16	21.5
X2	1	30.25	100	210.25	462.25	4	20.25	100	256	462.25

Continued

Antibiotic	Amoxicillin against A2M-K					Amoxicillin against A3M-E				
Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3
Inhibition zone (mm)	R	R	23	35	41	R	R	11	17	23
X	R	R	9	15	18	R	R	3	6	9
X2	R	R	81	225	324	R	R	9	36	81

Antibiotic	Ampicillin + Cloxacillin against A2M-K					Ampicillin + Cloxacillin against A3M-E				
Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3
Inhibition zone (mm)	R	9	15	21	29	R	R	R	R	R
X	R	2	5	8	12	R	R	R	R	R
X2	R	4	25	64	144	R	R	R	R	R

Antibiotic	Cephradine against A2M-K					Cephradine against A3M-E				
Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3
Inhibition zone (mm)	4	13	31	39	45	R	R	R	R	R
X	-0.5	4	13	17	20	R	R	R	R	R
X2	0.25	16	169	289	400	R	R	R	R	R

Antibiotic	Ciprofloxacin against A2M-K					Ciprofloxacin against A3M-E				
Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3
Inhibition zone (mm)	R	R	11	26	40	11	18	19	26	35

X	R	R	3	10.5	17.5	3	6.5	7	10.5	15
X2	R	R	9	110.25	306.25	9	42.25	49	110.25	225
Antibiotic	Oxytetracycline against A2M-K					Oxytetracycline against A3M-E				
Continued										
Antibiotic	Amoxicillin against A3M-K					Amoxicillin against O1M				
Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3
Inhibition zone (mm)	R	R	19	28	36	15	21	28	35	45
X	R	R	7	11.5	15.5	5	8	11.5	15	20
X2	R	R	49	132.25	240.25	25	64	132.25	225	400
Antibiotic	Ampicillin + Cloxacillin against A3M-K					Ampicillin + Cloxacillin against O1M				
Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3
Inhibition zone (mm)	R	12	18	26	34	11	18	26	34	40
X	R	3.5	6.5	10.5	14.5	3	6.5	10.5	15.4	17.5
X2	R	12.25	42.25	110.25	210.25	9	42.25	110.25	210.25	306.25
Antibiotic	Cephradine against A3M-K					Cephradine against O1M				
Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3
Inhibition zone (mm)	5	16	21	37	48	3	17	21	25	35
X	0	5.5	8	16	21.5	-0.4	6	8	10	15
X2	0	30.25	64	256	462.25	0.16	36	64	100	225
Antibiotic	Ciprofloxacin against A3M-K					Ciprofloxacin against O1M				
Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3
Inhibition zone (mm)	R	12	24	31	42	8	16	22	35	44
X	R	3.5	9.5	13	18.5	1.5	5.5	8.5	15	19.5

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	X2	R	12.25	90.25	169	342.25	2.25	30.25	72.25	225	380.25	
	Antibiotic		Oxytetracycline against A3M-K					Oxytetracycline against O1M				
	Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3	
	Inhibition zone (mm)	12	19	21	36	47	R	R	R	15	21	
	X	3.5	7	8	15.5	21	R	R	R	5	8	
	X2	12.25	49	64	240.25	441	R	R	R	25	64	
Continued												
	Antibiotic		Amoxicillin against O2M					Amoxicillin against O3M				
	Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3	
	Inhibition zone (mm)	11	15	21	25	29	R	R	R	R	R	
	X	3	5	8	10	12	R	R	R	R	R	
	X2	9	25	64	100	144	R	R	R	R	R	
	Antibiotic		Ampicillin + Cloxacillin against O2M					Ampicillin + Cloxacillin against O3M				
	Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3	
	Inhibition zone (mm)	R	R	R	R	R	15	27	34	42	55	
	X	R	R	R	R	R	5	11	14.5	18.5	25	
	X2	R	R	R	R	R	25	121	210.25	342.25	625	
	Antibiotic		Cephadrine against O2M					Cephadrine against O3M				
	Log of Conc. (µg/ml)	-1	0	1	2	3	-1	0	1	2	3	
	Inhibition zone (mm)	R	R	15	23	37	R	R	R	R	R	
	X	R	R	5	9	16	R	R	R	R	R	
	X2	R	R	25	81	256	R	R	R	R	R	
	Antibiotic		Ciprofloxacin against O2M					Ciprofloxacin against O3M				

Log of Conc. ($\mu\text{g/ml}$)	-1	0	1	2	3	-1	0	1	2	3
Inhibition zone (mm)	R	R	R	R	R	R	R	11	17	28
X	R	R	R	R	R	R	R	3	6	11.5
X2	R	R	R	R	R	R	R	9	36	132.25
Antibiotic	Oxytetracycline against O2M					Oxytetracycline against O3M				
Log of Conc. ($\mu\text{g/ml}$)	-1	0	1	2	3	-1	0	1	2	3
Inhibition zone (mm)	R	R	R	R	R	R	8	17	22	34
X	R	R	R	R	R	R	1.5	6	8.5	14.5
X2	R	R	R	R	R	R	2.25	36	72.25	210.25

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Table 6. MIC value of Antibiotics at five different concentrations against bacterial isolates.

Antibiotics	Sample ID	Concentrations ($\mu\text{g/ml}$)					R^2	MIC
		0.1	1	10	100	1000		
Amoxicillin	A1M	7	12	18	29	38	0.87	0.2238
	A2M-E	R	R	R	13	15	0.675	0.4466
	A2M-K	R	R	23	35	41	0.989	3.5481
	A3M-E	R	R	11	17	23	0.98	6.3095
	A3M-K	R	R	19	28	36	0.994	2.4547
	O1M	15	21	28	35	45	0.926	0.0794
	O2M	11	15	21	25	29	0.98	0.0602
	O3M	R	R	R	R	R	-	-
Ampicillin + Cloxacillin	A1M	12	16	28	36	45	0.939	0.1318
	A2M-E	8	19	22	26	35	0.907	0.083
	A2M-K	R	9	15	21	29	0.922	1.1481
	A3M-E	R	R	R	R	R	-	-
	A3M-K	R	12	18	26	34	0.947	0.575
	O1M	11	18	26	34	40	0.968	0.0199
	O2M	R	R	R	R	R	-	-
	O3M	15	27	34	42	55	0.931	0.724
Cephradine	A1M	7	16	28	38	41	0.961	0.1096
	A2M-E	6	21	28	34	44	0.943	0.0457
	A2M-K	4	13	31	39	45	0.963	0.1479
	A3M-E	R	R	R	R	R	-	-
	A3M-K	5	16	21	37	48	0.869	0.2238
	O1M	3	17	21	25	35	0.884	0.1174
	O2M	R	R	15	23	37	0.919	6.4565
	O3M	R	R	R	R	R	-	-

Continued

Antibiotics	Sample ID	Concentrations ($\mu\text{g/ml}$)					R^2	MIC
		0.1	1	10	100	1000		
Ciprofloxacin	A1M	R	R	15	25	35	0.98	5.0118
	A2M-E	15	28	38	44	54	0.98	0.0512
	A2M-K	R	R	11	26	40	0.967	6.3095
	A3M-E	11	18	19	26	35	0.859	0.0977
	A3M-K	R	12	24	31	42	0.955	0.5128
	O1M	8	16	22	35	44	0.899	0.1862
	O2M	R	R	R	R	R	-	-
	O3M	R	R	11	17	28	0.905	8.1283
Oxytetracycline	A1M	7	16	15	34	48	0.871	0.1995
	A2M-E	9	14	25	37	48	0.899	0.1995
	A2M-K	9	14	23	29	43	0.84	0.1949
	A3M-E	8	17	24	38	40	0.921	0.1148
	A3M-K	12	19	21	36	47	0.854	0.1584
	O1M	R	R	R	15	21	1	63.0957
	O2M	R	R	R	R	R	-	-
	O3M	R	8	17	22	34	0.872	1.2022

Inhibition zones of all samples created by different Antibiotics at each concentration ($\mu\text{g/ml}$) were measured in mm. All inhibition zones created on a particular sample at each concentration were used to calculate MIC for that sample. Higher R^2 values indicated stronger linear associations [52].

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Table 7. Antibiotic susceptibility testing against bacterial isolates.

Sample ID	A1M					A2M-E					A2M-K				
Organism	<i>Streptococcus spp.</i>					<i>Enterococcus spp.</i>					<i>Lactobacillus spp.</i>				
Antibiotic	Amoxicillin					Amoxicillin					Amoxicillin				
Conc. (µg/ml)	0.1	1	10	100	1000	0.1	1	10	100	1000	0.1	1	10	100	1000
Inhibition zone (mm)	7	12	18	29	38	0	0	0	13	15	0	0	23	35	41
Interpretation	R	R	I	S	S	R	R	R	R	I	R	R	S	S	S
Antibiotic	Ampicillin + Cloxacillin					Ampicillin + Cloxacillin					Ampicillin + Cloxacillin				
Conc. (µg/ml)	0.1	1	10	100	1000	0.1	1	10	100	1000	0.1	1	10	100	1000
Inhibition zone (mm)	12	16	28	36	45	8	19	22	26	35	0	9	15	21	29
Interpretation	R	I	S	S	S	R	I	S	S	S	R	R	I	S	S
Antibiotic	Cephradine					Cephradine					Cephradine				
Conc. (µg/ml)	0.1	1	10	100	1000	0.1	1	10	100	1000	0.1	1	10	100	1000
Inhibition zone (mm)	12	16	28	36	45	8	19	22	26	35	0	9	15	21	29
Interpretation	R	I	S	S	S	R	I	S	S	S	R	R	I	S	S
Antibiotic	Ciprofloxacin					Ciprofloxacin					Ciprofloxacin				
Conc. (µg/ml)	0.1	1	10	100	1000	0.1	1	10	100	1000	0.1	1	10	100	1000
Inhibition zone (mm)	0	0	15	25	35	15	28	38	44	54	0	0	11	26	40
Interpretation	R	R	I	S	S	I	S	S	S	S	R	R	R	S	S
Antibiotic	Oxytetracycline					Oxytetracycline					Oxytetracycline				
Conc. (µg/ml)	0.1	1	10	100	1000	0.1	1	10	100	1000	0.1	1	10	100	1000
Inhibition zone (mm)	7	16	25	34	48	9	14	25	37	48	9	14	23	29	43
Interpretation	R	I	S	S	S	R	R	S	S	S	R	R	S	S	S

Continued

Sample ID	A3M-E					A3M-K					O1M				
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Organism	<i>Enterococcus spp.</i>					<i>Lactobacillus spp.</i>					<i>Bifidobacteria spp.</i>				
Antibiotic	Amoxicillin					Amoxicillin					Amoxicillin				
Conc. (µg/ml)	0.1	1	10	100	1000	0.1	1	10	100	1000	0.1	1	10	100	1000
Inhibition zone (mm)	0	0	11	17	23	0	0	19	28	36	15	21	28	35	45
Interpretation	R	R	R	I	S	R	R	I	S	S	I	S	S	S	S
Antibiotic	Ampicillin + Cloxacillin					Ampicillin + Cloxacillin					Ampicillin + Cloxacillin				
Conc. (µg/ml)	0.1	1	10	100	1000	0.1	1	10	100	1000	0.1	1	10	100	1000
Inhibition zone (mm)	0	0	0	0	0	0	12	18	26	34	11	18	26	34	40
Interpretation	R	R	R	R	R	R	R	I	S	S	R	I	S	S	S
Antibiotic	Cephradine					Cephradine					Cephradine				
Conc. (µg/ml)	0.1	1	10	100	1000	0.1	1	10	100	1000	0.1	1	10	100	1000
Inhibition zone (mm)	0	0	0	0	0	5	16	21	37	48	3	17	21	25	35
Interpretation	R	R	R	R	R	R	I	S	S	S	R	I	S	S	S
Antibiotic	Ciprofloxacin					Ciprofloxacin					Ciprofloxacin				
Conc. (µg/ml)	0.1	1	10	100	1000	0.1	1	10	100	1000	0.1	1	10	100	1000
Inhibition zone (mm)	11	18	19	26	35	0	12	24	31	42	8	16	22	35	44
Interpretation	R	I	I	S	S	R	R	S	S	S	R	I	S	S	S
Antibiotic	Oxytetracycline					Oxytetracycline					Oxytetracycline				
Conc. (µg/ml)	0.1	1	10	100	1000	0.1	1	10	100	1000	0.1	1	10	100	1000
Inhibition zone (mm)	8	17	24	38	40	12	19	21	36	47	0	0	0	15	21
Interpretation	R	I	S	S	S	R	I	S	S	S	R	R	R	I	S
Continued															
Sample ID	O2M					O3M									
Organism	<i>Enterococcus spp.</i>					<i>Lactobacillus spp.</i>									
Antibiotic	Amoxicillin										Amoxicillin				

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Conc. ($\mu\text{g/ml}$)	0.1	1	10	100	1000	0.1	1	10	100	1000
Inhibition zone (mm)	11	15	21	25	29	0	0	0	0	0
Interpretation	R	I	S	S	S	R	R	R	R	R
Antibiotic	Ampicillin + Cloxacillin					Ampicillin + Cloxacillin				
Conc. ($\mu\text{g/ml}$)	0.1	1	10	100	1000	0.1	1	10	100	1000
Inhibition zone (mm)	0	0	0	0	0	15	27	34	42	55
Interpretation	R	R	R	R	R	I	S	S	S	S
Antibiotic	Cephadrine					Cephadrine				
Conc. ($\mu\text{g/ml}$)	0.1	1	10	100	1000	0.1	1	10	100	1000
Inhibition zone (mm)	0	0	15	23	37	0	0	0	0	0
Interpretation	R	R	I	S	S	R	R	R	R	R
Antibiotic	Ciprofloxacin					Ciprofloxacin				
Conc. ($\mu\text{g/ml}$)	0.1	1	10	100	1000	0.1	1	10	100	1000
Inhibition zone (mm)	0	0	0	0	0	0	0	11	17	28
Interpretation	R	R	R	R	R	R	R	R	I	S
Antibiotic	Oxytetracycline					Oxytetracycline				
Conc. ($\mu\text{g/ml}$)	0.1	1	10	100	1000	0.1	1	10	100	1000
Inhibition zone (mm)	0	0	0	0	0	0	8	17	22	34
Interpretation	R	R	R	R	R	R	R	I	S	S

Table 8. Correlations among antibiotics.

		MIC-AMOX	MIC-AMC	MIC-CED	MIC-CFX	MIC-OT
MIC-AMOX	Pearson Correlation	1	.978**	-.339	-.031	-.419
	Sig. (2-tailed)		.004	.510	.954	.409
	N	7	5	6	6	6
MIC-AMC	Pearson Correlation	.978**	1	.541	.637	-.465
	Sig. (2-tailed)	.004		.347	.174	.353
	N	5	6	5	6	6
MIC-CED	Pearson Correlation	-.339	.541	1	.084	-.100
	Sig. (2-tailed)	.510	.347		.893	.873
	N	6	5	6	5	5
MIC-CFX	Pearson Correlation	-.031	.637	.084	1	-.334
	Sig. (2-tailed)	.954	.174	.893		.464
	N	6	6	5	7	7
MIC-OT	Pearson Correlation	-.419	-.465	-.100	-.334	1
	Sig. (2-tailed)	.409	.353	.873	.464	
	N	6	6	5	7	7

** . Correlation is significant at the 0.01 level (2-tailed). Other values of Pearson Correlation are significant at the 0.05 level (2-tailed).

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Table 9. Independent samples test comparing the means of conventional and organic cow's raw milk.

		Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	Df	Sig. (2- tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper	
MIC_AMOX	Equal variances assumed	3.256	.131	- 1.352	5	.234	-2.5267400	1.8694804	-7.3323924	2.2789124
	Equal variances not assumed			- 2.262	4.001	.087	-2.5267400	1.1172654	-5.6285853	.5751053
MIC_AMC	Equal variances assumed	.020	.894	-2.262	4	.806	-.1125250	.4291051	-1.3039118	1.0788618
	Equal variances not assumed			-2.262	2.063	.817	-.1125250	.4302543	-1.9103620	1.6853120
MIC_CED	Equal variances assumed	10517.144	.000	1.625	4	.180	3.1552000	1.9417481	-2.2359570	8.5463570
	Equal variances not assumed			.995	1.000	.501	3.1552000	3.1697686	-37.0942869	43.4046869
MIC_CFX	Equal variances assumed	5.522	.066	.571	5	.593	1.7606500	3.0859733	-6.1720968	9.6933968
	Equal variances not assumed			.420	1.241	.736	1.7606500	4.1944568	-32.3693509	35.8906509
MIC_OT	Equal variances assumed	5961249.963	.000	1.953	5	.108	31.9755300	16.3755043	-10.1190439	74.0701039
	Equal variances not assumed			1.033	1.000	.490	31.9755300	30.9467544	361.2397357	425.1907957

Information is provided in two parts: (A) Levene's Test for Equality of Variances and (B) t-test for Equality of Means. Here, F is the test statistic of Levene's test, *t* is the computed test statistic, Sig. is the p-value corresponding to this test statistic, and *df* is the degrees of freedom. *Std. Error Difference* describes the standard error of the

IMPACT OF *TRICHODERMA ASPERELLUM* FERMENTED AGRICULTURAL WASTE-BASED FEED ON BROILER CHICKEN GROWTH AND MEAT QUALITY

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ABSTRACT

The demand for poultry products is increasing due to the rising global population. This has led to high prices and inadequacy of traditional poultry feed ingredients, creating a need to explore alternative feed sources like agricultural wastes. The present study was conducted to assess the effect of *Trichoderma asperellum*-fermented agricultural waste-based feed on the growth and meat quality of broiler. Wheat bran and rice husk were selected as agricultural waste-based substrates for solid state fermentation (SSF). A total of 25 one-day old broiler chicks were distributed into 5 treatment groups randomly: group 1 was fed completely on basic feed mixture (control), group 2 was fed on diet with 10% unfermented wheat bran (WB), group 3 was fed on diet with 10% fermented wheat bran (FWB), group 4 was fed on diet with 10% unfermented rice husk (RH) and group 5 was fed on diet with 10% fermented rice husk (FRH). The experiment was conducted for 5 weeks. Nutrient analysis showed that solid-state fermentation (SSF) improved the protein and carbohydrate content of the substrates. Statistical analysis of growth performance showed that there was a significant ($P < 0.05$) improvement in body weight in the group fed on diet with 10% fermented wheat bran (FWB) compared to control group. The group fed on 10% fermented rice husk (FRH) maintained a similar body weight as compared to control group. The groups fed on 10% unfermented wheat bran (WB) and 10% unfermented rice husk (RH) had lower body weight than control group. No significant changes were observed in meat quality parameters among all the treatment groups. In conclusion, fermenting the wheat barn through fungus *Trichoderma asperellum* not only improved the nutrient content by degrading complex components but also had a positive effect on chicken growth. This study suggests that wheat bran fermented by *Trichoderma asperellum* can be added to broiler diets at up to 10% without any deleterious effect on growth and meat quality.

1. INTRODUCTION

The poultry industry is experiencing feed price fluctuations due to rising costs of key feed ingredients prompting poultry nutritionists to explore alternative feed sources (Sugiharto et al., 2017). Some developing nations are producing various alternative ingredients such as wheat bran, rice bran, cottonseed meal, palm kernel cake and copra meal derived from agricultural wastes and byproducts. These feedstuffs are more affordable compared to main grain crops. However, their high fibre contents limit their usefulness for monogastric animals (Oguri et al., 2013; Chu et al., 2017).

For example, wheat bran and rice husk are among such ingredients with restricted applications in monogastric diets. The outer coat of seed from flour mills is known as wheat bran (Saini et al., 2023). It has the potential to replace yellow corn in poultry diets partially or completely (Alshelmani et al., 2021). WB includes a significant quantity of protein (160 g/kg), complete dietary fibre (451 g/kg), carbohydrates (177 g/kg), minerals (61.5 g/kg) and fat (47g/kg) (Teng et al., 2017; Junejo et al., 2019). It has high fibre content but low metabolizable energy (ME), thus its use in poultry diets is limited. Its nutrient content can be improved by microbial fermentation. It has been found that replacement of 10% basal diet with fermented wheat

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bran can be incorporated to broiler diets without any deleterious effect (Chu et al., 2017).

Rice husk is an agricultural waste generated during rice grain milling. Currently, large piles of rice husks continue to accumulate in many milling areas, where they are often burned for heat or discarded as waste. This disposal method poses significant environmental risks, including pollution and resource wastage. The recent trend in livestock farming is to utilize agricultural waste as substitute feed ingredients in broiler and other animal feeding trials. Although rice husk has long been recognized as a potential feed ingredient, its high silica content, high fiber levels, and harsh texture make it difficult to degrade, limiting its application in animal feed (Ikpe and Oko, 2019). Fermentation is a technique that enhances nutritional value of feed ingredients by reducing crude fiber and antinutritional contents (Predescu et al., 2024). To improve the nutrient content of agricultural wastes and by-products, researchers have been using fungal inoculums in recent years (Sharma and Arora, 2011; Choudhary et al., 2016). Recent studies have employed various *Trichoderma* species to ferment wheat bran and rice husk, breaking down complex compounds such as cellulose, hemicellulose, lignin, and non-starch polysaccharides into simpler forms. This process enhances their digestibility for broilers and improves their nutrient content (Aderolu et al., 2007; Chu et al., 2017).

Trichoderma are free living and filamentous fungi. They are usually found on decomposing plants, wood, and in the plant rhizosphere, where they provoke general tolerance against pathogens (Afzal et al., 2021; Jangir et al., 2017). Species of *Trichoderma* are good manufacturers of cellulases. They have strong penetration ability into non-soluble substrates and are therefore considered highly suitable for fermenting materials containing lignin and cellulose (Elmasry et al., 2017). Chiang et al. (2009) described that solid state fermentation enhanced the digestibility of nutrients, increased lactobacilli count in the large intestine, improved the gut morphology of broiler chickens and reduced the cost of poultry farming. Alshelmani et al. (2017) stated that broilers fed on feed compositions accompanied by *Paenibacillus polymyxa*-fermented palm kernel cake exhibited improved growth rates and meat quality. Likewise, Chu et al. (2017) pointed out that *Trichoderma pseudokoningii*-fermented wheat bran supplemented diets improved the growth rate and intestinal morphology of broilers. Similarly, Gungor and Erener (2020) showed that broilers given a diet supplemented with 1% *Aspergillus niger*-fermented sour cherry kernel improved weight gain and feed conversion ratio

in broilers. (Aderolu et al., 2007) indicated that *Trichoderma viride* fermented rice husk showed improved degradation of cellulosic content and increased energy content. Elmasry et al. (2017) indicated that wheat bran fermented with *Trichoderma longibrachiatum* as 10% feed additive improved the growth performance and feed consumption of broilers than control group. According to Hatta et al. (2014) broilers fed diets containing copra meal fermented with *Trichoderma viride* exhibited similar growth performance to the control group. Similarly, Marcinčák et al. (2018) described that corn meal fermented with *Umbelopsis isabellina* improved the growth and quality of meat of broilers fed on it as compared to control group.

Only a few microbes have been investigated so far to improve the nutritional value and digestibility of agricultural waste-based feed ingredients. In the current study, the potential of a lignocellulose degrading fungus *Trichoderma asperellum* was evaluated to improve the nutritive value of agro-waste products and its effects on broiler growth and meat quality were assessed.

2. MATERIALS AND METHODS

Collection and culturing of fungus

The previously isolated and identified fungus, *Trichoderma asperellum*, was procured from the Department of Biology, Lahore Garrison University. Potato dextrose agar (PDA) was utilized as the growth medium. The media was prepared and poured into petri plates under sterile conditions and allowed to solidify. The fungal inoculum was inoculated onto PDA plates with the help of sterile inoculating loop and incubated at 25-30°C for 7 days.

Collection of substrate samples

Wheat bran and rice husk were selected as substrates for solid state fermentation. Both were purchased from the local market and stored under ambient conditions until fermentation.

Pretreatment, Solid state fermentation (SSF), and Nutrient analysis

Aluminum boxes each containing 100 grams of wheat bran and rice husk were filled with deionized water to achieve 50% moisture content, mixed thoroughly, packed, and autoclaved. The boxes were then cooled to room temperature (Chu et al., 2017). For solid-state fermentation, a sterile saline solution was added to cultured PDA plates, and fungal spores of *Trichoderma asperellum* were harvested and suspended at a concentration of 1×10^5 to 1×10^7 spores per ml. Each kilogram of wheat bran and rice husk was

inoculated with 200 ml of spore suspension, packed, and incubated at 30°C for two weeks.

Post-incubation, the samples were autoclaved, washed, sun-dried, and stored at room temperature (Elmasry et al., 2017). For nutrient analysis, the fermented and unfermented samples were mixed with phosphate buffer, ground, and centrifuged. The supernatants were collected for protein and carbohydrate quantification. Protein content was measured using the Lowry method, with absorbance recorded at 660 nm (Satpathy et al., 2020), while carbohydrates content was determined using the Anthrone test, with absorbance recorded at 630 nm. Results were expressed as milligrams of protein or carbohydrate per gram of substrate.

Experimental birds, Housing, and Categorization

Twenty-five healthy one-day broiler chicks were separated from the flock and housed in a local animal shed in Gujranwala city. The chicks were weighed and randomly assigned to five dietary groups: Group 1 was fed a basic feed mixture (control), Group 2 received a diet with 10% unfermented wheat bran (WB), Group 3 received a diet with 10% fermented wheat bran (FWB), Group 4 was fed a diet with 10% unfermented rice husk (RH) and Group 5 received a diet with 10% fermented rice husk (FRH). The experiment was conducted over 35 days, during which the chicks had ad libitum access to feed and water.

Statistical analysis

The data for total protein and carbohydrate content was subjected to two sample T-test, assuming equal variances, in SPSS software to evaluate the statistical effect of microbial fermentation on wheat bran and rice husk. Data on body weight and meat quality were subjected to one-way ANOVA using SPSS software to assess the effects of experimental diets on the treatment groups. Significant differences between treatment groups were considered at a significance level of $P < 0.05$.

3. RESULTS AND DISCUSSION

Protein analysis

The protein content of unfermented and *Trichoderma asperellum*-fermented wheat bran and rice husk was analyzed through Lowry method, with results presented in Table 1. Fermentation with *T. asperellum* significantly increased the protein content of wheat bran from 19.06±0.13 mg/g to 54.51±0.09 mg/g, and rice husk from 10.56±0.06 mg/g to 10.80±0.06 mg/g. According to the t-test analysis, the increase in protein concentration for both fermented wheat bran and fermented rice husk compared to their unfermented

counterparts was statistically significant ($P < 0.05$), as shown in Table 1.

Carbohydrate analysis

The carbohydrate content of unfermented and *Trichoderma asperellum*-fermented wheat bran and rice husk was analyzed through anthrone test, with results presented in Table 2. Solid-state fermentation with *Trichoderma asperellum* increased the carbohydrate content of wheat bran from 16.45±0.07 mg/g to 22.58±0.14 mg/g and rice husk increased from 6.45±0.06 mg/g to 6.50±0.12 mg/g. According to t-test analysis, the increase in carbohydrate concentration for fermented wheat bran compared to unfermented wheat bran was statistically significant ($P < 0.05$). However, for rice husk, the increase in carbohydrate concentration was not statistically significant ($P > 0.05$), as shown in Table 2.

Effect of designed feed mixtures on the growth performance

The effects of the designed feed mixtures on the body weight of broiler chickens are presented in Table 3. One-way ANOVA revealed statistically significant differences in the body weights of chicks among the treatment groups at weeks 2, 3, 4, and 5 ($P < 0.05$). A least significant difference (LSD) post hoc test showed that the group fed a diet containing standard feed with 10% fermented wheat bran (FWB) exhibited significantly higher body weight compared to the control and other treatment groups after five weeks. This result highlights the positive effect of fermented wheat bran. Figure 1 provides a graphical representation of the effects of the designed feed mixtures on the body weight of broiler chicks from week 1 to week 5.

Effect of designed feed mixtures on the meat quality

The parameters used to measure meat quality were pH and colour with results presented in Table 4. The L*, a*, and b* values were used as indicators of breast meat colour. Chicken meat colour primarily depends on lightness values. One-way ANOVA revealed no significant differences in breast meat colour and pH among the treatment groups, indicating that the feed supplements did not result in any significant changes in meat quality. Figures 2 and 3 provide graphical representations of the pH and color differences between the treatment groups. The current study assessed the effect of fungal fermented agricultural waste-based feed on broiler chicken growth and meat quality, using the fungus *Trichoderma asperellum* (previously isolated and identified). Fermentation of wheat bran with *T. asperellum* increased protein content from 19.06 mg/g to 54.51 mg/g, and rice husk

protein content increased from 10.56 mg/g to 10.80 mg/g. Fungi grow on substrates and secrete various cellulose-degrading enzymes that convert non-starch polysaccharides into simple sugars. These sugars are absorbed by the fungus to stimulate metabolic activities and biosynthesis, leading to an increase in protein content (Chu et al., 2017). Furthermore, the fungi's ability to produce enzymes capable of hydrolyzing starch and non-starch polysaccharides into monosaccharides contributes to increased protein proportion, as monosaccharides are easily converted into protein (Bayitse et al., 2015). Carbohydrate content (simple sugars) of wheat bran increased from 16.45 mg/g to 22.58 mg/g after solid-state fermentation (SSF) with *T. asperellum*, while rice husk only showed a minor increase from 6.45 mg/g to 6.50 mg/g. The enzymatic activity during SSF efficiently breaks down lignocellulosic material (crude fiber) into reducing sugars, which are more digestible for poultry than crude fiber (Lin et al., 2018).

These findings align with previous studies, such as Chu et al. (2017), who reported that *T. pseudokoningii*-fermented wheat bran increased by reducing sugars and crude protein content. Similarly, Omwango et al. (2013) observed that pineapple waste fermented with *T. viride* enhanced crude protein content while reducing crude fiber. Ezekiel and Aworh (2013) showed that *T. viride*-fermented cassava peel significantly improved crude protein content while reducing crude fiber. Aderolu et al. (2007) demonstrated that *T. viride*-fermented rice husk reduced NDF and ADF (crude fiber) content. SSF was employed to ferment wheat bran and rice husk, chosen based on substrate characteristics. SSF provides better yield and simplifies downstream processing compared to submerged fermentation (Sugiharto and Ranjitkar, 2019). Cellulose, starch, and lignin are commonly used in SSF as carbon sources, with bacteria and yeast typically growing on the substrate surface, while fungi invade the substrate through mycelium (Srivastava et al., 2019).

In this study, the treatment group fed a diet with 10% fermented wheat bran (FWB) showed a significant increase in body weight (BW) after 5 weeks, reaching 1380 g/bird, compared to the control group (1304 g/bird) and the group fed 10% unfermented wheat bran (WB) (1296.2 g/bird). These results align with Elmasry et al. (2017), who reported a significant increase in BW in broilers fed on wheat bran fermented with *T. longibrachiatum* (SF1). Gungor and Erener (2020) also found improved growth in broilers fed sour cherry kernel fermented with *Aspergillus niger*. However, Hatta et al. (2014) reported that the growth rate of broilers fed fermented wheat bran was

like the control group. Similarly, Chuang et al. (2019) found no significant change in the growth of broilers fed a diet with *Aspergillus oryzae* and *Saccharomyces cerevisiae*-fermented wheat bran.

In contrast, the treatment groups fed 10% rice husk (RH) and 10% fermented rice husk (FRH) did not show significant changes in body weight compared to the control group. The group fed 10% rice husk (RH) had a slightly lower body weight (1293.4 g/bird) than the control group (1304 g/bird), but this difference was not statistically significant. The group fed 10% fermented rice husk (FRH) had a body weight (1311 g/bird) like the control group (1304 g/bird). These results are consistent with Ikpe and Oko (2019), who reported no significant change in body weight in groups fed rice husk fermented with molasses syrup. Post-slaughter, muscle pH is an important factor influencing meat quality traits, such as color, water holding capacity, and tenderness. The normal pH for breast muscle is above 5.70 (Alshelmani et al., 2016). In the present study, no significant differences were observed in pH between treatment groups (10% WB, 10% FWB, 10% RH, and 10% FRH) and the control group. However, the pH of the 10% rice husk group was slightly lower than the control group. The pH range was from 5.58 to 5.82 across all treatment groups. These findings are in line with Alshelmani et al. (2016), who reported no significant difference in breast muscle pH when palm kernel cake was treated with *Paenibacillus polymyxa* (ATCC 842). Meat color can be influenced by various factors, including nutrition, sex, age, strain, processing methods, intramuscular fat, and pH. A notable relationship exists between pH and meat color, where a decrease in pH can lead to protein denaturation, resulting in pale color. Lightness (L^*) values are commonly used to assess meat color. In the present study, breast meat color across all treatment groups fell within the normal range ($56 \leq L^* \leq 62$), indicating no significant differences in color among the groups. These results are consistent with Ashayerizadeh et al. (2018), who reported no significant effect on breast meat color and pH in broilers fed rapeseed meal fermented with *Aspergillus niger*, *Lactobacillus acidophilus*, and *Bacillus subtilis*.

4. CONCLUSION

It was concluded that *Trichoderma asperellum*-fermented wheat bran and rice husk can serve as effective feed ingredients in poultry diets. *Trichoderma asperellum*-fermented wheat bran can be included in broiler diets up to 10% without negatively affecting growth or meat quality.

5. CONFLICT OF INTEREST

All authors declare that they have no conflict of interest regarding the publication of this article.

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Table 1. Protein content of fermented and unfermented substrates

Substrate samples	Protein concentration (mg/g)	P-value
Unfermented wheat bran	19.06±0.13 mg	0.000
<i>Trichoderma asperellum</i> fermented wheat bran	54.51±0.09 mg	
Unfermented rice husk	10.56±0.06 mg	0.049
<i>Trichoderma asperellum</i> fermented rice husk	10.80±0.06 mg	

Values symbolize the average (mean) of 3 replicates of each treatment group along with standard error.

Table 2. Carbohydrate content of fermented and unfermented substrates

Substrate samples	Carbohydrate concentration (mg/g)	P-value
Unfermented wheat bran	16.45±0.07 mg	0.000
<i>Trichoderma asperellum</i> fermented wheat bran	22.58±0.14 mg	
Unfermented rice husk	6.45±0.06 mg	0.718
<i>Trichoderma asperellum</i> fermented rice husk	6.50±0.12 mg	

Values symbolize the average (mean) of 3 replicates of each treatment group along with standard error.

Table 3. Effect of designed feed mixtures on average weekly live body weight of broiler chickens

	DESIGNED FEED MIXTURES					P-value
	T1	T2	T3	T4	T5	
Weeks	Control	Diet with 10% WB	Diet with 10% FWB	Diet with 10% RH	Diet with 10% FRH	
week 0	43.14±0.51	43.60±0.42	43.54±0.48	43.34±0.54	43.56±0.59	0.965
week 1	138.8±3.60	134±1.70	135.6±1.50	131.6±2.48	130.6±3.76	0.317
week 2	337.4 ^{ab} ±5.89	323.2 ^b ±14.0	370.8 ^a ±14.7	306.2 ^b ±16.7	310.6 ^b ±7.15	0.011
week 3	618 ^b ±8.60	616.4 ^b ±11.7	672 ^a ±8.60	606.2 ^b ±19.0	614.2 ^b ±13.3	0.012
week 4	900.4 ^b ±21.7	943.4 ^{ab} ±22.4	989.2 ^a ±11.7	931.4 ^b ±11.8	928.4 ^b ±11.4	0.019
week 5	1304 ^b ±23.3	1296.2 ^b ±17.3	1380 ^a ±11.4	1293.4 ^b ±16.5	1311.8 ^b ±15.1	0.011

Values symbolize the average (mean) of 5 models of each treatment group along with standard error. Means within same rows with different superscript letters (ab) are significantly different ($p < 0.05$). WB: wheat bran, FWB: fermented wheat bran, RH: rice husk, FRH: fermented rice husk.

Body weight week 1 to 5

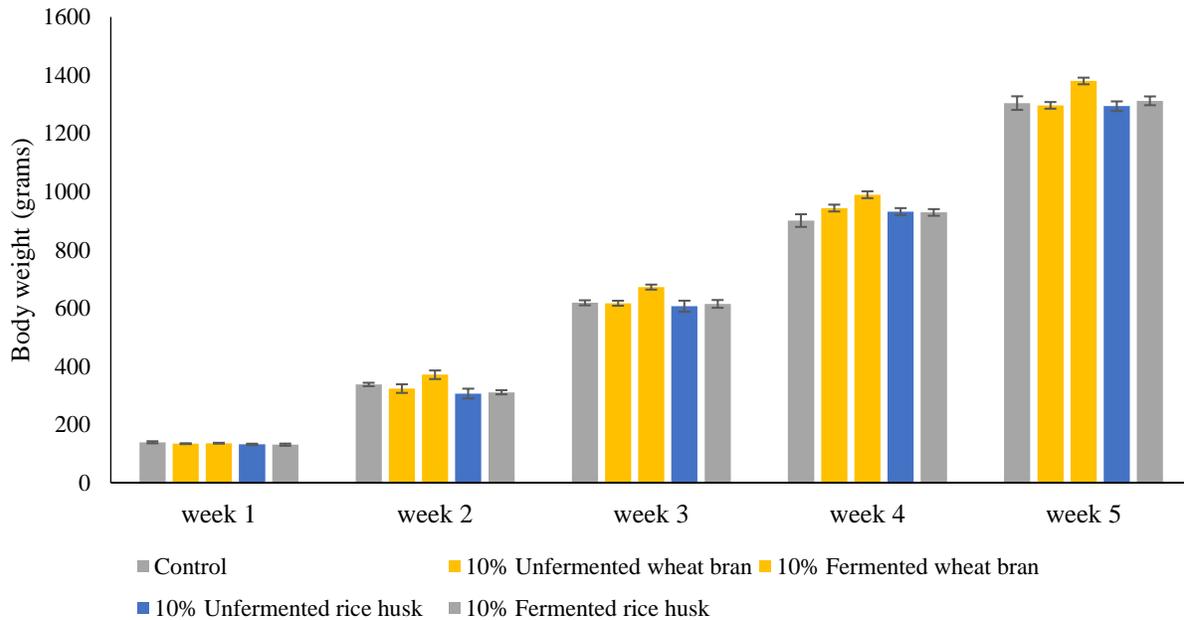


Figure 1. Effect of designed feed mixtures on the body weight of broiler chicks during the experiment study Each bar represents average body weight along with standard error (n=5). Fermented wheat bran-based feed improved the body weight in each successive week shown with yellow bar.

Table 4: Effect of designed feed mixtures on breast meat colour and pH of broiler chicken

Parameters	DESIGNED FEED MIXTURES						P-value
	Control	WB	FWB	RH	FRH	SEM	
Breast pH	5.82	5.71	5.77	5.58	5.71	0.021	0.261
L* (Lightness)	56.97	57.22	57.36	56.29	58.39	0.239	0.083
a* (redness)	3.74	3.78	4.09	3.94	3.76	0.120	0.890
b* (yellowness)	6.49	6.42	6.84	7.26	6.54	0.112	0.092

Values symbolize the average (mean) of 5 models of each treatment group. WB: wheat bran, FWB: fermented wheat bran, RH: rice husk, FRH: fermented rice husk. SEM: standard error of mean.

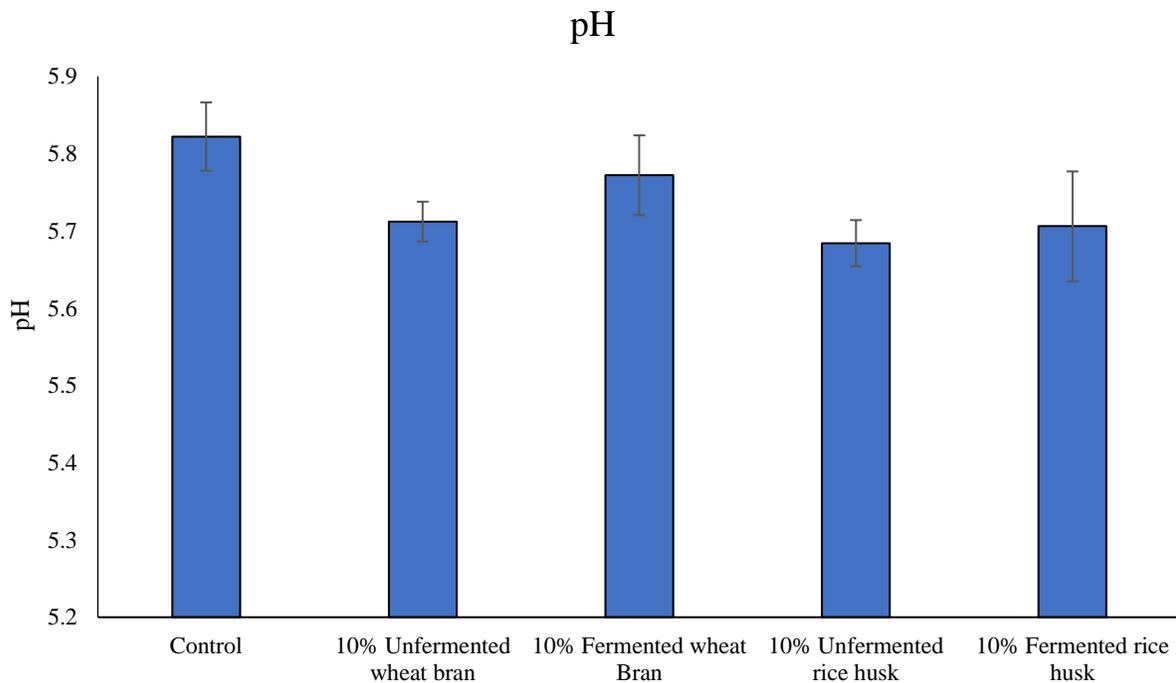


Figure 2. Effect of experimental diets on the pH of broiler chicken. Each bar represents average pH along with standard error (n=5). No significant difference was detected between treatment groups (Least significant difference, P<0.05).

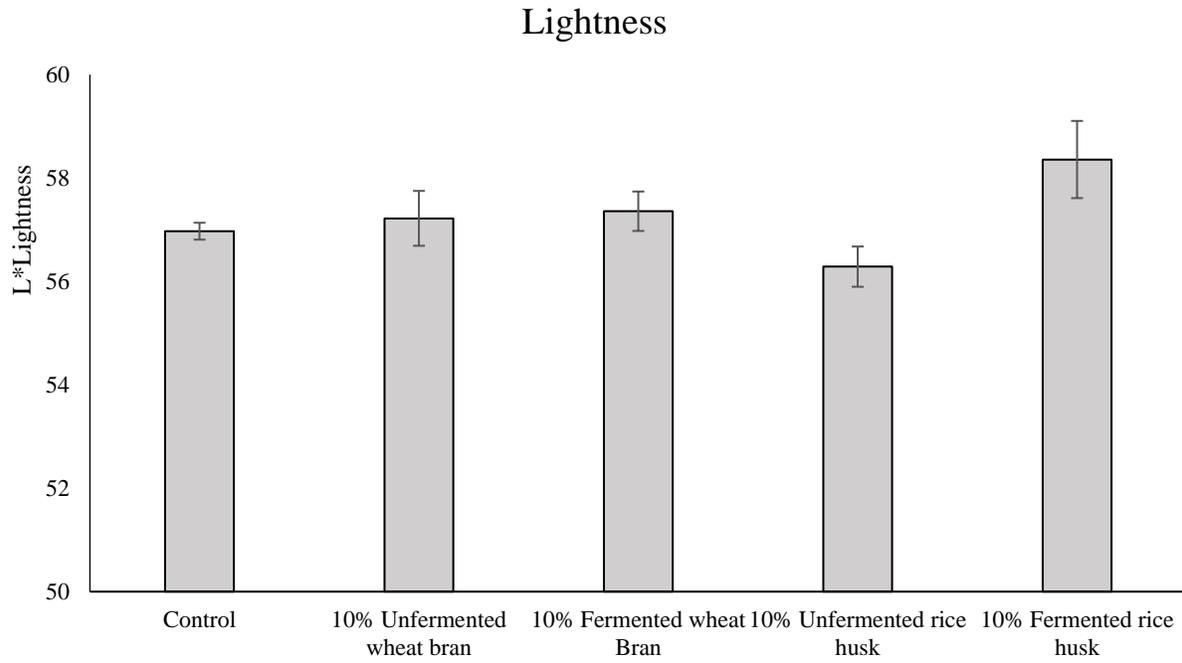


Figure 3. Effect of experimental diets on the breast meat colour (L*) of broiler chicken. Each bar represents average values of lightness along with standard error (n=5). No significant difference was detected between treatment groups (Least significant difference, P<0.05).

STUDIES ON SOME PHENOTYPIC TRAITS OF WEST AFRICAN DWARF GOATS IN DERIVED SAVANNAH ZONE OF NIGERIA

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ABSTRACT

In the Ejigbo Local Government Area of Osun State, a Nigerian derived savanna zone, a study was carried out to examine certain phenotypic characteristics of West African dwarf goats. Three hundred ninety-nine (399) West African dwarf goats were sampled in order to determine their live weight and body measurements. Analysis was done on data pertaining to 194 bucks and 205 does. These were categorised into six age groups (milk teeth, 2-teeth, 4-teeth, 6-teeth, 8-teeth, and worn teeth) and recorded by sex. In order to characterise the goat breeds in the trial region, the goal was to give comprehensive information on linear body measurements. For both sexes, body measurement values rose significantly ($P < 0.05$) with age. For bucks, the mean body weight was 5.32 ± 0.15 to 29.63 ± 1.88 kg, while for does, it was 5.75 ± 0.13 to 30.29 ± 1.22 kg physical weight and other physical characteristics were discovered to differ by sex. In animal genetic resources, body weight and linear body measurements are economically significant features, particularly for small ruminants like goats during selection and to give precise weight determination in remote locations without a scale. Linear measurements and body weight are important traits in meat animals. Quantitative measurements of the ideal body size and form are obtained from the analysis of body measuring data, which enables the computation of genetic parameters for these features and their incorporation into breeding plans. Given the high correlation and regression coefficients of linear body measurements in West African dwarf goats, the results of this study suggest that live weight can be predicted using certain body measurements. Lastly, there was a significant and favourable correlation between body weight and body measurements. Given this, it might be applied as a criterion for selection.

1. INTRODUCTION

Goats are the most prolific livestock species produced in Africa, where they make up to 35% of the global population [Olutogun et al., \(2023\)](#). This may be as a result of the important responsibilities that goats play in helping families in the majority of rural Africa, as well as their contributions to employment, money creation, soil fertility management, nutrition, and food security. In Nigeria, they are present in all of this genetic part of the country. Their geographical locations depend on their relative abilities to adapt to a particular environmental condition and stress.

The resources, which are contingent upon access to variety, as the breeder must work with the amount of variation present in the population [Idowu & Adelabu \(2018\)](#). A thorough understanding of phenotypic diversity is thus required, as it serves as an objective foundation for any improvement program, necessitating the research of breed-important features. Variation can occur from either a phenotypic or genetic basis. The former contained quantitative and qualitative features, whereas the latter included structural loci (electrophoretic variation) and DNA variation, respectively [Khan et al., \(2006\)](#).

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Domestic animal estimation has been a disaster for breeders and researchers. Previously, this was primarily regarded as an art of estimation based on the actual or alleged relationship between the conformation and productive qualities of the animals [Alefe \(2014\)](#). With the advent of body measurements towards the end of the last century, the evaluation of domestic animals gained significant technical and scientific importance. When it was realised both phenotypic as well as genotypic assessments of animal species were necessary for successful selection programs, which is one of the tools a breeder must use, the evaluation of morphological types by merely examining the relationship between body measurements and production was rightfully revalued over the past century [Zehnder et al., \(1997\)](#).

Goat populations from several sub-regions of Sub-Saharan Africa have remained genetically separate, with significant variety in size and coat colors [Morruppa and Ngera \(1986\) & Osinowo et al., \(1989\)](#). Morphometric data are critical for evaluating the characteristics of animal breeds and providing critical insight on the appropriateness of animals for sustainable breeding and conservation [Slippers et al., \(2000\) & Pace and Wakeman \(2003\)](#).

When approximated, an observable or measurable difference among individuals within a population for a specific characteristic provides a viable direction in creating a long-term genetic improvement program. [Mohammed and Amin \(1996\)](#) observed that the beginning points for defining a quantitative attribute in a population is the mean of that population; the remainder of the description may then include how many people ranked above or below the population mean. The phenotypic variance among West African dwarf goat populations in the derived savanna zone of Nigeria is the focus of this study. This investigation will contribute to the Nigerian small ruminant genetic resources databank and complete some of the systematic evaluation of size and conformation for the populations under study, in addition to offering an objective foundation for focussing future development initiatives. The goal of this project is to create a database of genetic resources for small ruminant animals in Nigeria.

2. MATERIALS AND METHODS

Location of the study

The study was conducted in the rural regions of six LGAs in Oyo and Osun State, Nigeria: Ogbomoso North, Ogbomoso South, Ogo-Oluwa, Surulere, Orire, and Ejigbo. Situated at latitude 8.1227° North of the equator and longitude 4.2436° East of the Greenwich

Meridian, the research regions were part of Nigeria's derived savanna region. It is between 300 and 6000 meters above sea level, and it receives 1247 mm of rainfall and 27°C in temperature annually [Idowu and Adelabu \(2018\)](#). The dry season, which lasts from November to February and has an average rainfall of less than 25 mm, is typically followed by a strong rainfall period from March to October. During the peak of the dry season, vegetable growth slows down, which lowers the amount of feed available for growth. In the regions, cattle, goats, sheep, and poultry are the most common native livestock.

Experimental animals and management

For the study, a sample of 399 West African dwarf goats in various age categories was selected. Depending on the owner's financial situation and the availability of crop residues, grains, seasons, and kitchen wastes, these goats were traditionally raised using extensive or semi-intensive husbandry. These changes were typically made to complement the primary feed source, which is forage browsing along major roads, fallow plots, and backyard areas. They were typically not intentionally fed, and supplements were typically given based on availability.

In the roaming range, flocks of numerous owners typically lacked boundaries, and male and female animals ran together in the flocks. Thus, numerous flocks could be considered one flock. The animals were not recorded. Not many owners brought their animals to graze on verdant pastures. Generally speaking, ethno-veterinary medicine was widely practiced. They were not improved by deliberate breeding and selection.

Age determination

The ages of the animals were generally unknown due to the nature of management and the lack of animal records. As a result, the primary function of dentition was to estimate the animals' age range. Dentition [Pace and Wakeman \(2003\)](#) was nevertheless utilised to place the animals into predetermined age brackets in the few instances where the owner was unaware of the true ages of his animals. Table 1 shows the number of pairs of permanent incisors or their absence in the lower jaw.

Duration/periods of data collection

The measurements were taken between November 2013 and May 2014, a span of twenty-seven weeks. With the exception of Ejigbo, the sixth local government area, where sixty-nine goats were measured, sixty-six goats were selected at random and measured in each of the five local governments. For precise measurements, which are accessible in the morning and evening, each local government area was

visited fifteen times. The tattooing technique was utilised to distinguish the prior sample.

Sample size

In all, 399 West African dwarf goats—194 males and 205 females—were sampled for quantitative characteristics (body measurements). These were documented based on the six age categories and sex. The following were the sample sizes for milk teeth, two teeth, four teeth, six teeth, eight teeth, and wither teeth: 29 (12 males and 17 females), 30 (11 males and 19 females), 18 (10 males and 8 females), 68 (47 males and 21 females), 39 (8 males and 31 females), and 215 (106 males and 109 females) were all present.

Body measurements

An attendant hired for the purpose of the study properly restrained the animals in a standing position while taking detailed measurements. Live weight (LW), height at withers (WH), rump height (RH), shoulder width (SW), rump width (RW), heart girth (HG), body length (BL), foreleg length (FL), tail length (TL), face length (FL), and rump length (RL) are among the ten metric features that are measured on each animal. For each animal sampled, the corresponding age group and sex were noted. The linear body measures' anatomical points of reference followed the procedures of [Greyling et al \(1994\)](#), [Krausgrill et al \(1996\)](#) & [Searle et al \(1989\)](#). Live weight was measured using an electronic Camry scale of model TCS-100-JE62ZB, sensitivity of 50g with a capacity of 100kg of class 111 with serial number:210400549 [Raymond et al \(1982\)](#).

A flexible tape was used to take measurements of the length and circumference, and a measuring stick was used to measure the height [Khan et al., \(2003\)](#) & [Hassan and Ciroma \(1992\)](#)

The parts measured were as follows:

Withers height (WH): The separation between the ground and the most cranial, perceptible spinous. The distance between the top of the pelvic girdle and the floor is known as the "rump height" (RH). Shoulder width (SW), commonly known as the broadest point over the intra-spinatus muscle, is measured as the horizontal distance between the two shoulders.

When viewed from the upper surface or from the acetabulum at the femoralanticalation, the width across the fusion of the animal's sacral vertebrae and pelvic bone towards the posterior end is known as the "rump width" (RW).

The measurement of the body's circumference at the narrowest point, directly below the shoulder, perpendicular to the body axis, was called the heart girth (HG).

Rump length (RL): The separation between the pin bone (Tuber ischi) and the pelvic girdle (Tuber coxa). To make measurement easier, two assistants held each animal in an unforced position. Body length (BL): Measured diagonally between the pin bone and the scapular lateral tuberosity. The distance between the mid-lateral point of the coronet and the proximal extremity of the olecranon process is known as the foreleg length (FL). Tail length (TL): The distance between the base of the tail and the coccygeal vertebrae's end.

Statistical analysis

The [SAS \(2003\)](#) statistical program was used to calculate descriptive statistics for each parameter, including mean, standard deviation, standard error, and coefficient of variation. Additionally, a General Linear Model was used to analyse the data. Age group and sex were the variables that were incorporated into the model as sources of variation. The statistical model used was:

$$Y_{ijk} = \mu + t_i + b_{ij} + \epsilon_{ijk}$$

Where Y_{ijk} – Individual observation for the j th treatment

μ = General mean

t_i = Effect of i th (age-group)

b_{ij} = Effect of j th (sex)

ϵ_{ijk} = Experimental error

Under the following presumptions:

- The effects of sex and age group were cumulative.
- The experimental errors had a shared variance, NID (0, σ^2), and were independently, randomly, and normally distributed with a mean of zero. To examine the discrepancies between means, Duncan's Multiple Range test was employed.

3. RESULTS AND DISCUSSION

Live weight, wither height, and rump height

Live weight changes of West African Dwarf goats are presented in Table 1. The results showed that mean live weight increased steadily from 5.31 ± 0.51 to 29.63 ± 1.88 kg in buck and from 5.75 ± 0.13 to 30.29 ± 1.22 kg in the does. The standard deviation within the group did not follow any particular trend. Coefficient of variation ranges from 3.77 to 29.22% in buck and 4.41 to 23.55% in does within the age-group. Age-group significantly ($P < 0.05$) influenced live weight as it increased from zero teeth to wither age.

Sex did not show any significant difference ($P > 0.05$) within the age-group.

Descriptive statistics of wither height are presented in Table 2. mean value ranges from 29.39 ± 0.43 to 55.77 ± 2.04 cm in male and 29.60 ± 0.33 to 56.61 ± 1.3 cm in female. Wither height increased significantly ($P < 0.05$) with age in both sexes. There were no significant differences ($P > 0.05$) between sexes in 0 and 2 teeth ages for this parameter, significant differences ($P > 0.05$) were not observed for 4 teeth, 6 teeth, 8 teeth and worn teeth (Table 2). Standard deviation did not follow any definite pattern, coefficient of variation ranges from 4.39 to 15.05% in male and 5.36 to 11.81% in female, respectively.

Results obtained for rump height are presented in Table 3. Mean value ranged from 32.00 ± 0.43 to 59.09 ± 1.89 cm in male and 32.98 ± 0.31 to 59.74 ± 1.24 cm in female, the result indicates that mean value increased significantly ($P < 0.05$), with age in both sexes and their standard deviation did not follow any definite pattern. Coefficient of variations is within the range of 3.60 to 13.89% in male and 5.90 to 9.89% in female. Males were generally more variable than female, and they were significantly ($P < 0.05$) longer than females in all ages except worn out teeth age where no significant difference was observed.

Shoulder width, rump width, and heart girth

Trends in the growth of shoulder width are summarized in Table 4 with respect to sex; and the mean value showed that male WAD goats were within the range of 13.17 ± 0.94 to 24.27 ± 0.73 cm and female were within the range of 13.54 ± 0.10 to 23.26 ± 0.39 cm. Mean value increased ($P < 0.05$) with age in both sexes. Coefficient of variation ranged from 7.37 to 9.94% and 3.87 to 7.28% in male and female WAD goats, respectively. The trait showed no sexual dimorphism.

Rump width (Table 5) was within the range of 11.42 ± 0.11 to 21.82 ± 0.89 cm in male WAD and 11.86 ± 0.11 to 21.79 ± 0.63 cm in female WAD goats. Males were significantly longer ($P < 0.05$) at 0 teeth, 4 teeth, and wither age than females. The mean value generally increased with age in both sexes ($P < 0.05$). Coefficients of variation are within the range of 3.08 to 13.57 in males and 3.97 to 12.55% in females.

Results also revealed that heart girth (Table 6) mean value ranged from 41.83 ± 0.61 to 86.14 ± 2.16 cm and

42.84 ± 0.49 to 87.13 ± 1.97 cm in male and female WAD goats respectively. Coefficients of variations are within the range of 4.14 to 14.91% in male and 4.41 to 9.89% in female. No significant differences ($P > 0.05$) were observed between sexes at all ages, however, the trait increased with age in both sexes while the highest value was observed for wither age.

Body length, foreleg length and tail length

Changes in body length of the studied WAD goats are presented in Table 7. The result showed that mean body length measured from 26.58 ± 0.45 to 43.27 ± 0.27 cm in male and 26.94 ± 0.37 to 43.53 ± 0.33 cm in female. Significant differences ($P < 0.05$) were observed between ages in both sexes. Co-efficient of variation ranged between 1.37 to 17.49% and 2.23 to 14.18% in male and female WAD goats respectively. Changes in foreleg length of the studied WAD goats are presented in Table 8. The results showed that mean foreleg length increased from 19.20 ± 0.27 to 32.88 ± 0.68 cm in male and 19.51 ± 0.23 to 33.19 ± 0.61 cm in female WAD goats. Significant differences ($P < 0.05$) were observed between ages in both sexes. Coefficient of variation ranged between 2.36 to 14.65% and 3.82 to 12.29% in male and female WAD goats, respectively.

Descriptive statistics of tail length (Table 9) showed that the mean value ranged from 6.41 ± 0.13 to 9.0 ± 0.01 cm for male and 6.61 ± 0.12 to 9.0 ± 0.01 cm for female, the value increased significantly ($P < 0.05$) with respect to age in both sexes. Coefficients of variation were within the range of 5.79 to 20.51% for male and 6.03 to 19.34%. Both sexes were generally variable, and standard deviation did not follow any definite trend.

Face length and rump length

Mean face length of WAD goat population is presented in Table 10 with respect to sex. The value ranged from 11.45 ± 0.21 to 20.27 ± 0.19 cm in male and 11.19 ± 0.16 to 20.00 ± 0.25 cm in female. The value increased significantly ($P < 0.05$) with age in both sexes (Table 10). Significant differences ($P < 0.05$) were also observed between sexes in favour of female WAD goat. Coefficient of variation ranged from 1.96 to 18.40% for male and 2.98 to 16.79% for the female.

Results also revealed that the mean values ranged from 14.29 ± 0.18 to 21.64 ± 0.20 cm in male and

14.9±0.12 to 21.53±0.47 cm in female. Age significantly ($P<0.05$) affects the growth of the trait as it increases with the age in both sexes. Coefficients of variation are within the range of 4.4 to 15.08% in male and 4.89 to 18.15% in female populations. Sex significantly affects the growth of the trait in favor of male WAD goats.

Mean values rump length ranged from 14.29±0.18 to 21.64±0.20 cm in male and 14.9±0.12 to 21.53±0.47 cm in female. Age significantly ($P<0.05$) affects the growth of the trait as it increases with the age in both sexes. Coefficients of variation are within the range of 3.12 to 15.08% in male and 4.89 to 18.15% in female populations. Sex significantly affects the growth of the trait in favour of male WAD goats.

Live weight

Live weight is the most often used size measurement in ruminant production. [Morruppa and Ngere \(1986\)](#) indicated that live weight of Red Sokoto goats ranged from 6.3 to 7.0 kg at milk teeth age; however, the values found in this study ranged from 5.3 to 5.75 kg for male and female goats, respectively. An animal's physical measurements are heavily influenced by its age. Individual components of bodily size are growing at a disproportionate rate. The study found that the age group had a substantial effect on linear body measurements. This finding was consistent with previous research on goats [Akpa \(1998\)](#) & [Mohammed and Amin \(1996\)](#). The lower value could be attributed to the herd's nutritional status as well as breed differences, as WAD goats are a small breed of goat. This is consistent with [Hall \(1991\)](#), who observed that sheep and goats in Southern Nigeria were significantly smaller than those in Northern Nigeria. The value obtained in this study differed slightly from that of [Osinowo et al., \(1989\)](#) for Red Sokoto goats but coincided with the findings of [Otoikhian \(2005\)](#). [Fajemilehin and Salako \(2008\)](#) showed comparable results for the same age group of WAD in Nigeria's forest zone.

[Slippers et al., \(2000\)](#) reported higher value in Nguni goats of South Africa. Values reported for other ages groups; 2 teeth age, 4 teeth age, 6 teeth age, 8 teeth age, worn wither teeth age are 10.38 kg, 13.81 kg, 16.47 kg, 20.48 kg and 29.63 kg for males and 10.22 kg, 13.62 kg, 17.31 kg, 21.50 kg and 30.29 kg for

female goats, respectively. [Khan et al., \(2006\)](#) reported that for 4-12 months, 12-18 months, 19-24 months and 25-36 months and above were 18.6±1.81 kg, 25.25±2.76 kg, 29.86±1.2 kg and 41.47±1.63 kg for male and 14.50± 1.19 kg, 21.0±3.47 kg, 24.00±1.21 kg and 33.95±4.97 kg for female goats, respectively. These variations may be due to better nutritional and environmental conditions, however, [Mukherjee et al., \(1986\)](#) reported values closer to the one observed in this study. [Ngere et al., \(1984\)](#) and [Egena \(2010\)](#) associated variation in body weight values to affect breed and environment, however, [Fajemilehin and Salako \(2008\)](#) in their study reported closer results for the same age group.

Withers height, rump, height and shoulder width

Mean wither height of West African Dwarf goat observed in this study showed a steady increase with age (Table 4.2). This is in line with reports of [Hassan and Ciroma \(1992\)](#) in both sexes that body measurements increased as the animals advanced in age, however, value reported for different age groups, 1-2 yrs, 3-4yrs and 5 yrs were 16.67±0.56, 24.01±0.62 and 30.30±0.10cm for males and 16.16±0.55, 21.65±0.68, and 29.75±1.26cm in females. For the present study, values obtained were 29.39±0.43 cm, 36.79±0.38, 41.75±0.65, 44.10±0.69, 50.00±1.13 and 55.77±2.04cm for male and 29.6±0.33, 35.62±0.77, 40.60±0.39cm, 46.25±1.51, 48.71±0.75 and 56.61±1.33cm for female. Male WAD goats showed superior values over female at zero teeth age, 2 teeth age, 4 teeth age and 8 teeth age, respectively. Female showed superior values at other age groups. From [Fajemilehin and Salako \(2008\)](#) it was observed that height at withers were within the range of 29.02±1.07 to 45.22±0.37 cm for both sexes, thus very similar to values obtained in this study. Mean value of rump height likewise increased with age. [Salako \(2004\)](#) reported values similar to the results of this study, however, there was a large variation when compared with the work of [Hassan and Ciroma \(1992\)](#) in similar age groups, where they reported 57.49±0.51 to 67.39±0.63 cm in both sexes compared to 32.00±0.43 to 59.74±1.24cm in both sexes in this study. These variations might be due to breed differences, effect of sex followed the same trend with high at wither.

The results for shoulder width in this study compared favourably well with the results of [Slippers et al. \(2000\)](#) who reported higher value in South African goats. The values obtained in this study for males range from 13.17±0.94 cm to 24.27±0.73 cm for male and 13.54±0.40 to 23.26±0.39cm for female in all age groups. [Morruppa and Ngera \(1986\)](#) reported closer

values for Red Sokoto goats of similar age groups. The differences observed in favour of the male goats agreed with earlier reports by [Ngere et al., \(1984\)](#).

Rump width, heart girth, body length, and foreleg length

Rump width, heart girth, body length, and foreleg length increased significantly ($P < 0.05$) in both sexes up to wither age, indicating that WAD goat had fully expressed their genetic potentials at this age groups and that selection for any particular trait can best be carried out at this age-group for breed improvement. For this research, heart girth value ranged from 41.83 ± 0.61 to 86.14 ± 2.16 cm for male and 42.84 ± 0.49 to 87.13 ± 1.97 cm for female. Sex differences in tooth age and wither age were noticeable but favored females; this result confirmed the study of [Fajemilehin and Salako \(2008\)](#), who observed values ranging from 38.36 ± 1.8 to 59.51 ± 0.21 cm in both sexes. They proposed that sex was a major source of variance for bodyweight and other body measures, and that does (females) outgrew bucks (males) because females grow faster. This observation was supported by the findings of [Devendra and Burns \(1983\)](#), [Ifut et al., \(1991\)](#) & [Akpa \(1998\)](#), as opposed to [Olutogun et al., \(2023\)](#). The tendency was also seen in rump width, foreleg length, and body length.

Tail length, face length and rump length

Tail length, face length, and rump length all grew at the same rate as the other body parameters studied in this study. Tail length ranged from 6.41 ± 0.13 to 9.00 ± 0.00 cm for males and 6.61 ± 0.12 to 9.0 ± 0.00 cm for females, while face length ranged from 11.45 ± 0.21 to 20.27 ± 0.19 cm for males and 11.19 ± 0.16 to 20.00 ± 0.52 cm for females. Male rump length ranges from 14.29 ± 0.18 to 21.64 ± 0.20 cm, while female rump length ranges from 14.91 ± 0.12 to 21.52 ± 0.47 cm. These findings were consistent with those of other researchers. [Morruppa and Ngera \(1986\)](#) claimed a greater value for Red Sokoto goats, however [Osinowo et al., \(1989\)](#) reported a lower value.

4. CONCLUSION

Based on linear body measures, estimating the body weight of West African dwarf goats is easy and precise. Linear measurements and body weight are important traits in meat animals. Quantitative measurements of the ideal body size and form are obtained from the analysis of body measuring data,

which enables the computation of genetic parameters for these features and their incorporation into breeding plans.

Any development program aimed at increasing goat meat yield should take into account the significant effects that both sex and age have on body measurements at various ages. Finally, body measures exhibited a substantial and positive connection with body weight. In light of this, it could be utilized as a selection criterion. Earlier investigations also revealed that selecting based on measures boosted meat production.

5. RECOMMENDATIONS

Changes in body weight require adjustments to linear body measures. Because different livestock species have distinct parameter connections, equivalent database information must be created for more livestock species to prevent mistakes from the past. The relationships between body weights and linear body measurements in all goat breeds at different ages and localities in West Africa require further study.

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7. CONFLICT OF INTEREST

All authors declared that there is no conflict of interests regarding the publication of this article.

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Table 1. Least square means, standard deviation, standard error, and coefficient of variation, for body weight (BW) of WAD goat at various age groups

Age group	Parameter	Male	Female	Total
0 teeth	N	106	109	215
	-	5.31	5.75	5.55
	\bar{X}	1.55	1.35	1.47
	S.D	0.15	0.13	0.10
	S.E	29.22	23.55	26.53
	CV			
2 teeth	N	47	21	68
	-	10.38	10.22	10.33
	\bar{X}	1.13	1.26	1.17
	S.D	0.17	0.27	0.14
	S.E	10.93	12.32	11.30
	CV			
4 teeth	N	8	31	39
	-	13.83	13.62	13.66
	\bar{X}	1.07	0.77	0.83
	S.D	0.38	0.14	0.13
	S.E	7.72	5.68	6.07
	CV			
6 teeth	N	10	8	18
	-	16.47 ^b	17.31 ^a	16.84
	\bar{X}	0.62	0.96	0.88
	S.D	0.20	0.34	0.21
	S.E	3.77	5.55	5.21
	CV			
8 teeth	N	12	17	29
	-	20.48 ^d	21.08 ^c	20.63
	\bar{X}	0.95	3.07	1.46
	S.D	0.28	1.08	0.27
	S.E	4.65	4.41	6.91
	CV			
Wither	N	11	19	30
	-	29.63	30.29	30.05
	\bar{X}	6.22	7.12	6.70
	S.D	1.88	1.63	1.22
	S.E	20.99	23.50	22.30
	CV			

Means with different superscripts within age group are significantly different ($P < 0.05$)

Table 2. Least Square Means, Standard Deviation, Standard Error and Coefficient of Variation of Wither Height (WH) for WAD Goats at Various Age Groups

Age group	Parameter	Male	Female	Total
0 teeth	N	106	109	215
	-	29.39 ^f	29.60 ^e	29.50
	\bar{X}	4.43	3.50	3.98
	S.D	0.43	0.33	0.27
	S.E	15.08	11.81	13.48
	CV			
2 teeth	N	47	21	68
	-	36.79 ^c	35.62 ^d	36.43
	\bar{X}	2.57	3.51	2.92
	S.D	0.38	0.77	0.35
	S.E	7.00	9.87	8.02
	CV			
4 teeth	N	8	31	39
	-	41.75 ^d	40.60 ^e	40.83
	\bar{X}	1.83	2.18	2.14
	S.D	0.65	0.39	0.34
	S.E	4.39	5.36	5.24
	CV			
6 teeth	N	10	8	18
	-	44.10	46.25	45.06
	\bar{X}	2.14	4.27	3.35
	S.D	0.69	1.51	0.79
	S.E	4.95	9.23	7.44
	CV			
8 teeth	N	12	17	29
	-	50.00	48.71	49.24
	\bar{X}	3.91	3.10	3.45
	S.D	1.13	0.75	0.64
	S.E	7.82	6.36	7.01
	CV			
Wear Worn teeth	N	11	19	30
	-	55.77	56.61	56.30
	\bar{X}	6.78	5.80	6.07
	S.D	2.04	1.33	1.10
	S.E	12.16	10.24	10.79
	CV			

Means with different superscripts within age group are significantly different ($P < 0.05$).

Table 3. Least Square Means, Standard Deviation, Standard Error and Coefficient of Variation (CV) of Rump Height (RH) of WAD Goats at Various Ages

Age group	Parameter	Male	Female	Total
0 teeth	N	106	109	215
	\bar{X}	32.00 ^f	32.38 ^e	32.19
	S.D	4.44	3.20	3.86
	S.E	0.43	0.31	0.26
	CV	13.89	9.89	11.99
2 teeth	N	47	21	68
	\bar{X}	39.57 ^d	38.64 ^e	39.28
	S.D	2.69	3.04	2.83
	S.E	0.39	0.67	0.34
	CV	6.80	7.97	7.20
4 teeth	N	8	31	39
	\bar{X}	44.38 ^c	43.08 ^d	43.35
	S.D	1.60	2.54	2.42
	S.E	0.56	0.46	0.39
	CV	3.60	5.90	5.58
6 teeth	N	10	8	18
	\bar{X}	47.60	49.25	48.33
	S.D	2.01	3.85	2.99
	S.E	0.64	1.36	0.70
	CV	4.22	7.81	6.19
8 teeth	N	12	17	29
	\bar{X}	53.71	53.00	53.29
	S.D	2.71	2.32	2.47
	S.E	0.78	0.56	0.46
	CV	5.04	4.38	4.63
Wither	N	11	19	30
	\bar{X}	59.09	59.74	59.50
	S.D	6.28	5.42	5.65
	S.E	1.89	1.24	1.03
	CV	10.63	9.08	9.50

Means with different superscripts within age group are significantly different (P<0.05).

Table 4. Least Square Means, Standard Deviation, Standard Error and Coefficient of Variation of Shoulder Width (SW) of WAD Goats at Various Age Groups

Age group	Parameter	Male	Female	Total
0 teeth	N	106	109	215
	-	13.17	13.54	13.36
	\bar{X}	0.97	1.00	1.00
	S.D	0.94	0.10	0.07
	S.E	7.37	7.42	7.51
	CV			
2 teeth	N	47	21	68
	-	14.40 ^f	14.43 ^e	14.11
	\bar{X}	0.95	0.93	0.93
	S.D	0.14	0.20	0.11
	S.E	6.58	6.42	6.48
	CV			
4 teeth	N	8	31	39
	-	16.25	16.35	16.33
	\bar{X}	0.71	1.14	1.06
	S.D	0.25	0.20	0.173
	S.E	4.35	6.42	6.49
	CV			
6 teeth	N	10	8	18
	-	17.70	18.25	17.94
	\bar{X}	0.48	0.71	0.64
	S.D	0.15	0.25	0.15
	S.E	2.73	3.87	3.56
	CV			
8 teeth	N	12	17	29
	-	19.92	21.12	20.62
	\bar{X}	0.90	1.05	1.15
	S.D	0.26	0.26	0.21
	S.E	4.52	4.99	5.56
	CV			
Wither	N	11	19	30
	-	24.27	23.26	23.63
	\bar{X}	2.41	1.69	2.01
	S.D	0.73	0.39	0.37
	S.E	9.94	7.28	8.50
	CV			

Means with different superscripts within age group are significantly different (P<0.05)

Table 5. Least Square Means, Standard Deviation, Standard Error and Coefficient of Variation of Rump Width (RW) of WAD Goat at Various Age Groups

Age group	Parameter	Male	Female	Total
0 teeth	N	106	109	215
	-	11.42	11.86	11.64
	\bar{X}	1.14	1.18	1.18
	S.D	0.11	0.11	0.08
	S.E	9.98	9.46	10.13
	CV			
2 teeth	N	47	21	68
	-	12.96 ^d	13.19 ^f	13.01
	\bar{X}	1.01	1.03	1.01
	S.D	0.15	0.22	0.12
	S.E	7.80	7.81	7.80
	CV			
4 teeth	N	8	31	39
	-	14.50	14.35	14.38
	\bar{X}	0.53	0.84	0.78
	S.D	0.19	0.15	0.13
	S.E	3.69	5.84	5.44
	CV			
6 teeth	N	10	8	18
	-	15.70	16.13	15.88
	\bar{X}	0.48	0.64	0.58
	S.D	0.15	0.23	0.14
	S.E	3.08	3.97	3.67
	CV			
8 teeth	N	12	17	29
	-	17.58	18.71	18.24
	\bar{X}	0.90	1.26	1.24
	S.D	0.26	0.31	0.23
	S.E	5.12	6.75	6.82
	CV			
Wither	N	11	19	30
	-	21.82	21.79	21.80
	\bar{X}	2.96	2.74	2.77
	S.D	0.89	0.63	0.51
	S.E	13.57	12.58	12.71
	CV			

Means with different superscripts within age group are significantly different (P< 0.05)

Table 6. Least Square Means, Standard Deviation, Standard Error and Co-efficient of Variation of Heart Girth of WAD Goats at Various Age Groups

Age group	Parameter	Male	Female	Total
0 teeth	N	106	109	215
	-	41.83 ^f	42.84 ^e	42.35
	\bar{X}	6.24	5.12	5.71
	S.D	0.61	0.49	0.39
	S.E	14.91	11.94	13.48
	CV			
2 teeth	N	47	21	68
	-	55.68 ^d	54.42 ^e	55.29
	\bar{X}	5.06	4.21	4.82
	S.D	0.74	0.92	0.58
	S.E	9.09	7.74	8.72
	CV			
4 teeth	N	8	31	39
	-	62.63 ^c	62.00 ^d	62.13
	\bar{X}	1.77	3.28	3.02
	S.D	0.63	0.59	0.48
	S.E	2.82	5.29	4.86
	CV			
6 teeth	N	10	8	18
	-	68.20 ^b	69.63 ^a	62.13
	\bar{X}	3.05	3.07	3.05
	S.D	0.96	1.08	0.72
	S.E	4.47	4.41	4.44
	CV			
8 teeth	N	12	17	29
	-	75.00	75.35	75.21
	\bar{X}	3.10	3.33	3.19
	S.D	0.90	0.81	0.59
	S.E	4.14	4.42	4.24
	CV			
Wither	N	11	19	30
	-	86.14	87.13	86.77
	\bar{X}	7.16	8.60	7.99
	S.D	2.16	1.97	1.46
	S.E	8.32	9.87	9.20
	CV			

Means with different superscripts within age are significantly different ($P < 0.05$).

Table 7. Least Square Means, Standard Deviation, Standard Error and Coefficient of Variation of Body Length (BL) of WAD Goats at Various Age Groups

Age group	Parameter	Male	Female	Total
0 teeth	N	106	109	215
	-	26.58 ^e	26.94 ^d	26.76
	\bar{X}	4.65	3.82	4.24
	S.D	0.45	0.37	0.29
	S.E	17.49	14.18	15.85
	CV			
2 teeth	N	47	21	68
	-	33.23	33.57	33.34
	\bar{X}	2.48	1.03	2.13
	S.D	0.36	0.22	0.26
	S.E	7.46	3.06	6.40
	CV			
4 teeth	N	8	31	39
	-	36.13 ^c	36.24 ^b	36.22
	\bar{X}	1.46	1.33	1.34
	S.D	0.52	0.24	0.21
	S.E	4.04	3.68	3.70
	CV			
6 teeth	N	10	8	18
	-	38.50 ^b	39.25 ^a	38.83
	\bar{X}	0.53	1.13	0.91
	S.D	0.17	0.40	0.21
	S.E	1.37	2.89	2.34
	CV			
8 teeth	N	12	17	29
	-	41.58 ^a	41.82 ^a	41.72
	\bar{X}	0.67	0.93	0.83
	S.D	0.19	0.23	0.15
	S.E	1.61	2.23	1.99
	CV			
Wither	N	11	19	30
	-	43.27 ^a	43.53 ^a	43.43
	\bar{X}	0.90	1.43	1.25
	S.D	0.27	0.33	0.23
	S.E	2.09	3.28	2.88
	CV			

Means with different superscripts within age group are significantly different ($P < 0.05$)

Table 8. Least Square Means, Standard Deviation, Standard Error and Coefficient of Variation of Foreleg Length (FL) of Male and Female WAD Goats

Age group	Parameter	Male	Female	Total
0 teeth	N	106	109	215
	-	19.20 ^d	19.51 ^c	19.35
	\bar{X}	2.81	2.40	2.61
	S.D	0.27	0.23	0.18
	S.E	14.65	12.29	13.48
	CV			
2 teeth	N	47	21	68
	-	24.71 ^c	24.45 ^d	24.63
	\bar{X}	1.62	1.48	1.58
	S.D	0.24	0.32	0.19
	S.E	6.57	6.06	6.40
	CV			
4 teeth	N	8	31	39
	-	27.13	27.48	27.41
	\bar{X}	1.73	1.41	1.46
	S.D	0.61	0.25	0.23
	S.E	6.37	5.11	5.32
	CV			
6 teeth	N	10	8	18
	-	27.75 ^c	28.25 ^b	27.97
	\bar{X}	1.59	1.98	1.74
	S.D	0.50	0.70	0.41
	S.E	5.71	7.02	6.21
	CV			
8 teeth	N	12	17	29
	-	30.54 ^a	29.94 ^b	30.19
	\bar{X}	0.72	1.14	1.02
	S.D	0.21	0.28	0.19
	S.E	2.36	3.82	3.38
	CV			
Wither	N	11	19	30
	-	32.88	33.19	33.08
	\bar{X}	2.24	2.64	2.47
	S.D	0.68	0.61	0.47
	S.E	6.82	7.96	7.46
	CV			

Means with different superscripts within age group are significantly different ($P < 0.05$)

Table 9. Least Square Means, Standard Deviation, Standard Error and Coefficient of Variation of Tail Length (TL) of WAD Goats at Various Age Groups

Age group	Parameter	Male	Female	Total
0 teeth	N	106	109	215
	-	6.41	6.61	6.51
	\bar{X}	1.32	1.28	1.30
	S.D	0.13	0.12	0.09
	S.E	20.51	19.34	19.92
	CV			
2 teeth	N	47	21	68
	-	7.95 ^a	7.86 ^b	7.92
	\bar{X}	0.53	0.69	0.58
	S.D	0.08	0.15	0.07
	S.E	6.72	8.80	7.37
	CV			
4 teeth	N	8	31	39
	-	8.56 ^c	8.40 ^d	8.44
	\bar{X}	0.50	0.51	0.62
	S.D	0.18	0.09	0.15
	S.E	5.79	6.03	7.29
	CV			
6 teeth	N	10	8	18
	-	8.50 ^e	8.38 ^f	8.44
	\bar{X}	0.53	0.74	0.62
	S.D	0.17	0.26	0.15
	S.E	6.20	8.88	7.29
	CV			
8 teeth	N	12	17	29
	-	9.00	9.00	9.00
	\bar{X}	0.00	0.00	0.00
	S.D	0.00	0.00	0.00
	S.E	0.00	0.00	0.00
	CV			
Wither		11	19	30
	N	9.00	9.00	9.00
	-	0.00	0.00	0.00
	\bar{X}	0.00	0.00	0.00
	S.D	0.00	0.00	0.00
	S.E			
CV				

Means with different superscripts within age group are significantly different (P< 0.05)

Table 10. Coefficient of Variation of Face Length (FAL) of WAD Goats at Various Age Groups

Age group	Parameter	Male	Female	Total
0 teeth	N	106	109	215.
	-	11.45	11.19	11.32
	\bar{X}	2.11	1.70	1.91
	S.D	0.21	0.16	0.13
	S.E	18.40	15.15	16.87
	CV			
2 teeth	N	47	21	68
	-	14.01 ^c	12.57 ^d	13.57
	\bar{X}	2.35	2.11	2.36
	S.D	0.35	0.46	0.29
	S.E	16.75	16.79	17.38
	CV			
4 teeth	N	8	31	39
	-	14.62 ^c	16.29 ^b	15.95
	\bar{X}	2.07	2.36	2.37
	S.D	0.73	0.42	0.38
	S.E	14.13	14.56	14.88
	CV			
6 teeth	N	10	8	18
	-	17.90 ^b	18.00 ^a	17.94
	\bar{X}	1.97	1.85	1.86
	S.D	0.62	0.65	0.44
	S.E	11.00	01.29	10.38
	CV			
8 teeth	N	12	17	29
	-	19.83	19.71	19.76
	\bar{X}	0.39	0.59	0.51
	S.D	0.11	0.14	0.09
	S.E	1.96	2.98	2.59
	CV			
Wither	N	11	19	30
	-	20.27	20.00	20.10
	\bar{X}	0.65	2.29	1.84
	S.D	0.19	0.52	0.37
	S.E	3.19	11.43	9.18
	CV			

Means with different superscripts within age groups are significantly different ($P < 0.05$),

Table 11. Least Square Means, Standard Deviation, Standard Error and Co-efficient of Variation of Rump Length (RL) of WAD Goats at Various Age Groups

Age group	Parameter	Male	Female	Total
0 teeth	N			
	-	106	109	215
	\bar{X}	14.29	14.91	14.60
	S.D	1.88	1.22	1.61
	S.E	0.18	0.12	0.11
	CV	13.15	8.19	11.00
2 teeth	N	47	21	68
	-	15.17 ^c	14.62 ^f	15.00
	\bar{X}	2.29	2.13	2.24
	S.D	0.33	0.47	0.27
	S.E	15.08	14.59	14.93
	CV			
4 teeth	N	8	31	39
	-	15.25 ^h	17.13 ^g	16.74
	\bar{X}	2.12	3.17	3.06
	S.D	0.75	0.59	0.49
	S.E	13.91	18.51	18.27
	CV			
6 teeth	N	10	8	18
	-	18.80	19.00	18.89
	\bar{X}	1.14	1.69	1.39
	S.D	0.36	0.60	0.32
	S.E	6.04	8.90	7.24
	CV			
8 teeth	N	12	17	29
	-	20.42 ^b	21.06 ^a	20.79
	\bar{X}	0.67	1.03	0.94
	S.D	0.19	0.25	0.17
	S.E	3.27	4.89	4.52
	CV			
Wither	N	11	19	30
	-	21.64	21.53	21.56
	\bar{X}	0.67	2.07	1.68
	S.D	0.20	0.47	0.31
	S.E	3.12	9.59	7.77
	CV			

Means with different superscripts within age groups are significantly different (P< 0.05)

PRECLINICAL STUDY OF *CELTIS OCCIDENTALIS* LEAVES EXTRACT ON PANCREATIC INJURY AND INFLAMMATION AGAINST ACETAMINOPHEN-INDUCED ACUTE PANCREATITIS IN RATS

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AM conceived the concept, wrote the manuscript, data analysis, while SN conducted experiment. SR, SK and MW design methodology.

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ABSTRACT

Acetaminophen is used as a painkiller and to alleviate fever. With or without a prescription, overdoses of acetaminophen lead to acute inflammation of the pancreas, cause degeneration of pancreatic tissues, and elevate the blood pancreatic enzyme level. This research aims to investigate the protective and anti-inflammatory potential of the methanolic extract of *Celtis occidentalis* leaves against acetaminophen-instigated acute pancreatitis. Thirty adult male Swiss albino rats were divided into five groups; each group has six individuals ($n = 6$). Group I is considered a control group without any treatment. Group II was given a single dose of acetaminophen (1000 mg/kg/b.w.) for fourteen days consecutively. Group III was given a dose of acetaminophen (1000 mg/kg/b.w.) before Imipenem (500 mg/kg/b.w.) administration. Groups IV and V were also given a single dose of acetaminophen (1000 mg/kg/b.w.) before the administration of methanolic extracts of low dose (150 mg/kg/b.w.) and high dose (300 mg/kg/b.w.). Results clearly indicate that the pancreatic enzymes (serum amylase and serum lipase) significantly increased in the acetaminophen treated group, which shows the induction of acute pancreatitis. Animals treated with methanolic extracts of low dose (150 mg/kg) and high dose (300 mg/kg) significantly ($P < 0.01$ and $P < 0.001$) reduced the serum pancreatic enzyme level. Histopathological assessment of the pancreatic tissues of different treatment groups also revealed that acetaminophen severely altered the pancreas morphology, but treatment with methanolic extract (150 mg/kg and 300 mg/kg) vigorously recovered the alterations as compared to the Imipenem (500 mg/kg) treated group. It should be concluded that the methanolic extract of *C. occidentalis* has bioactive compounds with strong antioxidant and anti-inflammatory properties that potentially regenerate histological alterations induced by acetaminophen in the pancreas.

1. INTRODUCTION

The pancreas is a vital organ of vertebrate animals and is associated with the digestive and endocrine systems. In the human body, it is located back to the stomach in the abdominal cavity and functions as a gland both endocrine and exocrine, used is combine term as heterocrine or mixed gland. The major portion of the pancreas is the exocrine region which is made up of acinar cells (99%) and produces exocrine secretions or

digestive enzymes (proteases, lipases, amylases, etc.) and sodium bicarbonate (NaHCO_3) (Leung and Leung 2010). These exocrine secretions are important in the digestion, metabolism and assimilation of foodstuffs that we're daily consuming. The endocrine part of the pancreas is made up of different types of secretory cells (α -cells, β -cells, delta/ δ -cells, and pancreatic polypeptide/PP-cells, etc.) which respectively secrete glucagon, insulin, somatostatin, and pancreatic polypeptides. All these endocrine secretions are responsible for glucose metabolism and regulate the storage and release of glucose. For secretions of the aforementioned exocrine and

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endocrine chemicals, regulatory machinery is involved that releases neurocrine, endocrine, paracrine, and also some intracrine that regulates the secretion of all pancreatic juice and pancreatic hormones. If alterations occur in the regulatory pathway by activating or inhibiting any mechanism, it leads to considerable changes or diseases (Leung and Leung 2010).

Inflammation is the most common illness in the pancreas also termed pancreatitis. The leading causes of pancreatitis include chronic alcohol abuse, stones in the gall bladder and, to some extent, the prescribed drug usage. Due to the mentioned leading causes, over secretion of digestive enzymes occurs, but still in the pancreas due to blockage in the ducts and ductules and a lack of regulatory mechanisms, irritations caused in pancreatic cells, and finally inflammation (Uchendu, Agu et al. 2017). One of the most prevalent types of inflammation is acute inflammation, or acute pancreatitis, which is clinically characterized by abdominal discomfort, severe pain in the stomach region, and an elevated level of serum pancreatic enzymes. Acute pancreatitis is recognized to be caused by a number of risk factors, mainly chronic alcohol consumption and gallstones. Although drug-induced pancreatitis accounts for less than 2% of cases, due to less background knowledge and complex mechanisms of interrelationship with other illnesses, this has not have been clearly confirmed (Chen, Lin et al. 2015, Uchendu, Agu et al. 2017). In a previous report between 1966 and 2009, the number of cases of first attack acute pancreatitis varied from 4 to 45 per 100,000 people per year. Although it has declined recently, the fatality rate is still in the range of 3.3% to 6% (Yadav and Lowenfels 2006, Shen, Lu et al. 2012).

Paracetamol (acetaminophen), a widely prescribed and unprescribed drug, is mostly used as painkiller and to alleviate fever. Because of its wide availability, it is used to relieve different illnesses and as a leading medication for intentional and unintentional overdoses (Chen, Lin et al. 2015). Overdoses of paracetamol lead to hepatotoxicity, which is ultimately associated with nephrotoxicity (Thanacoody 2023), because the paracetamol-degraded metabolites (sulphation and glucuronidation) that might be produced in the liver and removed from the body via the kidney during waste excretion induce kidney failure (Hussein, Kandeil et al. 2022). Acute pancreatitis is more common in cases of acetaminophen intoxication. Among others, less frequent extrahepatic signs of acetaminophen intoxication for which there are few

reports include acute pancreatitis (Chen, Lin et al. 2015). For the diagnosis of acute pancreatitis or when someone has symptoms of pancreatitis or other pancreatic disorders, amylase and lipase tests are used. A test used to monitor the concentration of these enzymes in blood while they are circulating (Ismail and Bhayana 2017).

Different treatment measures were used to cure various types of illnesses. For this purpose, different scientists worked on exploring novel compounds that have numerous therapeutic potentials (Chakraborty, Uddin et al. 2022, Sarwar, Hossain et al. 2022). Almost 80% of people primarily rely on natural products from medicinal plants for the recovery of different health issues (Riaz, Rasool et al. 2012, Atanasov, Zotchev et al. 2021). Medicinal plants, contain antimalarial compounds like quinine and digitoxin act as cardioactive medications, narcotic painkillers such as morphine; and anti-neoplastic treatments such as vincristine and vinblastine (Newman and Cragg 2020). One of the possible sources of therapeutic substances with promising ethnopharmacological characteristics is the genus *Celtis*. Nearly all of these plant parts (leaves, barks, roots, saps, etc.) have been used as traditional medicine to treat variety of illnesses, including diabetes, gastrointestinal, venereal, amenorrhea, pain, headaches, and fever (Krief, Hladik et al. 2005, Koduru, Grierson et al. 2007, Moffett 2010, Filali-Ansari, El Abbouyi et al. 2015).

Preliminary biological and medicinal studies of the extracts and other byproducts of the *Celtis* genus have uncovered a wide spectrum of biochemical activity. These include antioxidant, antidiabetic, analgesic, antifungal, anticancer, and anti-inflammatory as well as antibacterial features (Nchabeleng 2017, Ota, Višnjevec et al. 2017, Laryea and Sheringham Borquaye 2021, Baran, Keskin et al. 2022). The genus *Celtis* is commonly known as nettle trees or hackberries, placed in Cannabaceae family widely spread in Asia, Africa, northern Australia, and south and north America (Sattarian 2006, Bonner and Karrfalt 2008). The genus *Celtis* plants were formerly classified into two families; Ulmaceae and the newly formed Celtidaceae (Kozlowski 2021). This genus is particularly unique and can be distinguished from other genera in its family primarily by the features of its leaves, which are deciduous, alternating, and distinct with more than one vein. The flowers are small, greenish with single or both sexes. The ripened ovaries have one seed with a juicy nature (Duncan and Duncan 2000).

From the previously reported data, only 14 species from the genus *Celtis* have been assessed for

thorough studies considering the medicinal and phytochemical aspects of various bioactive compounds of those species. The previously reported species have different medicinal values and are used to treat a wide range of illnesses (Samadd, Hossain et al. 2024). The current study aims to evaluate the protective and ameliorative effects of *Celtis occidentalis* L. in male Wistar rats against acetaminophen instigated pancreatitis. This study offers a low-cost and natural therapy to prevent the pancreas from being toxic from an acetaminophen overdose.

2. MATERIALS AND METHODS

Chemical reagents used and preparation of methanolic extract

Methanol used as a solvent was purchased from Rahman Chemicals Lab in Barikot, Swat, KP Pakistan. Paracetamol and Imipenem (choice drug used against acute pancreatitis) were purchased from Sami Pharmaceutical Lab (Pvt.) Ltd. Lahore, Pakistan. For the detection of amylase and lipase enzymes, kits were acquired from Chughtais Lab (Pvt.) Ltd., Lahore. Leaves of the *Celtis occidentalis* plant were collected from Lal Qila Maidan Dir lower, KP Pakistan. The leaves were shade-dried for 20 days and then chopped by an electric rotor blender at the Department of Zoology, University of Malakand. The 200 grams powder were obtained and then soaked in 95% methanol for 10 days in shaker at room temperature. The obtained extracts were filtered through Whatman filter paper, and rotary evaporator was used for solvent evaporation under reduced pressure, and the obtained crude extract was kept in water bath at 40°C for 1 day to become dried.

Animals Model and Experimental design

Thirty adult male Swiss albino rats were obtained from the National Institute of Health (NIH) in Islamabad, weighing 150 grams. The animals were kept under standard conditions at 25±2°C temperature and 12 h light and 12 h dark cycles and were monitored for acclimation approximately ten days before to the start of the experiment. The animals handling and experimental protocol were approved by the ethical committee of the Department of Zoology, University of Malakand (E-SA-11-2009) and in accordance with the guidelines established by the American Physiological Society for Human and Animal Research (Association 2001). Thirty animals were divided into five groups with each group has six individuals ($n = 6$). The study was about the induction of acute pancreatitis or acute inflammation, and treatment was lasted for 14 days trail with some modifications (Ifeyinwa, Victor et al., 2023).

Group-I: (Control group): No treatment was given to this group.

Group-II: (Acetaminophen treated group): This group was treated with a single dose of acetaminophen (1000 mg/kg/b.w.) via oral route (o.r.) for 14 days.

Group-III: (Imipenem treated group): This group was treated with a single dose of acetaminophen (1000 mg/kg/b.w.) before treatment with daily administration of Imipenem (500 mg/kg/b.w, o.r.).

Group-IV: (Extract-150 treated group): This group was treated with a single dose of acetaminophen (1000 mg/kg/b.w.) before treatment with daily administration of methanolic extract (150 mg/kg/b.w.).

Group-V: (Extract-300 treated group): This group was also treated with a single dose of acetaminophen (1000 mg/kg/b.w.) before treatment with daily administration of methanolic extract (300 mg/kg/b.w.).

Induction of Acute Pancreatitis

For the induction of acute pancreatitis, the required dosage of 120 mg was calculated and thoroughly mixed in 0.9 ml of distilled water. A single dose of 0.9 ml of solution was given to each individual rat (i.e., 1000 mg/kg/b.w.) of the four experimental groups. The calculated dosage was continuously administered for 14 days.

Dissection of Animals and Sample Collection

On day 15th, all group animals were anesthetized with chloroform (inhalation of fumes from soaked cotton wool with chloroform) in a closed container. A blood sample (5 ml) was collected from each group of individuals through a cardiac puncture and centrifuged. Serum was separated and stored at 4°C in eppendorf tube. Pancreas was taken from each group stored in 10% formalin for further histopathological assessment.

Biochemical and Histopathological Analysis

Different tests were performed for the determination of serum amylase and serum lipase levels. For assessment of serum amylase and serum lipase using the standard method of colorimetric detection (Kumar, Gromski et al. 2021). For histopathology, the pancreas of each group was embedded in paraffin wax, and sections were made via the HC-202 laboratory rotary manual microtome at a thickness of 5 microns. Hematoxylin and eosin (H & E) dyes were used for staining purpose (Koivukoski, Khan et al. 2023). Slides were studied under Olympus research microscope, CH20i (Binocular version).

Statistical Analysis

Data on biochemical parameters were statistically analyzed using SPSS version 27.0. Results were reported as mean \pm SD (standard deviation), and one-way analysis of variance (ANOVA) was used to test the level of significance, followed by Tukey post hoc analysis. The levels of significance were considered below 0.05 ($p < 0.05$).

3. RESULTS AND DISCUSSION

Biochemical Data

Blood levels of biochemical markers (i.e. serum amylase and serum lipase) were evaluated for an assessment of pancreatic functioning. Figure 1 represents statistical data on serum amylase and serum lipase. Significant ($p < 0.001$ and $p < 0.01$) changes were observed in biochemical markers among different groups of animals. Results indicate that a single dose of paracetamol (1000 mg/kg) potentially induced acute pancreatitis compared to the normal control group. However, daily administrations of high dose methanolic extract of (300 mg/kg) mitigate acute pancreatitis, demonstrating that the methanolic extract of *C. occidentalis* has potent anti-pancreatic potential compared to the Imipenem (500 mg/kg).

Histopathological Results

Figure 2 represents the pancreas sections of different experimental groups. Histology of control group pancreas (A) shows normal architecture with clearly visible zymogen granules, capillaries, lobular ducts, and interlobular septum. The pancreas section of acetaminophen treated group (B) shows histological alterations in pancreatic hepatocytes, dilation of interlobular septum, degeneration of zymogen granules, and collapsed capillaries. The Imipenem administered group (C) shows normal histology with rare alterations in pancreatic hepatocytes and a sparsely dilated interlobular septum as compared to the acetaminophen-treated group. Histological architecture of pancreas treated with methanolic extract (150 mg/kg) (D) shows rare alteration in pancreatic hepatocytes, rarely found capillary collapse with little dilation. Other regions have no alterations to be found. Histology of pancreas treated with a methanolic extract (300 mg/kg) of *C. occidentalis* (E) shows morphologically normal, functionally active, and clearly visible pancreatic tissues, as compared to the control group pancreas. These pancreatic tissue sections clearly demonstrate that *C. occidentalis* has strong anti-inflammatory effects as compared to the Imipenem drug.

4. CONCLUSION

This research work revealed that administration of the *C. occidentalis* extract potentially reduced the deleterious effects induced by acetaminophen. The *C. occidentalis* provided defense against acute pancreatitis, prompting acetaminophen. The protection efficacy of the *C. occidentalis* extract was higher than when Imipenem was administered, which had a minimal effect. This study demonstrates how the *C. occidentalis* extract protects the pancreas from acute inflammation triggered by acetaminophen. Further research trials should be recommended to isolate compounds that actively decline serum lipase and serum amylase levels and regenerate histological alterations.

5. ACKNOWLEDGEMENTS

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6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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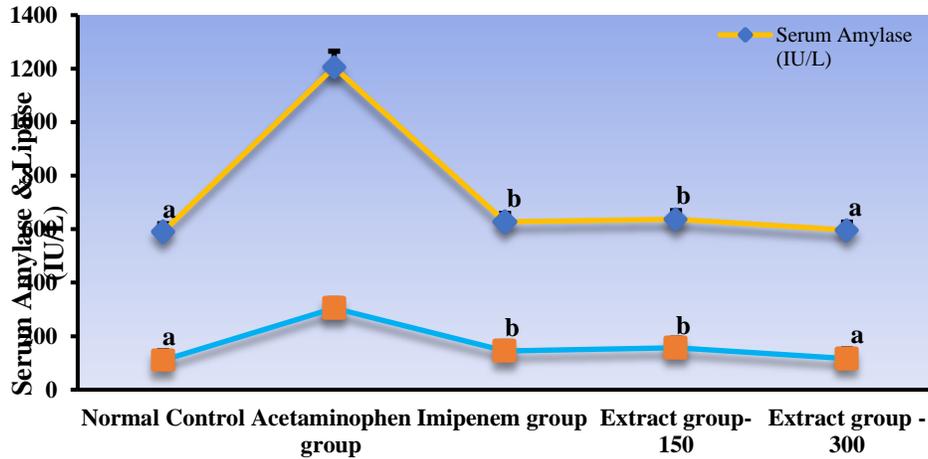


Figure 1: Represent the pancreatic enzymes (serum amylase and serum lipase) of different experimental groups. Data are presented in Mean \pm SEMs (standard error means). ap < 0.001 and bp < 0.01 is considered level of significance.

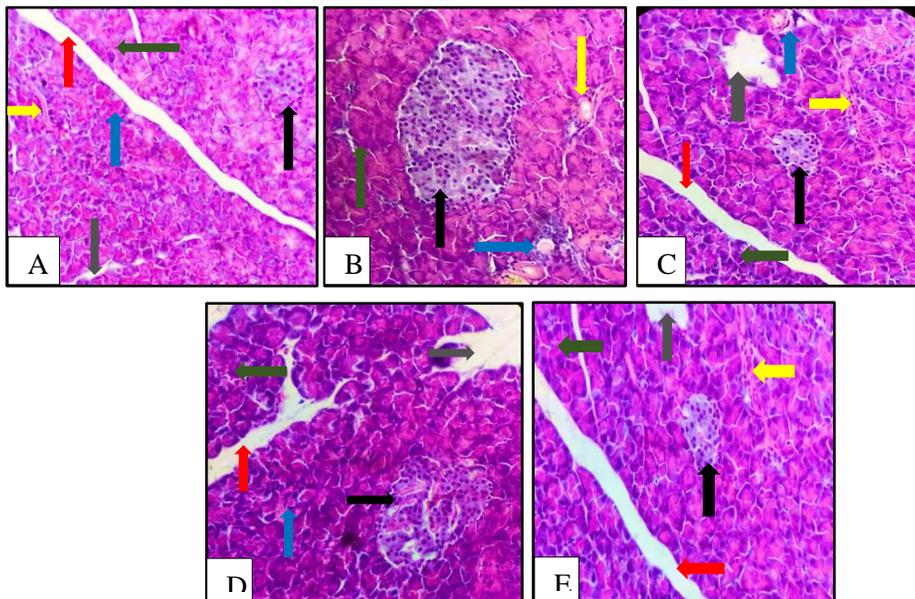


Figure 2. Represent pancreatic sections of different experimental groups. Photograph (A), pancreas section of the control group having normal histology. Photograph (B), pancreatic section of the acetaminophen group with altered histology. Photograph (C), pancreatic section of the imipenem group with rare alterations. Photograph (D), pancreas histology of the extract-150 group with rare alterations. Photograph (E), pancreatic section of the extract-300 group has normal histological architecture, same as the control group.

Keys: Islets of Langerhans-, Zymogen granules-, pancreatic duct-, Capillary-, pancreatic lobule-
 Interlobular septum-

COMPARISON OF DIABETES MELLITUS TYPE II BETWEEN MALE AND FEMALE PATIENTS OF CIVIL HOSPITAL KHAIRPUR MIR'S SINDH PAKISTAN

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ABSTRACT

The goal of present study was to evaluate the prevalence of Diabetes type II in Civil Hospital Khairpur Mir's, Sindh, Pakistan. The primary data was assembled from September 2023 to November 2023 through the hospital survey and secondary data based on hospital record was collected from January 2022 to November 2023. The patients were categorized into different age groups of 30 to 40, 41 to 50 and 51 to 60 years. The blood samples were collected from a total of 90 subjects randomly meantime the interviews related to age group, gender (sex), obesity (overweightness), exercise, diet, family history and monthly income were also conducted from the same subjects. Out of 90 subjects male and female 70 individuals were selected with diabetes while 20 non-diabetic individuals were selected. According to obtained results the ratio of type II diabetes in male patients was observed as (44.4%) and the ratio of female patients was (55.6%). About (32.0%) were found with an age of 30-40 years, 30.0% was calculated with an age of 41-50 years and (38.0%) was observed with an age of 51-60years. It was observed that about (17.8%) patients were with Primary education, (6.7%) with Middle Education, (10.0%) with Secondary Education, (8.9%) with Graduation and (56.7%) was observed uneducated. The results of monthly income showed that (54.4%) suspects have less than 10000 monthly incomes, (14.4%) have 11000-20000 monthly income, (13.3%) have 21000-30000 monthly income, (5.6%) have 31000-40000 monthly income and (12.2%) have a monthly income of more than 41000. About (40.0%) patients were observed with a physical activity for one hour daily, (15.6%) with two hours daily, (5.6%) with three hours daily and (38.9%) patients were observed with no physical activity. However, the obtained results of $p > 0.05$ for age and diabetes showed the non-significance association between age and diabetes, The education and monthly income showed the non-significant association $p > 0.05$ with diabetes while physical activity showed the statistically significant association $p < 0.05$ with diabetes. Meantime the symptoms such as too much hunger and blurry vision show statistically significant association $p < 0.05$ with diabetes. Since the results of secondary data of year 2022 showed that maximum ratio of male diabetic patients was observed as (9.2%) in the month of December and minimum ratio of male patients (7.0%) was observed in the month of February. While the maximum ratio of female patients (9.3%) was observed in the month of January and the minimum ratio of female diabetic patients (7.0%) was observed in April. Meantime the calculated results for the year 2023 showed that the maximal percentage of male subjects (10.6%) was examined in the month of January and least proportion of male patients (8.9%) was examined in the month of February. While the maximum ratio of female patients (10.5%) was observed in the month of October and the minimum ratio of female diabetic patients (9.4%) was observed in the month of February in Civil Hospital Khairpur, Sindh, Pakistan.

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1. INTRODUCTION

Diabetes mellitus (DM) is a disease in which the body has inability to make and use insulin. Insulin is a hormone which is generated by the pancreas that regulates the blood sugar levels in the body which supports the transfer of glucose from blood to the corpuscle of the body, where it is hydrolyzed and used for energy purposes. Without insulin people cannot survive because they can't maintain the blood glucose level in the body (ADA, 2007). Diabetics results in abnormal glucose levels in the bloodstream. A person could lead to serious short and long repercussions such as brain damage, amputations and also heart disease (ADA, 2007). DM considered a common diverse disorder of metabolism in which a subject face with chronic hyperglycemia. The reason of Diabetes is either lessened insulin secretion or impaired insulin actions or both (Kerner and Brückel, 2014). DM is acute fundamental metabolic disease, which not only extremely damage human health, but cause a huge socioeconomic problem (Wang et al., 2013; Ajami et al., 2006-2009). According to the modernized information from the international diabetes federation (IDF), expected global frequency of DM achieved 8.8% in 2015 and 12% of worldwide health outflow was due to DM in same year (Yates and Khunti, 2016).

Diabetes mellitus is rising as an epidemic of the 21st century. About 366 million people around the world have diabetes and it is probable to increase to 552million by 2030 in which midmost-income countries where more than 80% of diabetes death happened. Biologically male and female have similar diabetes popularity (International Diabetes Federation). Diabetic type I considered only for sectional of whole burden of diabetes in the age of earlier and found maximal in the well-to-do countries and grow equally in the rich and poor countries in globe. Additionally, a swing concerning to type I diabetes occurs in children at earlier age (Sicree et al., 2006). About 85 to 95% of diabetes type II are calculated as higher dominance range in most of advanced countries. It is thoroughly associated with inappropriate consumption of insulin by target cell and tissues. A presently extensive and severe health distress globally. According to WHO (1994), current cultural as well as social trends such as ageing population, growing urbanization, dietary changes, decreasing physical activity and undesirable life and behavioral patterns have aggravated these problems. Diabetes mellitus and milder types of glucose intolerance are primary impaired glucose tolerance and may now be found in almost every population on the planet and epidemiology information suggest that

without effective prevention and control program diabetes will likely continue to rise globally (WHO, 1994). In 2010 it was estimated that 285 million people aged 20 to 70 years ahead of having diabetes in worldwide levels, with 70% of them living in developing countries. By 2030, this figure is expected to rise to almost 438 million. In addition, the number of persons with IGT is predicted to rise to 472 million accounting for 8.4% of the adult population (Sicree et al., 2006). Various organ failure as well as growing metabolic disorders such as retinopathy, nephropathy, and neuropathy are among the unbearable symptoms of diabetes (Piero, 2006). One of the most frequent non-communicable diseases in the world is diabetes (Shaw et al., 2010).

Insulin is an anabolic hormone and the most successful glucose lowering agent existing. It is suggested for usage in T1DM as sole remedy and in T2DM patients weakening to control sugar levels with oral hypoglycemic drugs (Michael, 2012). As concern with metabolism of proteins, insulin developed for the transport of amino acid across membrane, trigger the protein formation, and prevent proteolysis. Integration of fatty acid from rotating triglycerides into adipose triglyceride as well as lipid formation is triggered by insulin lipolysis is introverted. Insulin participates to nucleic acid formation by stimulate the synthesise of DNA, ATP, and RNA (Cahill, 1971). Insulin has anabolic action and insulin signaling is dangerous for developing uptake, use and storage of main nutrients such as glucose, lipids, and amino acids. Insulin provokes glycogenesis, lipogenesis, and protein formation (Ahmed, 2002). Several factors affecting lifestyle which are important to be known for the development of type II DM. These factors are physical inactivity, sedentary lifestyle, cigarette smoking, obesity, hypertension, elevated cholesterol (combined hyperlipidemia), generous consumption of alcohol, stress, dehydration habits, too much hungry and genetically disorders (Hu et al., 2001). There are strong inheritable genetic connection in type II DM as having relative (especially first degree) with type II DM increases the risk of developing type II DM substantially (Rother, 2007).

2. MATERIALS AND METHODS

2.1 Samples Collection

A cross-sectional study was conducted for the analysis of blood sugar level in male and female diabetic and non-diabetic patients. This study was conduct in Civil hospital Khairpur Mir's, Sindh Pakistan. A total of 90 blood samples were collected from diabetic type 2 and non-diabetic patients. Out of 90 subjects 70 were diabetic and 20 were non-diabetic. Before collection

of blood sample each individual was informed regarding the purpose of sampling. The blood sample was collected with the help of a medical technician.

2.2 Analysis of samples in laboratory

These groups were further divided into three different age groups of (30-40), (41-50) and (51-60) years. The random blood sugar levels measured either in emergency conditions using gluco-meter or samples were sent to laboratory for analysis. Where samples were analyzed using micro lab 300 machine. In the laboratory the end point pip method was used for reading blood sugar level. The 3cc blood sample from the patient through the syringe was collected. After that the blood sample was transferred into Eppendorf tube. The sample was left at room temperature for 10 to 12 minutes for clotting of blood. After that the sample was centrifuged using centrifuge machine at about 4000 rpm (revolution per minute) for 2-5 minute for separation of serum. 10ul serum was taken in a separate Eppendorf tube and 1 ml of glucose reagent (1000ul) was added with the help of macro tips attached with the pipette of (100-1000ul) and the sample was kept for incubation at 37°C for 10 minutes. Before analysis the Micro lab was rinsed to prevent it from contamination and was set at Zero using water (blank). After that the sample was analyzed using Micro lab 300 of MERCK Company, the glucose testing was selected in the Micro lab and reading was noted.

2.3 Statistical Analysis

After the tabulations of information, the data analyzed by using the SPSS software sheets (IBM* SPSS statistics version 21). To know the total prevalence of diabetic patients with type 2 populations selected from Civil hospital Khairpur Sindh Pakistan. The data of total patients of diabetes males and females were collected from January 2022 to November 2023. For keeping records of data, a questionnaire applied having the information including (patients name, husband or father name, address, age, history of diabetes, test result of randomly blood sugar levels, smoking, cardiac patients, weight, diet, blood pressure, sign, and symptoms of patients etc.

3. RESULTS AND DISCUSSION

The present study was to evaluate the Comparison of diabetes type II between male and female in the civil hospital Khairpur Mir's Sindh. About Ninety patients were selected randomly for the diabetic tests from September to November 2023. The age of subjects varied from 30 to 40, 41 to 50, 51 to 60 years old. According to present results the percentage of males

and female's patients were observed to be 44.4% and 55.6% respectively, as shown in table 1.

We have differentiated the patients based on physical activities (exercise) in the daily routine of their life. According to our study we observed the 40.0% patients were doing exercise one hour, 15.6% were doing exercise two hours, 5.6% were doing exercise three hours, and 38.9% did not have any physical activities as represented in table 2.

During the research work we observed the effect of family history on the patients. According to our survey we concluded that the 54.4% subjects have diabetes, due to their family history because as we know there are some diseases are found in the grandfather or grandmother which transfer to their next generation, so diabetes is also one of the heritable diseases that cause some of serious effect on life of organism. As diabetes is the autosomal dominant mutation they can passed on to children when only one parent carries the gene for the disease, in this case mostly the parent who carry the gene has 50% chances to transfer it to their offspring. While 45.6% did not have consequences for transferring of disease because they are non-diabetic as signify in the table 2.

In our study, most diabetic patients were found to have frequent urination they cannot control the urine because they lose their control due to hyperglycemia. About 67.8% have dysuria that is abnormally frequent urination once or more than that in every hour, although 32.2% have not any dysuria because they have normal body glucose level, and they were performing normal urination as presented in the table 4.

In our study we examine the Patients that have distributed on the core of medication such as Glucophage, metformin, Glipizide tablets, and insulin injections. About 37.8% patients use the injection of insulin mostly in the case of type I diabetes but some time it is also injected in type II diabetes, while 40.0% were taking the medicines and has generally been used by type II diabetic patients as depend upon their condition and range of sugar level mostly, they used Glucophage tablets. These tablets play an important role in preventing organism from kidney damage, nerve problems, loss of limbs and sexual function problems. While it is also dependent upon the patients which type of diabetes, they have either type I or type II Diabetes. Others 22.2% were normal glucose levels because they were non-diabetic patients as shown in the table 3.

In the Civil hospital Khairpur, we found that 51.1% patients were having blurry vision due to diabetes that

cause the swelling in the eyes, temporary change in the vision but sometimes it damaged the back of the eye (retina) which cause blindness. A drug Metformin which has the effect of lowering the sugar level helped people lower the chances of developing vision loss. While 48.9% were normal eye vision as shown in table 6.

In this study it is observed that the diabetic subjects have different complications such as fatigue, frequent urination, hyperglycemia, thirst, too much hunger, stress, hypertension, physical activity, blurry vision, diet plan irregularity and various other factors that affect the lifespan of a patient, while non-diabetic patients involved in normal life, they do not have any complications.

To our knowledge, Diabetes mellitus is an endless disease in developing countries. In different provinces of Pakistan, a lot of work had been done in different aspects of diabetes mellitus. According to our study first one is to compare the prevalence of type II Diabetes mellitus between males and females. The overall prevalence of the study was found to be 26.1% in both males and females in three village of Khyber Pakhtunkhwa (Shera et al., 2007) and other provinces such as in Sindh 25% of prevalence (Shera et al., 1995), while in Baluchistan 22% (Shera et al., 1999). The maximum figures of abnormal glucose tolerance were found as 25.1% in men and 26.4% in women with the growing age, the prevalence of T2DM in Pakistan was recommended an increase from 4.3 million in 1995 to 14.5 million in 2025 as expected. This will include the Pakistan the fourth among top 10 diabetes reporting countries of the world (Wild 2004). Diabetes mellitus is attributable to many factors such as industrialization, urbanization, and a change in lifestyle (Ramachandran et al., 2003).

However, our present work was conducted in Civil Hospital Khairpur Mir's Sindh Pakistan. The results in this study showed the comparison of diabetes type II between male and female. We conducted our survey from September to November 2023. The current study shows the prevalence of diabetes type I and type II in gender, males as 44.4% and in females 55.5% in Civil Hospital Khairpur Mir's. While previous observation explained that the total 15.41% of males and 12.31% of females were found with diabetes (Zafar et al., 2011). Thus, the present ratio is increasing due to environmental factors affecting the sedentary lifestyle. The current study shows age wise comparison of diabetes mellitus type II was higher in the age group of 45-60 years as 44.3% and lower in age group of 30-40 years that was 9.9%. As observed in the previous literature age group was higher in 55-64 years that

denoted the same results in our study (Ahmed et al., 2011).

Our result showed that the Net monthly income of 54.4% of patients had less than 10,000 rupees, 14.4% had 11000-20000 rupees, while as 13.3% had 21000-30000 rupees, 5.6% had 31000-40000 rupees and 12.2% had more than 41000 rupees. In previous work shown 36.2% had less than 10000 rupees (Hydier et al., 2004). This increasing ratio indicates the poorness of people and unawareness about diabetes. The current work demonstrates that 54.4% of patients had diabetes due to inheritance, while 45.6% had not family history effect but they have developed diabetes due to environmental factors such as obesity, stress, imbalanced diet, and others. According to the association between family history with diabetes we found non-significant $p=0.651$ due to $p>0.05$. While previous work described the family history and hypertension, obesity and sedentary lifestyle were strongly associated with the presence of disease ($p<0.05$) as followed ($p=0.002$). (Khan et al., 2013). The current study shown the maximum values of random blood sugar levels reached between 280mg/dl and 300mg/dl in most of patients due to increasing blood pressure and less physically activity as significant $p<0.05$ ($p=0.000$). In previous work maximum number of patients 51% out of 100% had diabetes due to obesity, smoking, trauma to pancreas, physical sluggishness (Zuhaid, 2012).

4. CONCLUSION

The present study concludes that there was a significant association between physical activity and diabetes, while there was no significant association between monthly income and diabetes. While there was a significant association between marital status and diabetes. Meantime, the symptoms such as too much hunger, blurry vision and frequent urination showed statistically significant association with diabetes. However, the observed results of secondary data showed that there was an increased trend of diabetic patients from 2022-2023 in Civil Hospital District Khairpur Mir's, Sindh, Pakistan.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1. Frequencies and percentage of male and female subjects from civil hospital Khairpur Mir’s Sindh, Pakistan.

Gender	Frequency	Percentage
Male	40	44.4%
Female	50	55.6%
Total	90	100%

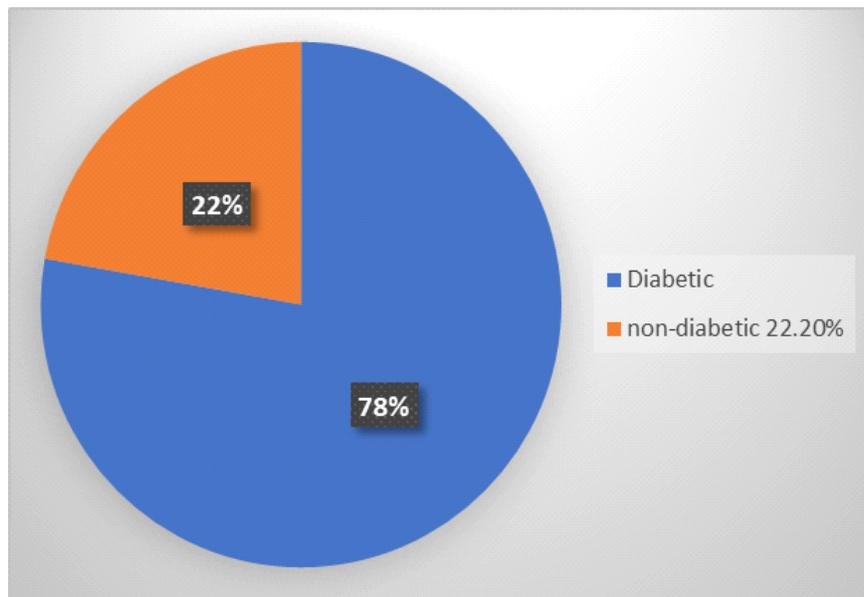


Figure 1. Percentage of male and female subjects from civil hospital Khairpur Mir’s Sindh, Pakistan shown that 78% subjects with diabetic while 22% were non-diabetics.

Table 2. Frequency and Percentage of subjects associated with Physical activity in Civil Hospital Khairpur Mir’s Sindh Pakistan.

Physical activities per hours	Frequency	Percentage
One hour	36	40.0%
Two hours	14	15.6%
Three hours	5	5.6%
No activity	35	38.9%
Total	90	100%

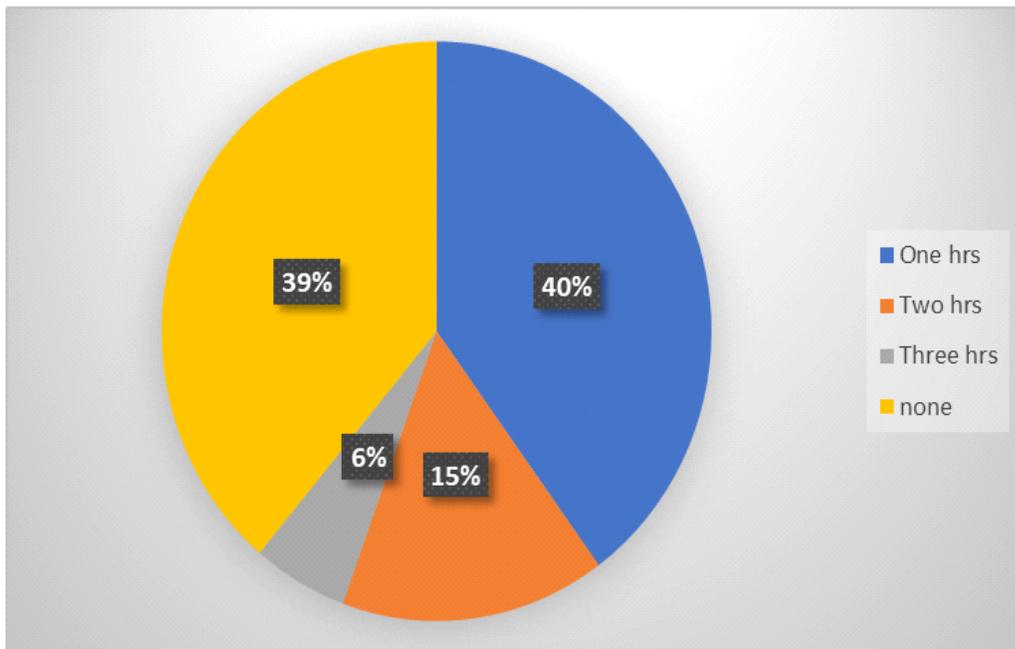


Figure 2. Percentage of physical activity in subjects from civil hospital Khairpur Mir’s Sindh, Pakistan.

Table 3. Shows high percentage of patients with diabetes rather than non-diabetic patients because of the genealogy arrangements.

Family history of subjects	Frequency	Percentage
Diabetic	49	54.4%
Nondiabetic	41	45.6%
Total	90	100%

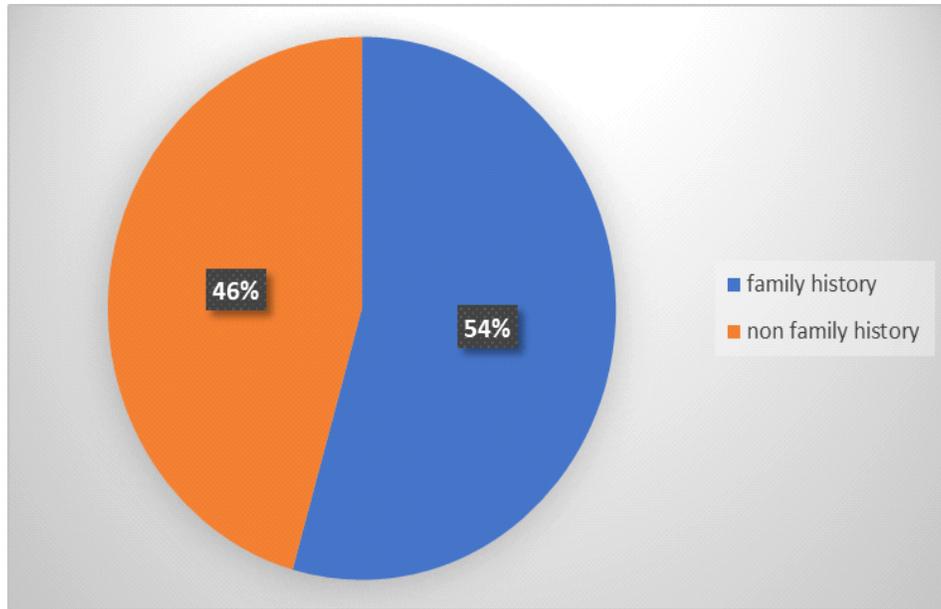


Figure 3. Percentage of family history concern with diabetic and non-diabetic patients in the Civil Hospital Khairpur, Mir’s Sindh, Pakistan.

Table 4. The frequency and percentages of patient having dysuria in the Civil Hospital, Khairpur Mir’s Sindh, Pakistan.

Frequent urinations	Frequency	Percentages
Dysuria	61	67.8%
Normal	29	32.2%
Total	90	100%

Table 5. Shown the frequency of patients and percentages that were elaborate the medication during observation.

Medication of patients	Frequency	Percentage
insulin	34	37.8%
Medicines	36	40.0%
Non diabetics	20	22.2%
Total	90	100.0%

Table 6. Frequency and Percentage of patients compare between blurry vision and normal in Civil Hospital Khairpur Mir's Sindh Pakistan.

Blurry Vision	Frequency	Percentages
Abnormal	46	51.1%
Normal	44	48.9%
Total	90	100%

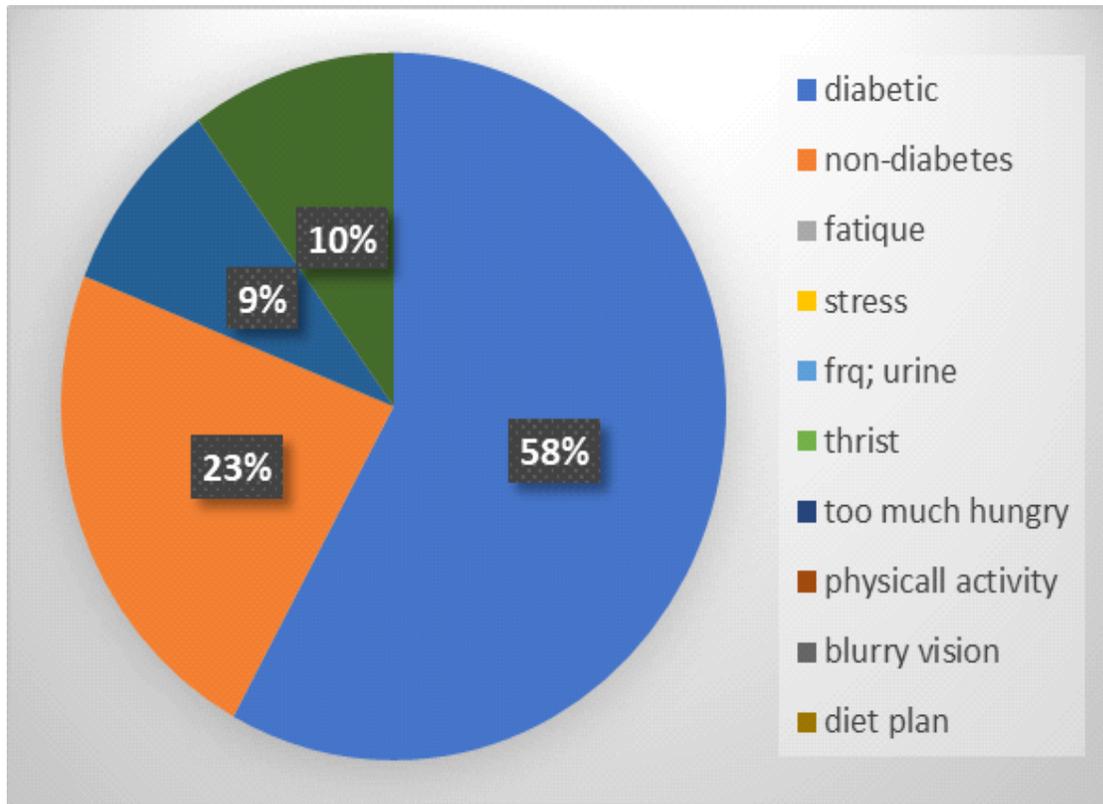


Figure 4. The whole Percentages of symptoms concern with Diabetic and Non-Diabetic subjects in the Civil Hospital Khairpur Mir's Sindh, Pakistan.

WASP FAUNA OF SUBFAMILIES EUMENINAE, VESPINAE AND POLISTINAE FROM SIBI, BALOCHISTAN, PAKISTAN

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ABSTRACT

This study identified Vespidae species from the subfamilies Eumeninae, Polistinae, and Vespinae in the Sibi district of Balochistan, Pakistan. A total of 9 species across 5 genera were recorded. Among them, four species belong to Eumeninae, three to Polistinae, and two to Vespinae. All species were documented for the first time from the Sibi district, with two species, *Anterhynchium (Anterhynchium) abdominale* and *Delta campaniforme campaniforme*, being reported for the first time from Balochistan province. Present study will serve as a foundation for future studies on Vespidae from Sibi and the wider Balochistan region.

1. INTRODUCTION

Vespidae is the largest family of order Hymenoptera with a cosmopolitan distribution and comprises more than 5000 species in 250 genera arranged in six subfamilies: Euparagiinae, Eumeninae, Masarinae, Polistinae, Stenogastrinae, and Vespinae (Carpenter, 1981 & 1982); Pickett & Carpenter, 2010). This family widely distributed in tropical Africa, South Africa, Asia, Australia and throughout temperate Eurasia and North to Central South America (van der Vecht, 1966). The vespidae fauna of Pakistan has significance due to its transitional position between the Palearctic and Oriental regions. The distribution of vespidae wasps in Pakistan have been well studied by many authors such as Nurse, 1903, 1904; Cameron, 1907; Meade-Waldo, 1910; Dover & Rao, 1922; Dover, 1925 [1924]; Kostylev, 1940; Chaudhry et al., 1966; Das & Gupta, 1984, 1989; Archer, 1989; Carpenter, 1996; Carpenter & Kojima, 1997; Gusenleitner, 2006, 2007, 2008;

Dvořák, 2007; Kumar, 2010; Bodlah et al., 2011, 2012, 2015; Mahmood et al., 2012, Siddiqui et al., 2015; Shah 2015; Faiz et al., 2016; Khan et al., 2017; Rasool et al., 2017; Rafi et al., 2017; Rauf et al., 2018; Durrani et al., 2018; Qasim et al., 2018a,b; 2022a,b.

Wasps have an important role in ecosystem as a biological control agent, effective pests, scavengers and pollinators (Fateryga, 2010). Many species of wasps are predators; they feed on spiders, crickets or immature of other insects and caterpillars (Goulet & Huber, 1993). However, almost all adult wasps feed on nectar but they also feed on juice of ripened fruits and nectar from the killed honey bees while solitary wasps paralyze their prey instead of killing it and store in nests for their larvae food (Spradbery, 1973). They also damage fruits and sting the people (Monceau et al., 2014). Stinging of hornet and yellow jacket wasps is a serious risk for general people, especially in late summer and fall when their colonies become maximum in sizes and the people who suffer

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from acute sensitivity to wasp venom become at risk (Galloway, 2008).

The biogeographical location of district Sibi is within the arid and semi-arid region of Pakistan. This area is characterized by its dry climate, limited vegetation, and adaptations of flora and fauna to survive in harsh desert-like conditions. The region's specific coordinates are approximately 27°55' and 30°38'N latitude and 67°17' and 69°50'E longitude lying at the bank of the River Bolan in the Balochistan, Pakistan. The district is well-known for its natural geographic beauty and mountain ranges. This present study aimed to explore the Vespidae wasp fauna of the district Sibi from different localities such as Luni, Kurak, Khajjak, Lehri, Sibi city, Mandai from Balochistan Pakistan.

2. MATERIALS AND METHODS

Vespid wasps were collected from various localities within the Sibi district of Balochistan, Pakistan, during the period of 2022-2023. The selection of collection sites was based on the diversity of habitats within the district to ensure a comprehensive representation of the local Vespidae fauna. Habitats included areas with abundant vegetation, flowering plants, and other environments favorable to wasps. Specimens were collected using aerial nets, a standard method for capturing flying insects. The nets were carefully used to capture wasps from flowers, plants, and other suitable environments. Collections were made during daylight hours when wasps were most active, and in varying weather conditions to capture a diverse range of species. After capture, the wasps were immediately placed in collection jars containing 70% ethanol, which served as a preservative. The collected specimens were identified to the species level using a Labomed Luxao 6Z Stereo Zoom Microscope. Identification was conducted based on morphological characteristics, using key literature references, including: (Das & Gupta, 1984; Chaudhry et al., 1966; Archer, 1989; Carpenter & Kojima, 1997; Carpenter, 1996; Gusenleitner, 2006, 2007, 2008; Dvorak, 2007; Kumar, 2010; Mahmood et al., 2012; Siddiqui et al., 2015; Rafi et al., 2017, Qasim et al., 2022a). After identification, the specimens were properly labeled with collection data (e.g., locality, date, collector name) and deposited in the

Biosystematics Laboratory at Balochistan Agriculture College, Quetta, Pakistan.

3. RESULTS

Family Vespidae Latreille, 1802

Subfamily Eumeninae Latreille, 1802 (Potter Wasps)

Genus: *Anterhynchium* de Saussure, 1863

1. *Anterhynchium (Anterhynchium) abdominale abdominale* (Illiger, 1802)

Material examined: Pakistan: Balochistan: District Sibi: Sibi city, 4.ii.2023. Leg. Mehmood Khan, 1 ♀; Kurak, 6.ii.2023, Leg. Mehmood Khan, 2 ♂.

Remarks: This is new record for Balochistan. Earlier this species was reported from Islamabad; Sindh: Karachi; Punjab: Faisalabad, Rawalpindi and Attock (Dover & Rao, 1922; Siddiqui et al., 2015; Rafi et al., 2017).

Distribution: India; Myanmar; Pakistan; Sri Lanka and Vietnam (Bingham, 1897; van der Vecht, 1963; Kumar & Sharma, 2014).

Genus *Delta* de Saussure, 1854–56

2. *Delta campaniforme campaniforme* (Fabricius, 1775)

Material examined: Pakistan: Balochistan: District Sibi: 20.vi.2022. Leg. Mehmood Khan, 2 ♂; Lehri, Leg. Mehmood Khan 1 ♀; Kurak 22.vi.2022, Leg. Mehmood Khan 2 ♂; Luni, 25.vi.2022

Remarks: New record for Balochistan. Already this species reported from Sindh: Ghotki (Khan et al., 2018).

Distribution: Australia; China; Cambodia; India; Indonesia; Guangdong; Laos; Myanmar; Nepal; Malaysia; Palawan; Philippines; Thailand, Papua New Guinea; Singapore, U. S. A. Vietnam and Pakistan (Nguyen, 2015).

3. *Delta dimidiatipenne* (de Saussure 1852)

Material examined: Pakistan: Balochistan: District Sibi: Sibi City, 13. v.2022. Leg. Mehmood Khan, 1 ♂; Kurak, 14.v.2022, Leg. Mehmood Khan 2 ♂; Luni, 17.v.2022, Leg. Mehmood Khan, 1 ♀.

Remarks: Earlier this species reported from Baluchistan: Quetta and Noshkey; Islamabad; Punjab: Attock, Chakwal, Gujranwala, Gujrat, Jhelum, Jhang, Faisalabad, Murree, Multan,

Khanewal, Khyber-Pakhtunkhwa: Abbottabad, Balakot, Battagram, Dir, Mansehra, Peshawar; Azad Jammu and Kashmir: Muzaffarabad, Poonch, Banjosa Rawalakot, Bagh; Gilgit-Baltistan: Astore, Ghizer and Hunza; Sindh: Dadu, Larkana, Sukkur, Nagarparkar, Hyderabad, Umarmkot, Korangi (Karachi) (Gusenleitner, 2006; Bodlah *et al.*, 2011; 2012; Mahmood *et al.*, 2012; Siddiqui *et al.*, 2015; Rafi *et al.*, 2017; Qasim *et al.*, 2022a).

Distribution: Afghanistan; Algeria; Chad; Djibouti; Egypt; Eritrea; Ethiopia; India; Iran; Jordan; Mauritania; Nepal; Niger; Morocco; Oman; Pakistan; Qatar; Saudi Arabia; Spain; Somalia; South Africa; Sudan; Syria; Tajikistan; Turkey; Turkmenistan; U.A.E.; Uganda; Yemen (Srinivasan & Kumar, 2010; Bodlah *et al.*, 2011; Siddiqui *et al.*, 2015; Rafi *et al.*, 2017).

4. *Delta esuriens esuriens* (Fabricius, 1787)

Material examined: Pakistan: Balochistan: District Sibi: Sibi City, 17.vi.2022. Leg. Mehmood Khan, 2 ♂; Khajjak, 20.vi.2022, Leg. Mehmood Khan, 3 ♂.

Remarks: This species already reported from Baluchistan: Quetta, Chiltan National Park Hazar Ghanji; Islamabad; Punjab: Attock Chakwal, Bakhar, Layyah, Multan, Shujabad, Mianwali, Muzafargarh, Head Fareed, Khanpur and Rawalpindi; Khyber-Pakhtunkhwa: Dir, Swat, Abbottabad, Balakot and Mansehra; Azad Jammu and Kashmir: Kohala, Kotli (Gusenleitner, 2006; Bodlah *et al.*, 2012; Mahmood *et al.*, 2012; Siddiqui *et al.*, 2015; Rafi *et al.*, 2017; Durrani *et al.*, 2018; Qasim *et al.*, 2022a).

Distribution: India; Indonesia; Iran; Iraq; Israel; Laos; Mauritius; Myanmar; New Caledonia; Oman; Pakistan; Philippines; Qatar; Saudi Arabia; Sri Lanka; Thailand; U. A. E.; Vietnam (Gusenleitner, 2006a; Srinivasan & Kumar, 2010; Bodlah *et al.*, 2012; Mahmood *et al.*, 2012).

Subfamily Polistinae Latreille, 1802 (Paper wasps)

Genus *Polistes* Latreille, 1802

5. *Polistes (Polistes) indicus* Stolfa 1934

Material examined: Pakistan: Balochistan: District Sibi: Luni, 7.ii.2023. Leg. Mehmood Khan, 1 ♂; Sibi City, 11.ii.2023, Leg. Mehmood Khan, 2 ♂; Mandai, 15.ii.2023, Leg. Mehmood Khan, 1 ♀.

Remarks: Already reported this species from Balochistan: Quetta, Chiltan National Park Hazar Ghanji; Punjab: Attock, Chakwal, Multan, Jhelum, Rawalpindi, Bahawalpur: Chak, 28 BC; Khyber-Pakhtunkhwa: Abbottabad, Alia, Ayubia, Baffa, Balakot, Battagram, Havelian, Oghi, Mansehra, Abad Khel, Kohat, Upper and Lower Dir, Peshawar; Sindh: Allahyar Pinhwar, Ghotki, Sukkur, Taj Mohammad Ruk; Gilgit-Baltistan: Chillas, Hunza, Gasdas, Ghizar valley, Jaglot, Thiee, Astore and Gilgit; Azad Jammu and Kashmir: Muzaffarabad (Dvořák, 2007; Gusenleitner, 2007; Mahmood *et al.*, 2012; Siddiqui *et al.*, 2015; Rafi *et al.*, 2017; Durrani *et al.*, 2018; Qasim *et al.*, 2022a)

Distribution: Afghanistan; Iran; Iraq; India; Oman; Pakistan; U.A.E. (Carpenter, 1996; Gusenleitner, 2007; Dvořák, 2007; Siddiqui *et al.*, 2015).

6. *Polistes (Gyrostoma) olivaceus* (DeGeer, 1773)

Material examined: Pakistan: Balochistan: District Sibi: Luni, 10.ii.2023. Leg. Mehmood Khan, 2 ♂; Sibi, 16.ii.2023, Leg. Mehmood Khan, 3 ♀; Khajjak, 17.ii.2023, Leg. Mehmood Khan, 2 ♂.

Remarks: Already this species reported from Islamabad; Gilgit-Baltistan: Nagar; Punjab: Rawalpindi, Attock, Chakwal and Jhelum. Khyber-Pakhtunkhwa: Mansehra, Naran, Balakot, Baffa, Oghi, Battagram, Allai, Abbottabad, Ayubia and Havelian; Azad Jammu and Kashmir: Muzaffarabad (Mahmood *et al.*, 2012; Siddiqui *et al.*, 2015; Shah, 2015; Rafi *et al.*, 2017).

Distribution: Afghanistan; Cambodia; China; Egypt; Fiji; French Polynesia; India; Indonesia; Iran; Japan; Laos; Madagascar; Malaysia; Marianas; Mauritius; Myanmar; Nepal; New Caledonia; Oman; Réunion; Samoa; Seychelles; Singapore; Sri Lanka; Taiwan; Tanzania: Zanzibar; Thailand; Tonga; Vietnam; introduced into Australia: Chile; Easter Is.; Hawaii; New Zealand (Das & Gupta, 1989; Mahmood *et al.*, 2012).

7. *Polistes (Gyrostoma) wattii* Cameron 1900

Material examined: Pakistan: Balochistan: District Sibi: khajjak, 6.ii.2023. Leg. Mehmood Khan, 3 ♂; Kurak, 8.ii.2023, Leg. Mehmood Khan, 2 ♂; Sibi city, 10.ii.2023, Leg. Mehmood Khan, 1 ♂.

Remarks: Already reported this species from Balochistan: Quetta, Chiltan National Park Hazar Ghanji; Islamabad; Punjab: Attock, Rawalpindi,

Abbaspur, Multan, Khanewal, Kabirwala, Kahrur Pakka, Lodhran, Shujabad, Jahanian, Mian Channu, Vehari, Bahawalpur (Chak 28 BC), Habib Massan; Murree, Taxila, Salt Range (Khewra); Sindh: Karachi. Allahyar Pinhwar, Drago, Ghotki, Rajab Ali Bharo, Sukkur, Kirthar National Park, Mirpur Khas, Umartot, Jacobabad,; Khyber-Pakhtunkhwa: Abbottabad, Dir, Kohat, Mansehra, Mardan, Peshawar, Warsak; Gilgit-Baltistan: Basha, Chillas, Dassu, Diamer, Gilgit, Sukkur, Juglot Ghizer, Hunza-Nagar and Skardu; Azad Jammu and Kashmir: Muzaffarabad, Poonch, Rawalakot (Das & Gupta, 1984; 1989; Gusenleitner, 2007; Mahmood et al., 2012; Siddiqui et al., 2015; Shah, 2015; Khan et al., 2017; Khan et al., 2018)

Distribution: Afghanistan; China; India; Iran; Iraq; Mauritius; Oman; Pakistan; Saudi Arabia; Tajikistan; Turkmenistan; U.A.E. (Das & Gupta, 1989; Gusenleitner, 2007; Kumar, 2010).

Subfamily Vespinae Linnaeus, 1758 (Yellow jackets and Hornets)

Genus *Vespa* Linnaeus, 1758

8. *Vespa orientalis* Linnaeus 1771

Material examined: Pakistan: Balochistan: District Sibi: Sibi city, 21.ii.2023. Leg. Mehmood Khan, 4♂; Kurak, 26.ii.2023, Leg. Mehmood Khan, 2♂; Luni, 28.ii.2023, Leg. Mehmood Khan, 3♀.

Remarks: Already reported this species from Balochistan: Quetta, Chiltan National Park Hazar Ghanji; Islamabad; Punjab: Multan, Khanewal, Jahanian, Kabirwala, Vehari, Abdul Hakim, Burewala, Lodhran, Dunyapur, Fort Abbas Attock, Chakwal, Murree, Rawalpindi; Sindh: Dadu City, Mirpur Mathelo, Kandhkot, Kirthar National Park, Mirpur Khas; Gilgit-Baltistan: Astore, Ghizer, Chillas, Dassu, Gilgit, Jaglot, Nomal; Khyber-Pakhtunkhwa: Bamboret valley, Brun and Chitral; Abbottabad, Bannuu, Charsada, Kohat, Mansehra, Mardan, Peshawar and Warsak, Jamrud, Chilas, Basha, Alai, Baffa, Balakot, Battagram, Dir, Havelian and Mansehra; Azad Jammu and Kashmir: Rawalakot, Topa, Thorar, Mandhol and Muzaffarabad (Das & Gupta, 1984; 1989; Gusenleitner, 2007; Dvořák, 2007; Mahmood et al., 2012; Siddiqui et al., 2015; Khan et al., 2017; Durrani et al., 2018; Khan et al., 2018; Qasim et al., 2022a).

Distribution: Afghanistan; Algeria; Albania; Bahrein; Bosnia and Herzegovina; Bulgaria; China; Croatia; Cyprus; Egypt; Ethiopia; Georgia; Greece; Italy; Iraq; Israel; Iran; India; Jordan; Kazakhstan; Kyrgyzstan; Lebanon; Libya; Macedonia; Malta; Montenegro; Nepal; Oman; Pakistan; Russia; Romania; Saudi Arabia; Somalia; Syria; Turkey; Turkmenistan; Tajikistan; Uzbekistan; U.A.E.; Yemen; introduced into Czech Republic; Spain; Madagascar; Mexico; U.S.A. (Das & Gupta 1984, 1989; Carpenter & Kojima, 1997; Dvořák 2007).

Genus: *Vespula* Thomson, 1869

9. *Vespula germanica* (Fabricius, 1793)

Material examined: Pakistan: Balochistan: District Sibi: Khajjak, 5.iv.2023. Leg. Mehmood Khan, 1♂; Leg. kurak, 8.iv.2023, Mehmood Khan, 1♂.

Remarks: Already reported from Balochistan: Ziarat, Kalat; Khyber-Pakhtunkhwa: Swat, Kalam, Balakot, Baffa, Chitral, Bamboret valley, Brun, Dir, Abbottabad, Mansehra, Havelian, Ayubia; Gilgit-Baltistan: Daimer, Chillas, Gulmit, Khyber, Khuramabad, Shish kot, Gulkin, Passu, Aliabad, Nagar, Denor, Sost, Misger, Ghizer, Skardu and Gilgit (Chaudhry et al., 1966; Dvořák, 2007; Mahmood et al., 2012; Shah, 2015; Faiz et al., 2016).

Distribution: Austria; Armenia; Algeria; Azores; Albania; Belarus; Belgium; Bulgaria; Canada; China; Croatia; Czech Republic; Denmark; England; France; Germany; Georgia; Greece; Hungary; Iran; Ireland; Israel; India; Kazakhstan; Korea; Luxembourg; Malta; Morocco; Mongolia; Norway; Netherlands; Pakistan; Poland; Portugal; Russia; Romania; Sweden; Scotland; Slovenia; Spain; Switzerland; Syria; Taiwan; Tajikistan; Turkey; Turkmenistan; Tunisia; Ukraine; Uzbekistan and U.K.; introduced into Ascension Island; Argentina; Australia; Canada; Chile; Iceland; New Zealand; South Africa; U. S. A. (Carpenter & Kojima 1997; Dvořák 2007; Mahmood et al., 2015).

4. DISCUSSION

The findings of this study highlight the rich diversity of Vespidae wasps in the Sibi district, Balochistan, Pakistan. The identification of 9 species across 5 genera within three subfamilies (Eumeninae,

Polistinae, and Vespinae) underscores the ecological significance of this region.

The documentation of four species belonging to the subfamily Eumeninae (*Anterhynchium* (*Anterhynchium*) *abdominale* *abdominale*, *Delta campaniforme* *campaniforme*, *Delta dimidiatipenne*, and *Delta esuriens esuriens*), three species from the subfamily Polistinae (*Polistes* (*Polistes*) *indicus*, *Polistes* (*Gyrostoma*) *olivaceus*, and *Polistes* (*Gyrostoma*) *watti*), and two species from the subfamily Vespinae (*Vespa orientalis* and *Vespula germanica*) is significant. Notably, all these species have been recorded for the first time from the Sibi district, indicating a gap in prior research in this area. Furthermore, two species, *Anterhynchium* (*Anterhynchium*) *abdominale* *abdominale* and *Delta campaniforme* *campaniforme*, were recorded for the first time from Balochistan province.

The presence of such a diverse array of Vespidae species is indicative of the ecological health of the Sibi district. Wasps, as both predators and pollinators, play crucial roles in ecosystems by contributing to the pollination of various plants. The identification of these species can provide valuable insights into the local ecosystem's dynamics and the role these wasps play in maintaining ecological balance.

Additionally, the findings suggest that the Sibi district, and potentially other underexplored areas of Balochistan, may harbor even more undiscovered species of Vespidae. This study serves as a foundation for future research, encouraging further exploration and documentation of wasp species in the region.

5. CONCLUSION

This study highlights the rich diversity of Vespidae wasps in the Sibi district, Baluchistan, Pakistan indicating a healthy ecosystem where wasps play key roles as predators and pollinators. The limited scope of the present study emphasizes the importance of further research in this region to find potentially undiscovered vespidae species. These findings would provide a valuable foundation for related future studies.

6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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HISTOLOGICAL COMPARISON OF SPLENIC HEMOSIDEROSIS TOXIC EFFECT OF BISPHENOL A (BPA) AND BISPHENOL S (BPS) IN MUS MUSCULUS

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ABSTRACT

Bisphenol A (BPA) and Bisphenol S (BPS) are phenolic organic compounds. These compounds are commonly used in the manufacturing of plastic containers, epoxy resins, food and drink cans, water pipes, electronic equipment, thermal paper, kitchen utensils, toys, and dental sealants. The general population exposed to bisphenol S, both directly (through oral and/or topical routes) and indirectly (through environmental pollution and/or food chain).? The main objective of the study was to evaluate the effect of Bisphenol A and Bisphenol S on the spleen of mice. Mice were equally divided into three groups, i.e., Control group (given water only), and dose group II (orally administrated 40mg/kg body weight, Bisphenol A for the period of 21 days) and dose group III (orally administrated 40mg/kg body weight, Bisphenol S for the period of 21 days) after dissection the spleen of the all mice were extracted and further processed for morphological, morphometric and histological analysis. The Bisphenol A treated group of mice shown that an evident ($p < 0.001$) increased in the body weight and the spleen weight of the Bisphenol A treated dose II treated group as compared to the control group, where significant ($p < 0.001$) decreased in the body and spleen weight were observed in the dose group III bisphenol S in comparison of with the mice of dose group II Bisphenol A. Histological analysis of dose group II Bisphenol A showed some abnormalities in the Spleen of mice like parenchymatic cells with an increased number of megakaryocytes, vacuolated cytoplasm, and irregular nuclei, thickening in the capsule, destruction of marginal zone red pulp and destruction in the white pulp. It concluded that the excessive and long-term use of Bisphenol A and Bisphenol S leads to impaired vital organs of albino mice, causing the impairment in blood parameters and splenic toxicity. This plastic product must be used with care, particularly for products underuse by children.

1. INTRODUCTION

According to estimates, toxicant exposures account for about 24% of human anomalies and disorders, with the possibility that these conditions could be passed on to subsequent generations even in the absence of direct exposure (Hou et al., 2012).

The mature phenotype and susceptibility to abnormalities/diseases in later life significantly influenced by environmental toxicant exposure during intrauterine life, postnatal life, early life, and/or germ cell, according to previous studies (Skinner et al., 2013). Analyzing the chemical toxicity of compounds like bisphenol A (BPA), cadmium, mercury, dioxin, and tri chloro ethane reveals that some of these toxins have an immediate impact on the ecosystem.

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Hemosiderosis is a general term used to describe the overload of iron in tissues and other organs. Increase in iron concentration results the epidermis tissue that turn bronze colored skin. Hemosiderin is a protein that store iron in the tissue (Chin et al., 2019). Iron excess occurs when iron intake increases indefinitely due to regular transfusions of whole blood and red blood cells or increased iron absorption through the gastrointestinal tract.

Bisphenol A also called endocrine disrupting agent because their ability to disrupt endocrine organs. Bisphenol A is component epoxy resins, which is use worldwide to produce plastic products such as drinking pipeline lining, paints, dental sealants, in paper industry production of paper, adhesives, food containers (Qiu et al., 2015). Bisphenol A was quickly removed from consumer products due to their harmful effects on environment and human health. Chemicals that are used as alternatives to bispheno. A have been reported toxic and with parallel physiological effects in living organisms (Rochesteri & Bolden, 2015).

Bisphenol S is used as an industrial product for production of thermal paper in which it is used as a cash receipt and also in cleaning agents (Siracusa et al., 2018). Bisphenol S used in chemical as well as food industry for different kind consumer product production its used as in a variety of application such as dental sealants, dental composite filling materials, varnishes, paints, coating flooring, pipe and tank lining (Cabaton et al., 2009). Exposure of Biphenol S through different route like dermal, inhalation, oral causes damaging effects on human health and cause environmental pollution (Yang, Guan, Yin, Shao & Li., 2014).

2. MATERIALS AND METHODS

Animal model Development

This research is based on the qualitative research method. The workplace was Animal House, School of Zoology, Minhaj University Lahore. Thirty mice aged 5-6 weeks with a body weight of 28-30 grams purchased from the University of Veterinary and Animal Sciences.

These mice were placed under well managed and controlled conditions, such as a 12-hour day/night

cycle, temperatures of $27 \pm 2^{\circ}\text{C}$, and humidity of 40-50. These were housed in iron cages 24 inches long and 12 inches wide. The animals received commercially prepared grain pills. (Chick Feed No.14 National Feeds, Lahore). The mice were fed a feed that was high in minerals, proteins, and multivitamins.

Experimental design

The experimentation was performed to contemplate the harmful impact of Bisphenol S and Bisphenol A on spleen of mice. Thirty mice aged 5-6 weeks with a body weight of 28-30 grams used to this experiment. Mice were be divided into three groups 3 groups; one was control group and other two presented as experimental group. Control group was labeled as (C), other experimental group 1 marks as BPS 40/mg/kg body weight +diet and group 2 mark as BPA 40/mg/kg body weight +diet. Dose of Bisphenol S was prepared on 40mg/kg b.w. Bisphenol.S was dissolved in 9ml distilled water in such way that each 0.1 ml contained the desired dose of Bisphenol S (40mg/kg) concentration of required dose. Dose of Bisphenol A was prepared on 40mg/kg b.w. 1ml of Bisphenol A was dissolved in 9ml distilled water in such way that each 0.1 ml contained the desired dose of Bisphenol.A (40mg/kg) concentration of required dose.

Dose Organization

The animal kept under control condition then animal was marked as experimental and control group. The experimental group further divided into two group 1 and group 2. The control group received clean water and first treated group received Bisphenol S o.1ml of 40mg/kg b.w. the second treated group receive Bisphenol A 0.1ml of 40mg/kg b.w. Dose were given orally for the period of 21 days with the help insulin.

Tissue processing and sampling

To evaluate the damage caused by Bisphenol A and S the spleen of mice and sample of spleen taken. After taking of spleen sample, they were put into sterile petri dish and wash it with normal saline solution for few minutes. (Hussein, A. J., 2007) protocol was follow for histopathological study of sample of spleen taken.

Morphological investigation

Sample of spleen taken from treated mice ready for morphological examination. Vernier caliper was used to determine the length and width of spleen of treated group of mice and control group of mice. A computerized weight measuring machine was used to determine the weight difference between the spleen of the treated group and control group. The difference between spleen weights were noted and found statistically significant difference (Morphometric anomalies were seen among control and experimental groups).

Histopathological Examination

For histopathology of mice spleen sample fixed. The spleen flap removed and washed with normal saline solution 0.75% NaCl. Then the flap of spleen tissue sample fixed in 10% formalin solution for the period of 24 hours. Then samples of spleen tissue were dehydrated by passing through different concentrations of ethanol and then cleared with liquid xylene. After dehydration spleen tissue was impregnated with the liquid paraffin then allowed tissue to solidify. After paraffin impregnation the wax block containing spleen tissue was trimmed and the section of spleen was made with the help of microtome. Then the temperature of the water bath maintained to 55 to 56% for stretching of ribbons. These section ribbons were placed on sterile glass slide with using of albumin 2 to 3 drops on slide. The slides firstly air dried then placed in incubator for 15 minutes at 50-55 °C. After removal of paraffin wax, slides immersed with the help of xylene 1 and 2 for the period of 10 minutes. Slides that contain spleen tissue were transferred into descending alcohol having concentration 100%, 90%, 70%, 50% for the period of 10 minutes and then with 5 minutes respectively. Slides immersed in hematoxylin for approximately for the period of 5 minutes. Slides washed with running tap water. Slide dipped into alcoholic solution (5%) for the period of 30 seconds. Slides were stained with eosin for one minute. Slides again washed with running tap water. After staining slides was again dehydrated. These prepared slides were observed under light microscope with different magnification 10x,40x,100x.

3. RESULTS AND DISCUSSION

Morphological analysis

The control group of mice showed that there was a regular appearance of spleen. The spleen and other organs look smooth and shiny. The red pulp of spleen and the White pulp of spleen standard no kind irregularities found spleen of control group of mice average in size and shape. Several kinds of abnormalities or deformities were found. e.g the spleen looks dull in color and also its shine of treated with Bisphenol A (BPA 40mg/kg). The spleen of treated with Bisphenol A (BPA 40mg/kg) shown malformation like colors and wrinkles on its surface. The spleen also showed that much dark stain on its surface and also dark stain representing the degradation of marginal zones that present between the white pulp and red pulp of spleen of treated group of mice with Bisphenol A (BPA 40mg/kg). On the contrary the spleen of the Dose group III Bisphenol S (BPS 40mg/kg) shown that was comparatively less damage than the spleen of Dose group II Bisphenol A (BPA 40mg/kg) and more damages than the spleen of control group

Morphometric analysis

The initial body weight of control group was noted that was $16.1 \pm 4.6g$, the final weight of the control group was noted after the administration of oral dose. It's found that the average body weight of mice found that $17.4 \pm 4.8g$. The calculated increase in the percentage of body weight was 51.5. The initial body weight of mice is noted to determine the percentage body weight gain mice after treatment of Bisphenol A (BPA 40mg/kg). The initial body weight of Bisphenol A (BPA 40mg/kg) treated group of mice was $16.1 \pm 4.6g$, the average final weight of mice noted that was $17.4 \pm 4.8g$. After calculating the percentage of body weight increased was 28.73g. The percentage of values shown that a significant loss in the percentage of weight increase as compared to control group. The initial body weight of Bisphenol S (BPS 40mg/kg) dose III treated group of mice was $18.1 \pm 5.3g$, the average final weight of treated mice group was $26.4 \pm 4.2g$, its shown that after calculation the percentage of body weight the gain in the body weight was 45.8 its determine that a significant higher than the percentage body weight of mice of Bisphenol A (BPA 40mg/kg) 28.73g.

Histological Analysis of spleen

Histological investigation shows that there was clear alteration of splenic tissue of treated groups of mice as compared to control group. splenic tissue of BPA and BPS treated group of mice showed that highly activated germinal centers of spleen white pulp with minimal apoptotic feature and prominent megakaryocytosis and lymphohistiocytic infiltrate of the red pulp, and comparatively increased the count of eosinophils and mature lymphocytes were noted detected. On other hand there was abnormalities also occurred in spleen due to occurrence and accumulation of iron in the spleen that increase in red pulp and microphage count increase in white pulp. There were apoptotic changes that occurred in the germinal center of white pulp of the spleen both BPA and BPS treated group of mice. Histological study also shown that the wall of central arterioles was ruptured and shown that increasingly narrow lumens.

4. CONCLUSION

Bisphenol A and S is used in many edible plastic products that used daily in life like baby bottles, baby feeders, plastic bottles, hard and soft plastic, water bottles, storage containers, food containers and paper industry, protective coating for pipeline. Overuse of these products such as bisphenol A and S has many healthcare issues to humans as well as affecting children's health. In the present study we try to find toxicological evaluation the harmful effects of bisphenol A and S spleen of the mice.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1. A Comparison of average weight of mice of dose II groups Bisphenol A (BPA 40mg/kg) and control group of mice

Parameter	Control group (mean ± SEM)	BPA II (BPA 40mg/kg) (mean ± SEM)
Initial Body weight (g)	16.1 ± 4.6	17.4 ± 4.8
Final body weight (g)	24.4 ± 4.8	22.4 ± 6.2***
Spleen weight	0.37 ± 0.43	0.13 ± 0.43***

Note: The results of table shown as ***= p<0.001

Table 2. A Comparison of average weight of mice of dose groups Bisphenol A (BPA 40mg/kg) and Bisphenol S (BPS 40mg/kg).

Parameter	BPA II (BPA 40mg/kg) (mean ± SEM)	BPA III (BPA 40mg/kg) (mean ± SEM)
Initial Body weight (g)	17.4 ± 4.8	18.1 ± 5.3
Final body weight (g)	22.4 ± 6.2***	26.4 ± 4.2***
Spleen weight (g)	0.13 ± 0.43	0.31 ± 0.43***

Note: Results were represented as ***p<0.001.

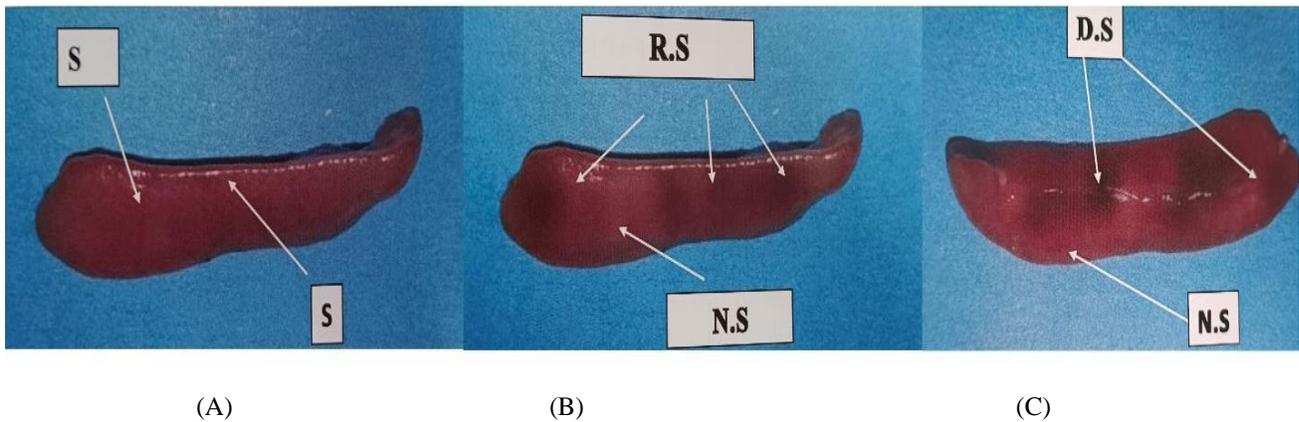


Figure 1. Spleen of control group shown (A) Bisphenol A treated group mice spleen (C) Bisphenol S treated group mice spleen

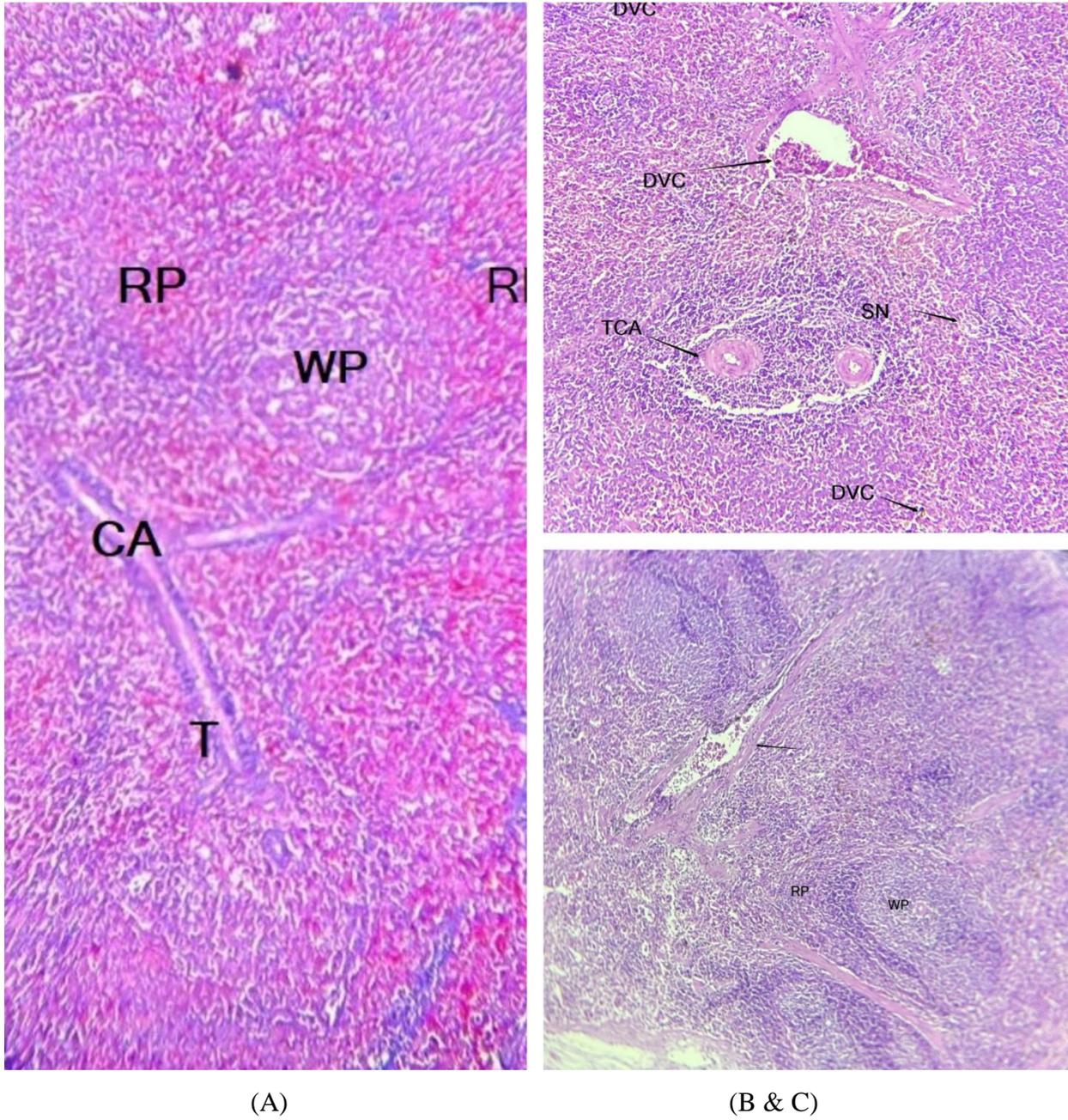


Figure 2. H & E-Stained spleen section of control, Bisphenol A and Bisphenol S treated group for period of 21 days. Photomicrographs were taken at 10x,40x 100x using *optika- B-350 microscope*.

THE MORPHOMETRICS, GROWTH AND LENGTH-WEIGHT RELATIONSHIP OF HILSA SHAD (*TENUALOSA ILISHA*) IN THE DOWN STREAM KOTRI BARRAGE INDUS RIVER, PAKISTAN

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ABSTRACT

Morphological characteristics and Length-Weight relationship of Hilsa Shad (*Tenualosa ilisha*) from the Down Stream Kotri Barrage Indus River, Pakistan are examined. A total of 25 specimens (*T. ilisha*) were collected from August 2019 to September 2020 and found multiple dots in their bodies. These dots were varying to some extent; few specimens have no dots while others had 12 dots. The dots consist of black color that appeared very first time in Pakistan and has no history in the past. The merits are similar but morphometric characters, body length and weight were found to be different. The maximum and minimum weight and length were noted as $254 \pm 0.7g$ and $31 \pm 0.6g$, $21.1 \pm 0.9cm$ and $15.5 \pm 0.8cm$, respectively. Only three specimens were found without dots present in their body followed by four dots and nine dots, however, only two specimens categorized as two dots and twelve dots, five specimens with eight dots and seven specimens with single dot. The growth of fish was found higher in one dot specimens followed by four, eight and twelve dot specimens. Results also revealed that almost all specimens exhibited an isometric growth and the Length-weight relationship was significant with $r^2 \geq 0.910$ while no statistical ($P > 0.05$) difference was found between female (A) and male fish (B). The Hilsa (*T. ilisha*) is a migratory fish from sea to river for breeding and spawning purpose but, unfortunately illegal fishing, local demand, environmental change and habitat destruction cause Hilsa fish to decline continuously in the Indus river of Pakistan. Therefore, it is recommended that stock assessment with genomic studies of Hilsa Shad will be taken to distinguish the actual number of species present in the Indus River of Pakistan.

1. INTRODUCTION

The tropical Hilsa shad (*Tenualosa ilisha*; Hamilton-Buchanan, 1822) belonging to the family Clupeidae is an anadromous, planktivore and euryhaline species and lives in pelagic and neritic waters (Riede 2004; Hossain et al. 2019, 2021). They are commonly found in many countries of Asia such as Bangladesh, Nepal, Sri Lanka, India, Pakistan, China, UAE, Myanmar, Iraq, Iran, Malaysia, Oman, Kuwait, Qatar, Saudi Arabia, Thailand and Vietnam (Freyhof, 2014; Hossain et al. 2021). This species is characterized as least concern in Asia countries mentioned in IUCN red list (Freyhof 2014) because they are the most commercially targeted species in freshwater bodies.

The starting 1900s, Hilsa has been of great socio-economic interest, and was generally harvested in the river Indus, Shatt Al-Arab (southeastern Iraq), Meghna, Irrawaddy, Ganges, Brahmaputra, Mahananda, Godavari, Krishna, Cauvery, and Euphrate river (Jafri, 1988; Hamilton-Buchanan, 1822; BoBP, 1985, Date, 1878, BoBLME, 2012). In Pakistan, Hilsa is a well-known traditional native species of Sindh region. In 1984 the annual catch of hilsa (*T. ilisha*) was recorded high 5469 tons but it is gradually decrease from the last two decades about 146 tons recorded in 2004 (Department of Fisheries, Karachi). The biggest threat to the fishery is the low run of fresh water during migration to the Indus River (Jafri, et al., 1988). Adult shads reach 57 to 61 cm (Al-Baz & Grove 1995; Amin et al. 2004) and fully mature at 19-30 cm within 7 to 12 months reported in

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Bangladesh, India and Kuwait (Jhingran & Natarajan 1969; Ramakrishnaiah, 1972; Halder, 2002; Mome & Arnason, 2008). Hilsa shad usually migrate to rivers from marine habitats for spawning and breeding purpose. They spend all their life in the sea except for breeding (Tint et al., 2019; Arai & Amalina, 2014; Blaber, 2000 AL-Baz & Grove, 1995; Pillay and Rosa, 1963). The period of hilsa migration for spawning is recorded differently in many countries such as February to March in Southeastern Iraq, May to June in Kuwait, October to November in Bangladesh, and February to July in Indus River (Hossain 1985, Al-Baz & Grove, 1995, Narejo et al. 2008, Bhaumik et al. 2011; Al-Dubakel 2011; Hossain et al., 2021). Growth and morphological studies are very helpful to classify the particular type of species and bring attention to its meristic trait in the field of fisheries sciences (Bagenal & Tesch 1978; Hossain 2010, Hossen et al. 2018; Santic et al. 2018; Rahman et al. 2019, Islam et al. 2020; Hossain et al., 2021). In addition to its economic significance, there is incomplete information on stock testing of palla fish in Indus River, while significant research work has recently been available on the migration pattern (Jafri, 1988, Bhuiyan and Talbot, 1968), annual development of small gonads, natural reproduction, height, weight, age, feeding and growth (Panhwar et al., 2011). However, no studies have been covered the meristic features and length-weight relationships of this species found in the Indus River of Pakistan. Therefore, purpose of this study was to determine the morphological characteristics with length and weight measurements of Hilsa shad in the Down Stream Kotri Barrage Indus River, Pakistan.

2. MATERIALS AND METHODS

The Indus River in Sindh range with the major landing areas were selected for collection of fish samples (Figure 1).



Figure 1 Study site is the Kotri Barrage on the Indus River, Sindh, Pakistan.

Sampled were collected from Ghulam Muhammad Barrage /Kotri Barrage and Ketti Bunder of river Indus from August 2019 to September 2020 one-year period.

Sampled were preserved in ice box and formalin and taken in laboratory for further identification. A total of six morphometric, determine (normal length, head length, fork length, total length, girth and eye width) and seven combined letters (precy pelvic scutes, anal fin radiation, rays of the dorsal fin, pectoral fin radiation, total number of scutes, pelvic fin radiation and post pelvic scircs) were selected for assessment. Although, TL (Total Length), FL (Fork Length), SL (Standard Length), HL (Head Length); SNL (Snout Length), ED (Eye diameter), DFL (Dorsal Fin Length), PFL (Pectoral Fin Length), VFL (Ventral Fin Length), AFL (Anal Fin Length), UCFL (Upper part of Caudal Fin Length), DCFL (Down part of Caudal Peduncle Length) and GR (Girth) were also measured showing figure 2.

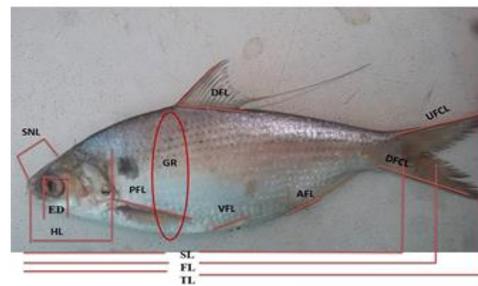


Figure 2 Shows thirteen morphometric measurements of Hilsa (*T. ilisha*) found in Indus River, Sindh-Pakistan.

All compliance calculations for morphometric measurements were performed by way of directed (Pillay et al., 1957). All grains were measured in millimeters nearby and measured in electric balance up to the nearest gram. The weight-bearing relationship with the condition of the related factor Kn is determined by the least square approaches as provided by Le Cren in 1951. The formula of length-weight relationship is $W = a L^b$. Generally, morphological characters that appear on the outside of the body of fish are used to identify the particular type of species (Figure 2). Countless numbers of fish are collectively referred to as morphometric characters and morphometric. These characters are superficial and very versatile and should be used with caution. Character letters have been widely used in fisheries biology to measure understanding and the relationship between the various tax categories. (Avsar, 1994, Corti et al., 1988; Villaluz et al., 1988).

3. RESULTS

The morphometric, length-weight relationship and meristic characteristic of Hilsa (*T.ilisha*) found in different areas of Indus River and specimen collected from August 2019 to September 2020. The body shape

of Hilsa Shad is fusiform and dorsoventrally compressed and the body colour is silver to light purple. The mouth is terminal in position and upper jaw with a distinct median notch is present (Figure 2). A total of 25 specimens of Hilsa (*T.ilisha*) were collected and found multiple dots in their bodies. These dots were varying to some extent; few of specimens have no dots while others had 12 dots in their bodies (Figure 3). The dots consist of black color that appeared very first time in Pakistan and has no history in past. The merits are similar but morphometric character, body length and weight found different. The maximum weight was 254 g and maximum length was 21 cm, minimum weight was 31 g and length were 15.5 cm respectively. Only three specimens were found whereas no dots were spotted in the body; the seven specimens were found which have single dot present. Two dots' specimens were found; three specimens presented four dots in sample. The five specimens were viewed eight dots whereas three specimens illustrated nine dots in body. The two specimens and twelve dots are present in the body. The high rate of weight was found in dots one, eight, four and twelve respectively.

During the year-round sampling, it was noticed that two separate populations of Hilsa, *T. ilisha* exist. To identify two different morphometric characters, one is spotted *T.ilisha* and the second without spotted *T .ilisha* we found. Table 2 described equation $\log (W) = \log a + b \log (L)$ (a: intercept and b: slope of the equation). N (sample size), Length (L) in cm- weight (W) in g. Minimum: (Min) and Maximum (Max) of L & W; SE is Standard error; CI (b): confidence intervals of b; r2: coefficient of determination, P is significance of regression with P significant at ≤ 0.05 relative condition factors (Kn) of the selected fish species with its range (Min-Max) and SE. t-test significance was conducted to verify if b is significantly different from the consensus $b = 3$. The growth behavior was deduced based on b. No statistical ($P > 0.05$) difference was found between female and male fish, mentioned in Figure 5 & 6.

4. DISCUSSION

Hilsa (*Tenualosa ilisha*) was found in different areas of Indus River and specimen collected from August 2019 to September 2020. A total of 25 specimens of Hilsa (*T.ilisha*) were collected and found multiple dots in their bodies. These dots were varying to some extent; few of specimens have no dots while others had 12 dots in their bodies. The dots consist of black color that appeared very first time in Pakistan and has no history in past. The merits are similar but

morphometric character, body length and weight found different. The maximum weight was 254g, maximum length was 21 cm, minimum weight was 31g and length was 15.5 cm respectively.

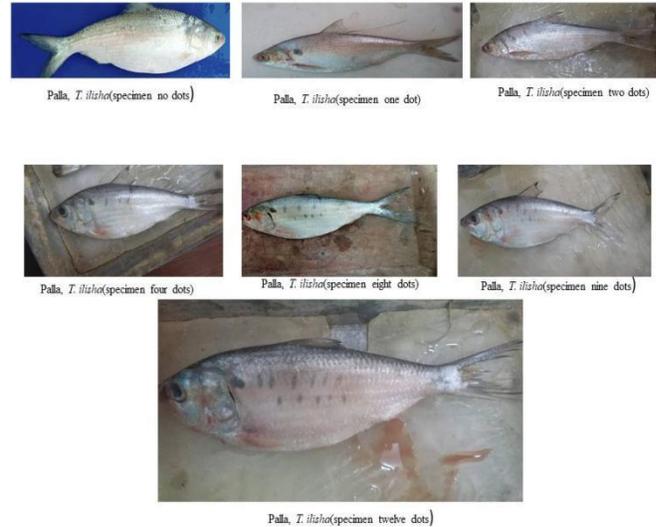


Figure 3. Photographs of Hilsa (*T. ilisha*) collected from Indus River, Sindh-Pakistan.

Only three specimens were found whereas no dots were spotted in the body; the seven specimens were found which have single dot present. Two dots' specimens were found; three specimens presented four dots in sample. The five specimens were viewed eight dots whereas three specimens illustrated nine dots in body. The two specimens and twelve dots are present in the body. The high rate of weight was found in dots one, eight, four and twelve respectively. On the basis of dots the further investigation in genome or chemical/ environmental effect on species (*T.ilisha*) or new species that found in genus *Tenualosa*. The previous research was a comparative study of two types of Hilsa (*T.ilisha*) in Kotri Barrage and Ketti Bunderat Indus River. Belong to type A and B intertype differences in six morphometric measurements (total length, standard length, fork length, head length, eye diameter and girth) and seven meristic characters (total number of scutes, pre pelvic scutes, post pelvic scutes, dorsal fin rays, pectoral fin rays, pelvic fin rays and anal fin rays). Length-weight relationship, condition factor values and GSI values were also different in summer and winter types of *T. ilisha* (N.T. Narejo, et al., 2008). The present research is initially in progress to further investigate different kinds of palla (*T.ilisha*) different kinds as well as other species in Indus River. Ongoing new species of genus *Tenualosa* is discovery or environment factor affecting Hilsa (*T.ilisha*) body as well as its growth.

Similarly, this research also illustrates low growth rate as compared to previous data was (57 cm) of Rahman et al., (1999) and Amin et al., (2002) in Bangladesh and Al-Baz & Grove (1995) in Kuwait (61 cm) of Amin et al., (2004), though Fish Base (Froese and Pauly, 2020) showed a maximum length of 60 cm. All other studies (Flura et al., 2015; Sarkar et al., 2017; Roomiani and Jamili, 2011; Bhakta et al., 2019; Mohanty and Nayak, 2017; Bhaumik et al., 2011) found the body length were greater than the current study. McConnell, et al., 1978 details on morphometric observation of fish and the learning of mathematical relationships between them are important for text work. In addition, to see the source of the stock, the classification of stocks or the identification of important commercial fish species, morphometric characters are regularly used (Godsil, et al., 1948; Schaffer, et al., 1948; Pillay, et al., 1957; Royce, et al., 1963; Kramholz and Cavanah, 1968). During the year sampling of the current research, it is known that the different people of the Hilsa, *T.ilisha* new were present in one.

On the basis of our findings, we suggest that it is necessary to take serious consideration to provide proper hilsa access to the Indus River during the breeding season, and to impose restrictions on fishing during the migration of the river and restrictions on sub-fishing for this traditional fishery. Due to the excessive exploitation and indiscriminate killing of Hilsa from inland waters especially from breeding areas (river harbor), the fishing area was severely depleted from previous years. The number of items responsible for this decrease includes:

- An obstacle to the migration of natural habitats due to the topography of the river
- Excessive fishing pressure at different stages of life
- River water pollution alters the natural boundaries of rivers
- Destruction of a dwelling.
- As a result, in order to preserve the Hilsa fishery it was necessary to develop a Hilsa Fishery Management Action Plan (HFMAP).

5. CONCLUSION

The Hilsa (*T.ilisha*) is a migratory fish from sea to river and its very tasty important fish in fisheries but due to environmental, over fishing and coastal or breeding ground destruction the Hilsa (*T.ilisha*) is decline our Indus River. This research supporting to identification differences in morphometric character and length-weight relationship all effect on species

environmental as well as overfishing and also observing number of dots present in bodies and it varies. Further to investigate genomic studies to distinguish that Hilsa (*T.ilisha*) has two or more species or single species present in our Indus river.

6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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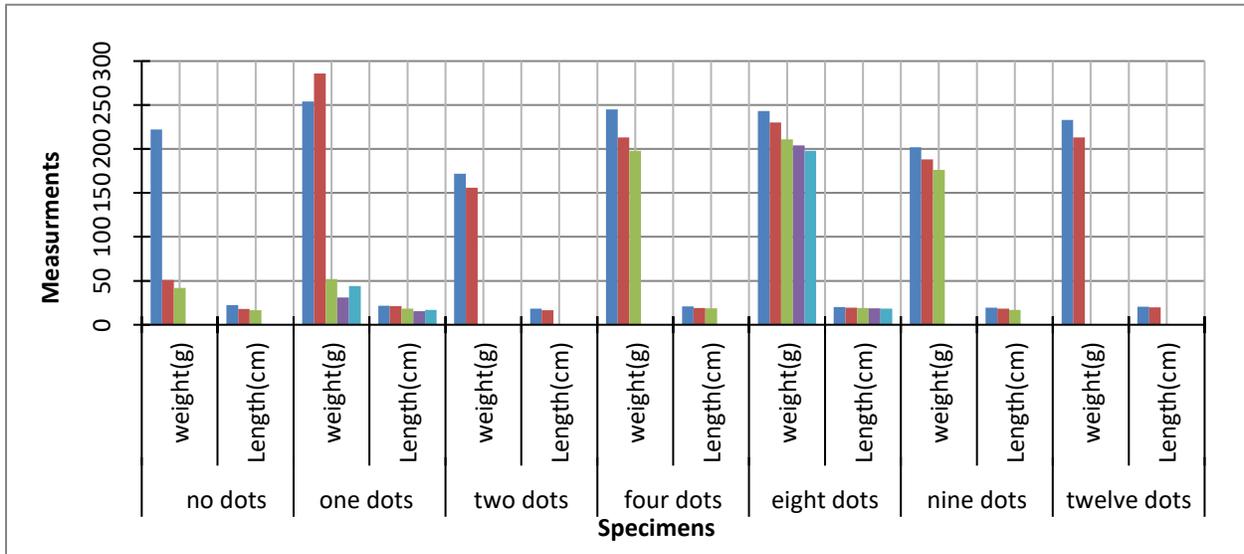


Figure 4. Graph showing the Length weight measurement of specimens *T. ilisha* collected from Indus River, Sindh, Pakistan

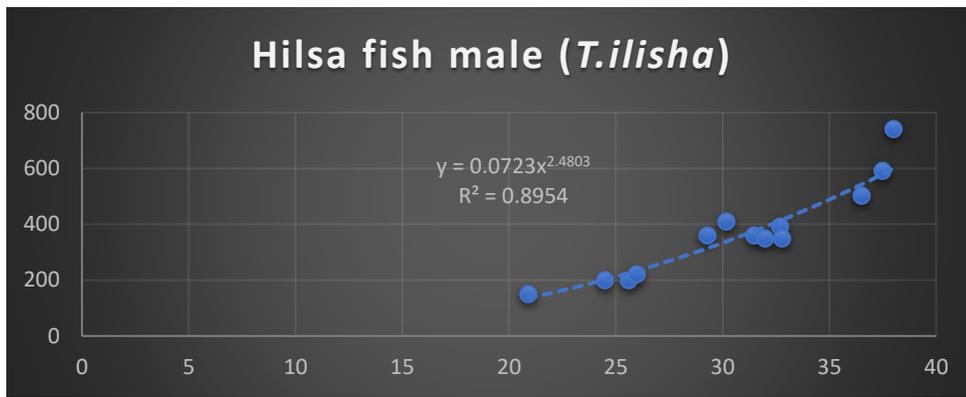


Figure 5. Regression analysis of Hilsa fish female.

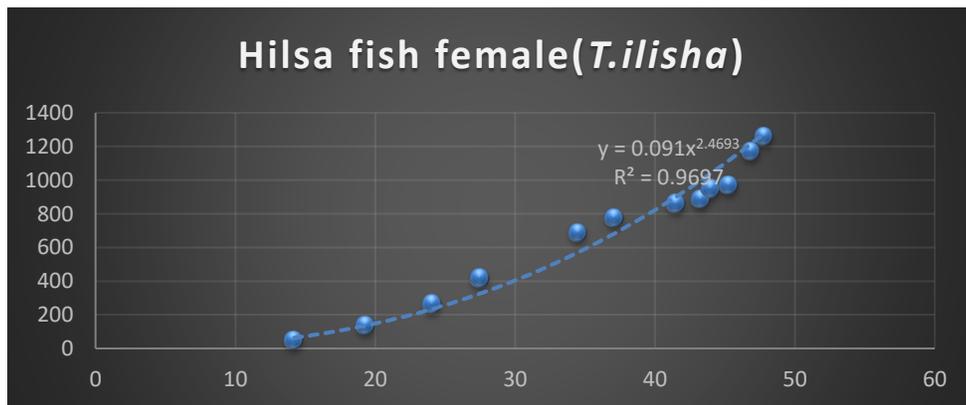


Figure 6. Regression analysis of Hilsa fish female.

Table 1. Morphometric analysis of Hilsa fish (*T. ilisha*).

S.N	WT(g)	TL(cm)	FL(cm)	SL(cm)	HL(cm)	HH(cm)	SnL(cm)	ED(cm)
One dot specimens								
1	150	20.9	18.4	17.5	4.9	3.5	0.9	1.1
2	200	24.5	18.2	16.9	4.2	4.5	0.9	1.2
3	200	25.6	16	15.1	4.3	4.2	0.9	0.9
4	220	26	13.2	12.6	3.7	3.1	0.7	0.8
5	360	29.3	14.5	13.9	4.2	3.8	0.8	0.8
6	410	30.2	13.3	12.9	3.9	3.7	0.7	0.9
7	360	31.5	14.3	13.6	4	3.8	0.9	0.8
Mean	271.4285714	26.85714286	15.41428571	14.64285714	4.171428571	3.8	0.828571429	0.928571429
Two dot specimens								
8	350	32	15.2	14.8	4.5	3.6	1	1.1
9	390	32.7	14.3	13.2	3.9	3.1	0.8	0.8
Mean	370	32.35	14.75	14	4.2	3.35	0.9	0.95
Four dot specimens								
10	500	36.5	17.3	16.3	5	5.3	1.2	1.1
11	590	37.5	16.8	15.1	4.5	4.8	1	1
12	50	36.5	16.4	14.9	4.1	4.2	1	1
Mean	380	36.83333333	16.833333	15.433333	4.5333333	4.766667	1.066667	1.033333
Eight dot specimens								
13	243	20.2	16.9	14.9	5.2	5.3	1	1.1
14	230	19.4	16.6	14.7	5	5.1	1	1.1
15	139.2	19.3	16.4	14.4	4.8	4.9	1	1
16	265	24.1	16.1	14.2	4.4	4.6	1	0.9
17	416.3	27.5	15.8	14	4.1	4.5	1	0.8
Mean	258.7	22.1	16.36	14.44	4.7	4.88	1	0.98
Nine dot specimens								
18	686	34.5	16.5	15.7	5.1	5.1	1.2	1
19	775.5	37.1	16.2	15.3	5	5	1	1
20	860	41.5	15.9	15.1	4.9	4.7	0.9	0.8
Mean	773.8333333	37.7	16.2	15.36667	5	4.933333	1.033333	0.933333
Twelve dot specimens								
21	887	43.3	17.6	16.7	5	4.9	1.4	1.1
22	950	44	17.1	16.3	4.9	4.8	1.2	1
Mean	918.5	43.65	17.35	16.5	4.95	4.85	1.3	1.05
No dot specimens								
23	970	45.3	16.8	15.6	5.1	4.8	1.1	1
24	1170.2	46.9	15.6	15	4.1	4	0.8	1
25	1260	47.8	13.9	13.4	4	3.7	0.6	0.8
Mean	1133.4	46.66666667	15.433333	14.66667	4.4	4.166667	0.833333	0.933333

*WT represents weight gain; TL (total length); FL (fork length); SL (standard length); HL (Head Length); HH (head height); SnL (Snout Length); ED (Eye diameter).

Morphometrics and Growth of Hilsa Shad at Kotri Barrage

Table 2. Length-Weight relationship of Hilsa shad (*T. ilisha*).

Sex (Male &female)	Male	Female
N	13	12
L min-max (cm)	20.9-38	14.2-47.8
W min-max (g)	740-150	1260-50
a	0.07230942	0.109462
b	2.480301413	2.46932
SE (b)	0.391599728	0.391599728
CI (b)	0.26216903	0.26216903
r ²	0.891903205	0.910437854
p	0.005326472	0.005326472
t-test sig	-3.543401943	-3.543401943
	Negative	Negative
K (fulton's condition factor)	1.245334342	1.459104178



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