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Conclusion should elucidate how the results communicate to the theory presented as the basis of the study and provide a concise explanation of the allegation of the findings.

ACKNOWLEDGEMENT

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Connecting Science and Public Trust

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Vaccine Development in Pakistan: Connecting Science and Public Trust

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Creating vaccines in Pakistan shows human ingenuity but also the challenge of building public trust. The biotech field is growing, but limited funding and poor communication is slowing the progress of vaccines in Pakistan. The COVID-19 pandemic showed how crucial vaccines are, but it also exposed gaps in explaining science to people. For Pakistan to lead in biotech, it must link vaccine innovation with public confidence through better dialogue.

The polio crisis in Pakistan explains this issue. Pakistan is one of the countries where, polio still exists despite many efforts. Lack of confidence is the problem, not lack of scientific expertise. It is considered that Vaccines cause infertility, which is a false notion. Authorities' health efforts are distrusted by rural populations, which are frequently disregarded. It will be success of science once these social and cultural problems are addressed.

The media shapes perceptions and trust of common people. The benefits of vaccines were contradicted by headlines during COVID-19. However, media can also restore trust. Scientists in Punjab who collaborated with local leaders reported a 20% increase in vaccine enrollment in 2021.

Not only just facts are built by understanding but also trust. Scientific topics should not be the main focus of the media as they are topic of scientists. It can be beneficial to have public conversations with scientists and religious leaders, see production of vaccines process, and encourage participation in science education initiatives in schools. Common platforms and large media sources must directly address public concerns and exchange knowledgeable viewpoints.

Following main actions are needed for Pakistan to become a vaccination leader:

1. Educate scientists on how to clearly communicate their work.

- Health-related subjects should be accurately covered without any exaggeration.
- Universities should train students to create effective communication and understanding between labs and societies.
- Provide immunizations to prevent local diseases, i.e hepatitis and dengue.
- Make sure trials are transparent by involving local people. This increases confidence and guarantees that solutions address real demands.
- There must be a team that includes social media, scientists, and religious leaders to solve false images build by people against Science.

In order to save lives and establish itself as a global health leader, Pakistan must combine research with attempts to gain trust. Collaboration among specialists, the media, and communities is essential for using scientific advancement for the common good.

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Review Article

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Overview of Marburg Viral Disease

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ABSTRACT

Marburg viral disease induced by the Marburg virus. It is a constituent of the Filovirus family, which also encloses the Ebola virus. The disease first came into light in 1967 during episodes of the virus in Marburg and Germany. After that, the infection caused high CFR in Angola, Uganda, and Congo. The disease includes the sudden outset of symptoms, including high fever, vomiting, intense headache, abdominal pain, and rash. In patients with severe infection, it leads to bleeding, jaundice, organ collapse, and eventually death. Fruit bats of genus Rousettus are considered to be the biological pool of the Marburg virus. This disease destroyed African countries with high death rates. The virus can be transferred from green African monkeys or fruit bats. It can be transmitted through human-to-human interactions via blood, body fluids of diseased people, and contaminated surfaces. MVD can be diagnosed by technical laboratory tests incorporating enzyme-linked immunosorbent assay (ELISA), reverse transcription-polymerase chain reaction (RT-PCR), and virus seclusion. Supportive maintenance has been done to demonstrate some efficacy in controlling the symptoms and improving the probability of survival. Adequate management and care have been taken to prevent the unusual spread of disease, such as the separateness of infected patients and suspected individuals, which should pertain to quarantine measures. Because of the high casualty rate and lack of a certified vaccine or any antiviral cure, the only way to keep MVD in check can be through prevention. This review briefly overviews the Marburg virus, including diagnosis, pathogenesis, transmission, and treatment.

INTRODUCTION

The Marburg virus causes Marburg viral disease. It is a member of the filoviridae family. It is a rod-like shape, as shown in Figure 1. This disease was first reported in 1967 when a hemorrhagic fever outbreak occurred in Germany. At that time, rephrase: thirty-one patients were prevalent, out of which seven patients developed severe disease with a fatal outcome. It has caused several outbreaks in sub-Saharan Africa and can cause illness with a high (88%) fatality rate [1]. The outbreak was reported initially in African countries like Kenya, Uganda, Angola, and South Africa. The primary sources of infection were African green monkeys (Chlorocebus aethiops) and various species of bats, which may include *Rousettus aegyptiacus* and *Hipposideros caffer*.The monkeys were imported from Uganda by scientists conducting experiments to produce polio vaccines [2]. The latest update of Marburg viral disease was reported from Ghana in 2022. This disease is highly associated with the destruction of the body organs, such as the liver, brain, and tissues of the renal system. The virus detected in the oral route and the fecal samples of bats proved that the Marburg virus can be transmitted from the infected bats to the in-contact bats. MARV is highly infectious; it can spread rapidly and cause death [3]. The infection can be transferred to humans and non-human primates (intermediate hosts) from the discharge of bats. The recent explosion of the Marburg virus was reported in

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Equatorial Guinea in January 2023, and the laboratory confirmed the victim of the Marburg virus in West Guinea on February 13, 2023. A total of 17 cases were reported, out of which 12 passed away (these cases were confirmed on 8 June 2023). Remove these lines or rephrase from an Indian point of view, the Marburg virus disease is a cue to prepare ourselves to tackle various infectious diseases because they have encountered the recent episode of the Nipah virus in 2018, which took several vitalities in the southern state of Kerala [4]. A virus becomes resistant as it can transform into different forms and has distinguishable effects in various areas. The highest CFR was recorded in Angola during the viral attack in 2005 because the Marburg viral Angola strain emerged as more pathogenic and invulnerable than other MARV strains when experimentation was done on non-human primates [5, 6]. Marburg virus is similar to the ebolaviruses in structure. It is an enveloped, non-segmented, single-stranded, and negative sense RNA virus that belongs to the *filovirus* family. One difference between the Marburg virus and the Ebola virus is that the Marburg virus is not immunosuppressive, grammatically wrong but the Ebola virus is. The Marburg virus has a single species, "Marburg Marburgvirus," which includes two viruses: Marburg (MARV) and Ravn virus (RAVV). There are two known variants of the Marburg virus: Marburg Musoke and Marburg Angola. The latter one is the most pathogenic and causes rapid illness in non-human primates. The glycoproteins on the cell surface are an essential feature of this virus and are the fundamental target for examining viral vaccines. The disease results in hemorrhagic fever along with the disability of organs like infection of the brain and spleen. The involvement of the central nervous system may result in confusion, excitability, and aggression in behavior [7]. It is a zoonotic disease transmissible to humans by bats, monkeys, and other intermediate hosts. Marburg virus can be transferred by direct contact with infected patients' blood and body fluids. The incubation period of the Marburg virus is two days to 3 weeks. The Ebola virus is also a member of the *et* family and resembles the Marburg virus's clinical features and transmission route. Presumably, the Marburg virus was thought to be less dangerous than the Ebola virus, which has caused two large explosions with high fatality rates. The first deadly outbreak happened in 1998-2000, which caused 128 deaths in the Democratic Republic of the Congo (DRC), and the case fatality rate was 83%. It was followed by the largest lethal population outbreak in Angola from 2004-2005, resulting in 329 deaths with a fatality rate of 88% [8, 9]. Different variants were associated with the spell of the Marburg virus infection in the DRC, but only one version caused disease in Angola [8, 10]. The investigation was done on miners who developed an infection after a visit to the Kitum Cave; the MARV was found in Egyptian fruit bats. Viral RNA was noticed in the liver, spleen, and lung tissue of a healthy female R. aegyptiacus (fruit bat) in July 2007. Phylogenetic variations of the viruses displayed that they were very different from the strains obtained from Kenya (Musoke and Ravn) but analogous to the cases unraveled in Europe in 1967. The strains of the Marburg virus isolated from bats and humans in 2007 belong to obscure lineages [11]. When histopathological inspection of the liver of the infected bats was done in the laboratory, there were no lesions that could cause the Marburg virus infection. On performing the immunohistochemical study, there were no traces of the Marburg virus. Diagnostics tests for evaluating the Marburg virus disease are reverse transcriptase polymerase chain reactions (RT-PCR) and enzyme-linked immunosorbent assay. Specific probes can be made for the sequencing of the MARV genome [12]. Indeed, the Marburg virus was unveiled about 50 years ago, but technicians have performed clinical trials to analyze it properly. Since there is no recent large-scale outbreak of MVD, less is known about it. The MARV outbreaks are unusual, which is why there is not enough information to evaluate and generate treatment options for MVD. Detailed disease studies and investigations may help in future medical trials and improve curative management of Marburg virus disease. Today, no authentic treatment is available for MARV infection. Therefore, supportive care can be done as a primary treatment for the patients of Marburg viral disease. Suspected patients of MVD can be managed by running their blood and urine tests. After confirmation of infection, patients must be quarantined. Proper precautionary measures should be taken to prevent infection (clean clothes and utensils). In poultry, several treatments have become successful with prolonged or increased survival rates on broiler chickens. The tested treatments may include cytokine inhibition and antibody transfer, but these treatments proved useless in the non-human primate (NHP) model. Drugs such as hydroxyzine and pentazocine are beneficial in monitoring. The treatment or therapy against the viral infection depends upon the host's immune response and the actions taken to control virus replication. Antibodies can be used for treatment because they target proteins of filovirus. An anti-EBOV convalescent serum with coordination of interferon and supportive care can be used for treating infection. However, the role of antibodies in serum for the patient's survival is anonymous [13]. Antibody-based treatment's success was first informed in 2012 when researchers used polyclonal immunoglobulin G. It was refined from the convalescent serum of NHPs,

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immunized with exploratory MARV vaccines that endured subsequent filovirus infection [14].The term "viremia" refers to the presence of viruses in blood, and post-exposure treatments are evolved against filoviruses, among which monoclonal antibody-based strategies have appeared. It displays an elevated level of protection as these monoclonal antibodies target the virus's glycoproteins. The first outbreak of the Marburg viral disease was in two areas of West Germany; "Marburg a der Lahn" and "Frankfurt am Main" [2]. The virus was destroyed for the first time in August 1967 and ceased in November 1967. At first, 29 people were diagnosed with the infection, out of which seven patients grammatically wrong surrendered to the deadly virus. As we all know, the Marburg viral disease is caused by the Marburg virus, which belongs to the Filovirus family. Before developing the disorder, all suspects may have a direct connection with the sources of infection, which may be green African monkeys or various species of bats. The eruption of MVD took place among the laboratory manufacturers attempting to produce vaccines for poliomyelitis. Some past studies highlighted that infection first occurred when researchers sought to develop green monkey cell cultures using their tissues. The workers became infected with the Marburg virus while dealing with the tissues of infected green monkeys imported from Uganda. In addition to the two areas of West Germany, the Marburg virus also caused devastation in Belgrade and Yugoslavia (Serbia), and the carriers were the same Ugandan primates. The Marburg virus rendered devastation worldwide. The morbidity and mortality rates depended upon the intensity of the spell and virus strain, but the standard case fatality rate was about 50%. According to the World Health Organization (WHO) analyses, the Marburg virus causes a highly virulent disease and can develop hemorrhagic fever in humans and animals. In the first outbreak of 1967, laboratory staffers were exposed to the Marburg virus through the meat and organs of sick green African monkeys *(Chlorocebusaethiops).* A young man traveled from Zimbabwe to Johannesburg and contracted the virus in February 1975 and developed a primary infection. Kenya experienced an outbreak of the Marburg virus disease in 1980 when a doctor developed an infection in Nairobi, resulting in brutal haematemesis. In 1987, a 15-year-old boy fell prey to the infection and passed away. He reportedly confronted the Ravn virus (responsible for MVD) in a cave colonized by fruit bats. After that, the Marburg virus caused devastation from 1998-2000 in the Democratic Republic of Congo, 2004 to 2005 in Angola, 2012-2017 in Uganda, and recently in August 2021 in Guinea [15]. Marburg is a dangerous zoonotic virus that can spread disease in humans and non-human primates. Clinical indications are sudden fever, diarrhea, nausea, chills, vomiting, and headache. The disease is divided into three primary grades: You have stated different information above in introduction the initial phase may continue for four days; next is the organ phase, which longs for 5-13 days; and at the end, the recovery phase, which continues for 13 days [16]. This virus can deteriorate the organs and occasionally cause jaundice. It may lead to a rash of the pancreas and strenuous weight loss. A person with MVD can experience pain in the limbs, and the temperature of the body falls slowly. A patient can develop a rash on the face that can progress to the trunk and limbs. Some of the victims develop conjunctivitis and photophobia as well, and swelling of lymph nodes is also observed in the cervical and axillary regions. The disease usually lasts for 15-20 days. During this period, the patient may feel nausea, tumult, disturbance in the autonomic nervous system, fainting, and eventually die in a deep coma. Prophylactic and protective policies must be considered while dealing with the Marburg virus [17]. We should use personal protective equipment to prevent contamination and work in a biosafety cabinet to maintain hygiene and exposure to self-contamination. The particular epidemic can be controlled by proper laboratory equipment and awareness of the many risk factors. In areas of the outbreak, avoiding contact with non-human primates is appreciated. Humans should avoid frequent visits to mines and caves as fruit bats (carriers of the virus) live there. Adequate care should be taken to dissuade contact with the person having indications of MVD. Infected areas should be decontaminated with disinfectants, and the affected neighborhoods' public should be educated about the precautions, signs, and symptoms. Training hospital staff and front-line workers is essential to tackle the disease more efficiently. Proper care should be taken in the case of a pregnant woman because a fetus can contract a virus from the mother, and high fetal casualty rates highlight the need to concentrate on the treatment of the pregnant mother. The virus can also be excreted in breast milk, and the affected mother should avoid breastfeeding for at least 15 days [18]. Explain that till today, there is no approachable treatment available for the Marburg viral disease. However, possible trials and investigations are carried out in laboratories to determine the safe and protective therapies. Some are being tried out on nonhuman primates, while other trials were sampled on humans. Antiviral drugs (Galidesivir and Favipiravir), monoclonal antibodies, and interferons may be remedies. Considering vaccines, various laboratory practices are executed for the vaccine production of MARV. Currently, ongoing investigations focus on the recombinant vectors to produce genes that provide immunity against the

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expression proteins of the Filovirus. The vaccine trials have been done on the Marburg virus by using inactivated Marburg virus particles, growing them in a Petri plate, and killing them with warmth or chemicals. So, several methods have been investigated to use a live-attenuated version of the Marburg virus as a vaccine. Protein-based vaccines that appear to be effective in the case of MVD and mRNA-1,360 have been formulated and produce adequate viral protein utilizing mRNA technology. In various in-vitro and in-vivo studies, black seeds *(Nigella sativa)* have proved to be antiviral, antioxidant, and immunomodulatory in treating patients infected with the Marburg virus disease. The anti-viral effects of N. sativa help to reduce the viral load in MARV patients, and it is also effective against other viral diseases such as HIV, HCV, HBV, and EBV. Primary prevention and management measures are essential to regulate the Marburg viral disease. Sanitation and hygienic steps should be followed to avoid contact with blood and other body saps. Wildlife animals should be carefully supervised with protective gloves [19]. Several plasma treatments have been experimented on clonal antibodies and demonstrated more promising results. In the future, MVD can be deterred and governed by influential diagnostic techniques, training of healthcare workers, and supplying helpful control criteria for infection. Scientists or researchers from all over the world should share information to enhance public awareness of the virus in public to control death rates in outbreaks. To recover from the harmful virus, supportive care is essential because people living in shallow areas cannot afford high-quality treatments and diagnoses. It usually includes maintenance of the body hydration, medication, physio-social consent, and nutrition. Drugs taken orally are beneficial to degrade the symptoms of infection, like nausea, fever, confusion, and headache. Patients can intake intravenous fluids to keep the body hydrated and vitamins to meet the body's nutritional requirements.

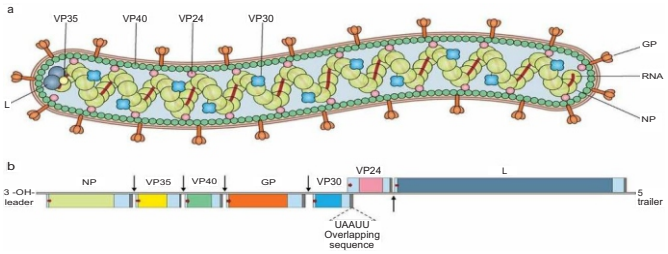

Figure 1: Virion Structure and Genome Organization of Marburg Virus

The Marburg virus structure, along with depicting the structural proteins. Bottom, an illustration of the genome organization of the Marburg virus. This seven-gene strain of the Marburg virus has been drawn roughly to scale. The light blue boxes indicate noncoding areas, and the colored box code regions for genes. The red arrows demonstrate the position of the transcriptional start signals, while the pale brown bars highlight conserved transcriptional stop signals. Intergenic regions segregate the genes, indicated using black arrows, except the overlapping sequence (black triangle) between VP24 and VP30. At the extreme ends, the 3' and 5' trailer sequence is shown in Figure 2 [20].

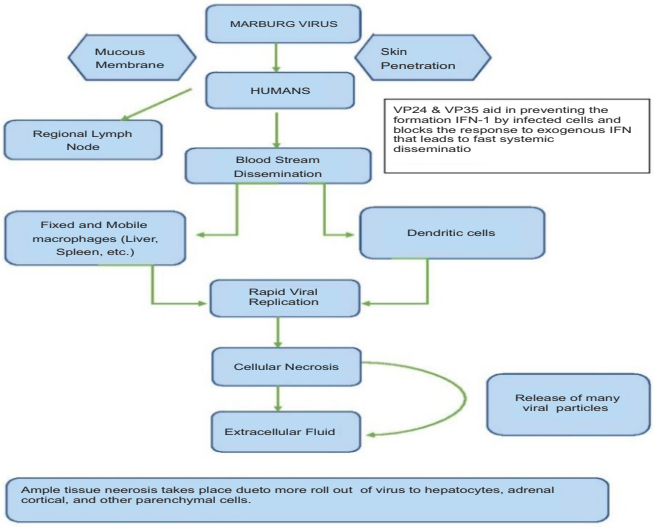

Figure 2: Pathogenesis is of Marburg Virus

Marburg virus can be transmitted from human to human and animal to human as well because it is a zoonotic disease, as shown in Figure 3.

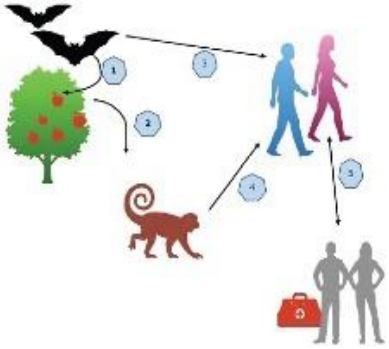

Figure 3: Mode of Transmission of Marburg Virus [19]

When a person gets infected by the Marburg virus, the manifestations are unclear and complicated to diagnose clinically. With time, the progression of the disease occurs. This information is wrong or wrongly written It becomes an epidemic with distinctive signs and symptoms [21]. At the onset of the infection, the patient feels severe headaches and bother in the lumber region. Besides this, the eyes of many patients become prudent, painful, and pressurized. Proper diagnosis and the isolation of patients are necessary because the virus can be transmitted easily from one person to another, like in healthcare workers and doctors. The headache and fever are pursued by chest pain,

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fatigue, puking, and diarrhea. The doctors may doubt Marburg's infection because the patient displays a papular rash and instantaneous cachexia, but the commencement of hemorrhagic fever confirms the disease. All these signs lead to considering Lassa fever, which includes sore throat and facial edema in the late stage of illness. The blood of infected individuals is examined, and it depicts malarial parasites associated with chills and headaches [22]. Patients also develop secondary infections like bacterial infections, typhoid fever, hepatitis, and yellow turmoil. Samples for diagnosis of the viruses are taken from the acute and convalescent phase of the incubation period and by performing liver biopsy and throat washing. After sampling, they are sent to a laboratory for serological and virological testing. The serum is collected from the infected person, and complement fixation tests are conducted for viral confirmation as an antigen. Immunohistochemical techniques are also used for the detection and identification of viral particles. In the diagnosis process, virus isolation is a sensitive method, and the Marburg virus shows enhanced growth in Vero cells and Vero E6 cells. Remove this One technique of RT-PCR formulated by researchers uses the dye SYBR green with a set of primers Filo-A (5`-ATCGGAATTTTTCTTTCTCATT-3`) and Filo-B (5`-ATGTGGTGGGTTATAATAATCACTGACATG-3`). They are concocted to intensify the L genes of the MARV virus. Another method of using real-time quantitative RT-PCR was evolved by investigators in which a fluorogenic TaqMan probe is tagged with dye 6-carboxyfluorescein at the 5` end of DNA and quencher tag at the 3` end. Transmission electron microscopy can be manipulated to detect the Marburg virus in tissues and body fluids. Viral antigens can also be diagnosed with the help of immunochemistry by examining tissue lacerations and determining vision configuration. Operators deduce by performing these techniques that viruses reside in pancreatic cells and hepatocytes. MARV is aimed at macrophages as fibroblasts, vital replication sites for the Marburg virus [23]. Although no known treatment is present for the Marburg virus disease, some therapies are considered to increase the survival rate of infected persons. Filoviruses are deadly viruses and have caused destruction worldwide. As we discussed earlier, the Angola strain of the Marburg virus causes 85% of death rates [24]. Viral infections resulting in hemorrhagic fever can be dealt with with various therapeutic schemes comprised of immunization, management of antibodies, and use of antiviral medications for therapy. Different classes of antivirals are used based on the molecule they target, specific proteins that destroy virus particles, interferon, and immunomodulatory substances [25]. In case of a proper remedy for MVD, the disease is overseen by diverse pharmacological aspects and supportive measures to prevent organ failure and prolong a patient's life. The cases of the Marburg virus disease can be regulated by strict guidance of preventive techniques and isolation. Rephrase or remove When a patient experiences septic shock, he/she is divulged to the ICU to get the intrusive antidote for prevention and surveillance of ailment. Sometimes, the patient can be treated with decent supportive maintenance and administering a distinct antiviral cure. In filoviruses, clinical strategies can be split into three grades, i.e., incubation of virus, pre-coagulopathy, and coagulopathy [26, 27]. In the foremost phage, the organism is exposed to a viral particle until it shows symptoms, and at this stage, the infected patient gets a vaccination and an antiviral treatment [28]. Next, in the second step, the virus begins to replicate and provoke diseases, and in this phase, virostatics are influential. The final and last phase is portrayed by coagulation irregularities caused by defects in the cytokine grid. At this set, interfering and proinflammatory cytokines are the only choices left to evaluate the infection. Non-human primates can be dealt with by using disease-modifying mechanisms such as human recombinant protein C (rhAAPC), d chemotherapeutic procedures are used to cope with coagulation disabilities and nematode dicoumarol protein c2 (rNAPc2) are clotting substances [29, 30]. It is valuable to obstruct the activity of tissue factor VIIa (a protein complex). PMOs (phosphorodiamidate morpholino oligomers) are inhibitory molecules that prevent the replication of filovirus particles. Positively charged PMOs and short interfering RNA (siRNA) can be used as a victorious vaccine to be administrated in non-human primates for persuasive restorative of filoviruses [31, 32]. siRNA marks specific genes through RNA machinery and inhibits viruses that induce hemorrhagic fever, such as MARV. Mannose is a sugar present in cells that has a receptor CD206 present on the exterior of dendritic cells and macrophages. siRNA combines with CD206, resulting in GalNAc-siRNA and mannose forming a complex with siRNA that is mannose-siRNA [33, 34]. These two complexes are studied in non-human mod that target the MARV proteins and provide security [35, 36].

DISCUSSION

Appropriate knowledge of each aspect of the deadly virus is essential as the published articles from pioneer writers are followed. The MARV infection has been disregarded for many years, but it has recently gained the interest of scientists and researchers because controlling it has become a challenge for them. The highest number of cases

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was monitored between 2004 and 2005 in Angola, where the case fatality rate was 90%. Different episodes of the Marburg viral disease were reported in Uganda from 2007 to 2017, where 100% CFR was recorded in 2014. Recently, this deadly virus played a game of demise in West Guinea in 2023. Disease-modifying narcotics and inhibitors of viral proteins have shown more promising consequences in patients [37, 38]. Viral RNA and IgG antibodies in vectors (bats) are detected by serological data and screening of bats by RT-PCR. The broad-spectrum drug "favipiravir" is beneficial as an intervention for filovirus conditions. Designation of the reservoir host should permit the development of risk-lessening parameters to mitigate the possibility of coming disease outbreaks. Control and proper management are necessary to prevent infection [39]. Formal governance and supportive therapies can treat some cases with fewer viral loads. Kalonji (black seeds) is beneficial in the treatment of the Marburg virus disorder because of its anti-inflammatory efficiency as a MARV attacks lymphocytes and macrophages, and these contaminated cells facilitate the synthesis of human necrotic factor and Nigella sativa (kalonji) destroy alpha-tumor necrotic factor [40].

CONCLUSIONS

After worldwide devastation caused by COVID-19, the Marburg virus appears to be a rising challenge in front of the world. Because of the high case fatality rates, MVD was confirmed to be a deadly infection challenges to manage due to the undersupply of vaccines and insufficient cures. Endeavors are made globally by researchers, healthcare professionals, and epidemiologists so that humanity can tackle the Marburg viral disease more effectively. Although Africa has been specified as the prospective source of the MARV, most outbreaks have been caused by animal spillage to the human population. Vaccine studies have been continuous for the past few years, and the virus is being investigated on various animal models, including NHPs, hamsters, poultry, and mouse samples. N. sativa is a spice used traditionally to treat different ailments. The discourses learned from the past outbreaks emphasized the significance of preparedness and creation to address the threat of MVD to public health. The explicitness and sensitivity of these diagnostics are beneficial in epidemiological analysis and examination of filovirus infections.

Authors Contribution

Conceptualization: RA, HAS, HURAK, AN, JS, HR, F, AH, AP, AS
Methodology: RA, HAS, HURAK, AN, JS, HR, F, AH, AP, AS
Formal analysis: RA, HAS, HURAK, AN, JS, HR, F, AH, AP, AS
Writing, review and editing: RA, HAS, HURAK, AN, JS, HR, F, AH, AP, AS

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Conflicts of Interest

All the authors declare no conflict of interest.

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REFERENCES

[1] Brauburger K, Hume AJ, Mühlberger E, Olejnik J. Forty-five years of Marburg virus research. Viruses. 2012 Oct; 4(10): 1878-927. doi: 10.3390/v4101878.

[2] Ristanović ES, Kokoškov NS, Crozier I, Kuhn JH, Gligić AS. A forgotten episode of Marburg virus disease: Belgrade, Yugoslavia, 1967. Microbiology and Molecular Biology Reviews.2020 May; 84(2): 10-128. doi: 10.1128/MMBR.00095-19.

[3] Abir MH, Rahman T, Das A, Etu SN, Nafiz IH, Rakib A *et al.* Pathogenicity and virulence of Marburg virus. Virulence.2022 Dec;13(1):609-33.doi:10.1080/21505594.2022.2054760.

[4] Thomas B, Chandran P, Lilabi MP, George B, Sivakumar CP, Jayadev VK *et al.* Nipah virus infection in Kozhikode, Kerala, South India, in 2018: epidemiology of an outbreak of an emerging disease. Indian Journal of Community Medicine.2019 Oct; 44(4): 383-7. doi: 10.4103/ijcm.IJCM_198_19.

[5] Blair PW, Keshtkar-Jahromi M, Psoter KJ, Reisler RB, Warren TK, Johnston SC *et al.* Virulence of marburg virus Angola compared to Mt. Elgon (Musoke) in macaques: A pooled survival analysis.Viruses.2018 Nov; 10(11): 658. doi: 10.3390/v10110658.

[6] Nicholas VV, Rosenke R, Feldmann F, Long D, Thomas T, Scott DP *et al.* Distinct biological phenotypes of Marburg and Ravn virus infection in macaques. The Journal of Infectious Diseases.2018 Nov; 218(suppl_5): S458-65. doi: 10.1093/infdis/jiy456.

[7] Muvunyi CM, Ngabonziza JC, Bigirimana N, Ndembi N, Siddig EE, Kaseya J *et al.* Evidence-Based Guidance for One Health Preparedness, Prevention, and Response Strategies to Marburg Virus Disease Outbreaks.Diseases.2024Dec;12(12):309.doi:10.3390/diseases12120309.

[8] Bausch DG, Nichol ST, Muyembe-Tamfum JJ, Borchert M, Rollin PE, Sleurs H *et al.* Marburg hemorrhagic fever associated with multiple genetic lineages of virus. New England Journal of Medicine. 2006Aug;355(9):909-19.doi:10.1056/NEJMoa051465.

[9] Qiu X, Wong G, Audet J, Cutts T, Niu Y, Booth S *et al.* Establishment and characterization of a lethal mouse model for the Angola strain of Marburg virus. Journal of Virology.2014Nov;88(21):12703-14.doi:10

DOI: https://doi.org/10.54393/fbt.v5i1.82

.1128/JVI.01643-14.

[10] Bertherat E, Talarmin A, Zeller H. Democratic Republic of the Congo: between civil war and the Marburg virus. International Committee of Technical and Scientific Coordination of the Durba Epidemic. Medecine Tropicale: Revue du Corps de Sante Colonial. 1999 Jan; 59(2): 201-4.

[11] Towner JS, Amman BR, Sealy TK, Carroll SA, Comer JA, Kemp A *et al.* Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. PLOS Pathogens. 2009Jul; 5(7): e1000536. doi: 10.1371/journal.ppat.1000536.

[12] Miraglia CM. Marburgviruses: an update. Laboratory Medicine. 2019 Jan; 50(1): 16-28. doi: 10.1093/labmed/lmy046.

[13] Emond RT, Evans B, Bowen ET, Lloyd G. A case of Ebola virus infection. British Medical Journal. 1977 Aug; 2(6086): 541-4. doi: 10.1136/bmj.2.6086.541.

[14] Dye JM, Herbert AS, Kuehne AI, Barth JF, Muhammad MA, Zak SE *et al.* Postexposure antibody prophylaxis protects nonhuman primates from filovirus disease. Proceedings of the National Academy of Sciences. 2012Mar; 109(13): 5034-9. doi: 10.1073/pnas.1200409109.

[15] Srivastava S, Sharma D, Kumar S, Sharma A, Rijal R, Asija A *et al.* Emergence of Marburg virus: a global perspective on fatal outbreaks and clinical challenges. Frontiers in Microbiology. 2023 Sep; 14: 1239079. doi: 10.3389/fmicb.2023.1239079.

[16] Islam MA, Adeiza SS, Amin MR, Kaifa FH, Lorenzo JM, Bhattacharya P *et al.* A bibliometric study on Marburg virus research with prevention and control strategies. Frontiers in Tropical Diseases. 2023 Jan; 3: 1068364. doi: 10.3389/fitd.2022.1068364.

[17] Chakraborty S, Chandran D, Mohapatra RK, Alagawany M, Yatoo MI, Islam MA *et al.* Marburg virus disease-a mini-review. 2022 Aug; 10(4).

[18] Albakri K, Al-Hajali M, Saleh O, Alkhalil AM, Mohd AB, Samain CA *et al.* Marburg virus disease treatments and vaccines: recent gaps and implications. Annals of Medicine and Surgery. 2023 Feb; 85(2): 328-30. doi: 10.1097/MS9.0000000000000163.

[19] Lawrence JA, Ul Rasool MH, Parikh C, Chowdhury S, Sueldo A, Sarwar S. Emergence of Marburg virus disease in West Africa amid COVID-19 and Ebola: efforts, challenges, and recommendations to prevent the next public health crisis. Journal of Infectiology and Epidemiology. 2022 Apr; 8: 259. doi: 10.23937/2474-3658/1510259.

[20] Prator CA, Dorratt BM, O'Donnell KL, Lack J, Pinski AN, Ricklefs S *et al.* Transcriptional profiling of immune responses in NHPs after low-dose, VSV-based vaccination against Marburg virus. Emerging Microbes & Infections. 2023 Dec; 12(2): 2252513. doi: 10.1080/22221751.2023.2252513.

[21] Saijo M, Niikura M, Ikegami T, Kurane I, Kurata T, Morikawa S. Laboratory diagnostic systems for Ebola and Marburg hemorrhagic fevers developed with recombinant proteins. Clinical and Vaccine Immunology. 2006 Apr; 13(4): 444-51. doi: 10.1128/CVI.13.4.444-451.2006.

[22] Elsheikh R, Makram AM, Selim H, Nguyen D, Le TT, Tran VP *et al.* Reemergence of Marburgvirus disease: update on current control and prevention measures and review of the literature. Reviews in Medical Virology. 2023 Sep; 33(5): e2461. doi: 10.1002/rmv.2461.

[23] Koundouno FR, Kafetzopoulou LE, Faye M, Renevey A, Soropogui B, Ifono K *et al.* Detection of Marburg virus disease in Guinea. New England Journal of Medicine. 2022 Jun; 386(26): 2528-30. doi: 10.1056/NEJMc2120183.

[24] Zhu W, Zhang Z, He S, Wong G, Banadyga L, Qiu X. Successful treatment of Marburg virus with orally administrated T-705 (Favipiravir) in a mouse model. Antiviral Research. 2018 Mar; 151: 39-49. doi: 10.1016/j.antiviral.2018.01.011.

[25] Daddario-DiCaprio KM, Geisbert TW, Ströher U, Geisbert JB, Grolla A, Fritz EA *et al.* Postexposure protection against Marburg haemorrhagic fever with recombinant vesicular stomatitis virus vectors in non-human primates: an efficacy assessment. The Lancet. 2006 Apr; 367(9520): 1399-404. doi: 10.1016/S0140-6736(06)68546-2.

[26] Kortepeter MG, Dierberg K, Shenoy ES, Cieslak TJ. Marburg virus disease: a summary for clinicians. International Journal of Infectious Diseases. 2020 Oct; 99: 233-42. doi: 10.1016/j.ijid.2020.07.042.

[27] Paragas J and Geisbert TW. Development of treatment strategies to combat Ebola and Marburg viruses. Expert Review of Anti-Infective Therapy. 2006 Feb; 4(1): 67-76. doi: 10.1586/14787210.4.1.67.

[28] Zhao F, He Y, Lu H. Marburg virus disease: a deadly rare virus is coming. Bioscience Trends. 2022 Aug; 16(4): 312-6. doi: 10.5582/bst.2022.01333.

[29] Iversen PL, Warren TK, Wells JB, Garza NL, Mourich DV, Welch LS *et al.* Discovery and early development of AVI-7537 and AVI-7288 for the treatment of Ebola virus and Marburg virus infections. Viruses. 2012 Nov; 4(11): 2806-30. doi: 10.3390/v4112806.

[30] Thi EP, Mire CE, Ursic-Bedoya R, Geisbert JB, H. Lee AC, Agans KN *et al.* Marburg virus infection in nonhuman primates: Therapeutic treatment by lipid-encapsulated siRNA. Science Translational

DOI: https://doi.org/10.54393/fbt.v5i1.82

Medicine.2014 Aug; 6(250): 250ra116-.doi:10.1126/scitranslmed.3009706.

[31] Cross RW, Fenton KA, Geisbert JB, Ebihara H, Mire CE, Geisbert TW. Comparison of the pathogenesis of the Angola and Ravn strains of Marburg virus in the outbred guinea pig model. The Journal of Infectious Diseases.2015 Oct; 212(suppl_2): S258-70. doi: 10.1093/infdis/jiv182.

[32] Zhang L, Lei S, Xie H, Li Q, Liu S, Liu Q *et al.* Screening and identification of Marburg virus entry inhibitors using approved drugs. Virologica Sinica.2020 Apr; 35: 235-9. doi: 10.1007/s12250-019-00184-3.

[33] Marzi A, Feldmann H, Geisbert TW, Falzarano D. Vesicular stomatitis virus-based vaccines for prophylaxis and treatment of filovirus infections. Journal of Bioterrorism & Biodefense. 2011 Sep; 9(4). doi: 10.4172/2157-2526.S1-004.

[34] Cheng K, Guo Q, Gu S, Wu H, Li C. Deadly Marburg virus outbreak received sustained attention: What can we learn from the existing studies?. International Journal of Surgery.2023 Aug; 109(8): 2539-41.doi: 10.1097/JS9.0000000000000443.

[35] Zumbrun EE, Garvey CB, Wells JB, Lynn GC, Van Tongeren S, Steffens JT *et al.* Characterization of the Cynomolgus Macaque Model of Marburg Virus Disease and Assessment of Timing for Therapeutic Treatment Testing. Viruses. 2023 Nov; 15(12): 2335. doi: 10.3390/v15122335.

[36] Cheng H, Lear-Rooney CM, Johansen L, Varhegyi E, Chen ZW, Olinger GG *et al.* Inhibition of Ebola and Marburg virus entry by G protein-coupled receptor antagonists. Journal of Virology.2015 Oct; 89(19): 9932-8. doi: 10.1128/JVI.01337-15.

[37] Valmas C, Grosch MN, Schümann M, Olejnik J, Martinez O, Best SM *et al.* Marburg virus evades interferon responses by a mechanism distinct from ebola virus. PLOS Pathogens.2010 Jan; 6(1): e1000721. doi: 10.1371/journal.ppat.1000721.

[38] Hartman AL, Towner JS, Nichol ST. Ebola and marburg hemorrhagic fever. Clinics in Laboratory Medicine.2010Mar;30(1):161-77.doi:10.1016/j.cll.2009.12.001.

[39] Simpson DI and World Health Organization. Marburg and Ebola virus infections: a guide for their diagnosis, management and control. World Health Organization; 1977.

[40] Maideen NM. A review of inherent beneficial effects of black seeds (Nigella sativa) in Marburg virus disease management. Food Health.2023; 5(6). doi: 10.53388/FH2023006.

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Review Article

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The Role of Lifestyle in Modulating the Gut Microbiome

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ABSTRACT

The human gut, a dynamic and diverse ecosystem of trillions of microorganisms, plays an essential role in the host's health and disease. This review explores the influence of lifestyle choices like diet, stress, physical activity, and environmental factors on gut microbiome and their broader societal implications. Studies have reported that plant-based and Mediterranean diets enhance microbial diversity. At the same time, a sedentary lifestyle, chronic stress, processed foods, and alcohol consumption badly impact on the gut microbial composition and lead to many diseases like dysbiosis, obesity, and cardiovascular diseases. Geographic and ethnic factors also influence the gut microbiome. The consumption of fermented food and a diet high in fiber has a positive impact on the gut microbiome. The gut microbiome also has many societal implications, and the targeted intervention can help to reduce economic losses and public health costs and improve the overall health of everyone. This comprehensive review focuses on the links between lifestyle, gut microbiome, and societal well-being and suggests integrative strategies to promote sustainable health practices.

INTRODUCTION

An abundant and diverse microbial community resides in the gastrointestinal tract of humans. Greater than 100 trillion microbes and about 2000 species have been reported within the intestine [1, 2]. Both pathogenic and symbiotic microorganisms are inhabitants of the human intestine, while around 1014 microbes reside in the colon of humans, making it one of the most populated habitats [3]. More than 3 million genes are encoded by these microbes, which are known to produce a large number of bioactive compounds, and these compounds play a vital role in human health [4] (Figure 1). Various beneficial activities have been reported in the gut microbiome for host health, like producing short-chain fatty acids and vitamins, immune homeostasis, digestion, and activity against pathogens. Alteration of gut microbiome badly affects human health and leads to diseases like type II diabetes, inflammatory bowel syndrome, and cardiovascular diseases [5, 6]. Short-chain fatty acids (SCFA) like propionate, butyrate, and propionate, produced by gut microbiome as a byproduct, are the primary energy source for epithelial cells of the intestine and strengthen the mucous layer. Studies on germ-free mice have shown that gut microbiome enhances the immunity of the intestine by affecting antigen-presenting cells, expression of toll-like receptors, lymphoid cells, and differentiated T cells [7] and by altering systematic antibody expression and immunity via raised splenic CD4+ cells [8]. Because of these facts about the gut microbiome, researchers are getting more

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interested in studying gut microorganisms. The gut microbiome changes quickly in response to the diet a person is consuming. For instance, if a person consumes a plant- or animal-based diet, the gut microbiome will change within 24h according to the diet [9].

The present study aimed to summarize the current knowledge on impact of lifestyle choices on gut microbiome and the implications of changes in gut microbial diversity because of lifestyle.

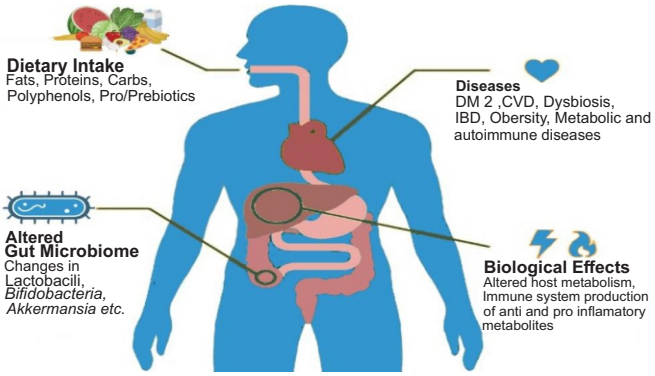

Figure 1: An Overview of Diet's Impact on Human Health, Diseases, and Gut Microbiome

There are many lifestyle choices that directly or indirectly influence the gut microbiome. Few of them are described below:

Geography and Ethnicity

Gut comparison analysis of individuals from European and African children's samples Lee *et al.*, reported that different countries gut microbiomes are not similar and have different compositions [10]. They also compared the USA and Korean twin pairs fecal microbiota and found different gut microbiome compositions between the two countries. This study revealed that environmental factors and diet influence the gut microbiome. They further reported a massive difference in microbiota in families consuming different diets and environments while staying within the same country. A significant difference in the microbial composition of lean and obese patients was observed during their study in the USA and Korea. Fecal samples of 2084 persons from various ethnic groups residing in the same area were collected and analyzed for gut microbiome composition. The differences in the microbial composition indicate that ethnicity is one of the gut microbiota influencers Although other factors like person-specific genetic profile and some environmental factors might involve this variation, diet is the main contributor of gut microbial variation in these individuals [11]. Rothschild *et al.*, reported a study in which they surveyed 1046 healthy persons sharing the same environment and belonging to different ancestors [12]. Their analysis revealed that the person-specific genetic profile has a limited role in determining the composition of the gut microbiome. The impact of country of residence on gut microbial composition has been studied in individuals who migrated to the USA from Thailand. The individuals who emigrated from Thailand to the USA faced many challenges like obesity, metabolic diseases, and quick changes in gut microbial composition were observed among them. The gut microbiome changes observed in these individuals include loss of microbial diversity, native gut species loss, and Bacteroidetes increased in the place of Prevotella species with the passage of time [13]. The reason for these changes can be a sedentary lifestyle, the adaptation of a Western diet, and food insecurity [14].

Pet-Friendly Living

Pet friendly life can be an environmental factor affecting gut microbial composition. Samples of 332 individuals living without and with pets were analyzed, and results showed no significant difference in microbial diversity. Still, the abundance of specific phyla, such as Firmicutes, was significantly higher [15]. A link between the gut microbiome profile of infants and furry pets has been observed, with the abundance of Oscillospira and Ruminococcus. Both phyla are responsible for various allergies and obesity [16]. As little literature is available on the relationship of pets and gut microbiome, more research must be conducted in this field.

Physical Activity

Regular physical activity/exercise plays an important role in the regulation of gut microbiome and its functionality. It can protect from the microbes associated with CVD and many other metabolic disorders. Therefore, physical exercise is a polypill for many chronic diseases/infections [17]. Now, researchers are taking more interest in studying the impact of physical activity on the gut microbiome. They are looking for ways to improve physical health by modulating the gut microbiome, which can help fight microbial infections and various chronic diseases and delay aging with the help of gut microbiome. The relationships between microbiome, nutrition, and physical exercise have been focused on in adults and professional and non-professional athletes. Physical exercise can enhance microbial diversity, which is evidence of good health [17]. But the problem is that persons with regular exercise or athletes use different kinds of diets and have entirely different routines for sex sports periodization and might depend on a particular sport. That's why their routine is different from that of the public. Therefore, the link between diet and gut microbial diversity in individuals who practice regular physical activity is strenuous to be established [18]. A possible role of some gut microbial species has been established in physical activity. For

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instance, an increase in the abundance of Prevotella copri has been observed in the individuals following regular exercises. This bacterium plays a role in gene expression of genes involved in L-lysine metabolism. L-lysine is an amino acid not produced within our body and plays a role in muscular integrity [19]. Similarly, in a study, Scheiman *et al.*, reported an increase in Veillonella atypica in marathon runners. This bacterial species is known to play a role in muscle recovery by degrading lactate produced during physical activity [20]. Likewise, in another study, Morita et al., reported an increase in Bacteroidetes uniformis in male long-distance runners, enhancing their performance [21]. These studies open new fields of opportunities to develop prebiotics, probiotics, and various symbiotic combinations to improve physical activities via food supplementation [22].

Diet

Diet plays a key role in maintaining optimal gut microbiome. The composition and functionality of gut microbes depend on dietary patterns and the availability of micro and macro-nutrients in the intestine. Several studies have been reported clinically and preclinically on the diet and stated that diet significantly influences the gut microbiome. The Western diet normally consists of simple sugars, and saturated fats tend to increase Bacteroidetes sp. This diet promotes bile-tolerant microbes like Bacteroidetes sp. and Alistipes sp. and tends to decrease Firmicutes sp. in the gut. Although main bacterial species remain dominant, a change in the diet influences the gut microbiome within 24h. [23]. Animal-based diets have less diverse gut microbiomes as compared to plant-based diets. The plant-based diet has high fiber levels, promoting the growth of fiber-fermenting bacteria. These fermenting microbes increase fermented products like short-chain fatty acids (SFA) and improve blood circulation in the gut [24]. It is now an established fact that dietary interventions affect and change the gut microbiome, so changes in gut microbiome based on diet are not surprising anymore. For instance, the keto diet (KD) is known to influence the gut microbiome. Ma *et al.*, stated that there was an increase in the Lactobacillus and A. muciniphila population, and relative abundance was observed for Turicibacter and Desulfovibrio in individuals given a ketogenic diet [25]. Both Lactobacillus and A. muciniphila are commensal bacterium known for SCA production [26, 27]. Many other studies have also reported a decrease in gut microbial diversity based on observed taxa and Shannon index among the persons given with KD [28, 29]. However, in a study, Swidsinski *et al.*, reported the change in gut microbiome based on KD if biphasic, indicating that it reduces the gut microbial diversity initially, but when a person receives KD diet for extended periods of time, then the microbial diversity increases automatically [30]. However, gut diversity is not similar to that of individuals who have intermittent fasting compared to individuals with KD [31]. Long-term dependency on the Mediterranean diet (MD) impacts on gut health. An increase in the concentration of Prevotella, Prevotellaceae, and Bacteriodetes and a decrease in Lachnospiraceae and Firmicutes has been observed in the individuals given Mediterranean diets [32]. Furthermore, in the individuals who used to eat MD for a longer duration, an increase in the butyrate and propionate was observed. These SCFA were associated with higher microbial diversity when a comparison was established with the Western diet [33]. The utilization of omega-3 fatty acids found in fish oil is associated with a higher level of docosahexaenoic acid (DHA) in the blood. This increased DHA level is associated with higher levels of Ruminococcaceae and Lachnospiraceae bacterial families. These families are involved in the dietary fiber fermentation, resulting in the production of SCFAs in the human gut [34]. Fecal metabolite N-carbamylglutamate can also be influenced by gut bacteria through dietary interventions [35]. The microbial community shaping can be managed by the dietary intake of vitamins, minerals, micronutrients, and polyphenols [36]. As gut microbial composition can be influenced by nutritional interventions, the gut microbiome can be modified easily by diet modification. Thus, a modified gut can achieve many physical and general health benefits (Table 1).

Table 1: Some of the gut microbes with associated diseases

Bacteria	Key Characteristics	Linked Disease Conditions	Related Physiological Changes	References
Akkermansia muciniphila	Gram-negative, oval-shaped, nonmotile obligate anaerobe	Reduced presence in IBD, obesity, and psoriatic arthritis	Exhibits anti-inflammatory effects	[37]
Escherichia coli	Gram-negative, rod-shaped facultative anaerobe	Overabundance linked to IBD, UTIs, gastroenteritis, and meningitis	Activates TLR pathways	[38]
Faecalibacterium prausnitzii	Gram-positive, nonmotile, rod-shaped obligate anaerobe	Lower levels associated with IBD and obesity	SCFA production and anti-inflammatory properties	[39]
Enterococcus sp	Gram-positive, cocci-shaped facultative anaerobe	Includes pathogenic species causing UTIs, endocarditis, or bacteremia	Induction of anti-inflammatory response	[40]
Eubacterium sp	Gram-positive, rod-shaped obligate anaerobe	Reduced presence linked to IBD	Produces SCFAs and beneficial phenolic acids	[41]
Roseburia sp	Gram-variable, curved, motile obligate anaerobe	Decreased levels noted in IBD	SCFA production	[42]

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Clostridium sp	Gram-positive, rod-shaped obligate anaerobe; spore-forming	Associated with diseases like tetanus, botulism, gas gangrene, and pseudomembranous colitis	Supports TH17 cell generation	[43]
Bilophila sp	Gram-negative, obligate anaerobe; urease-positive, bile-resistant, catalase-positive	B. wadsworthia linked to colitis, liver abscesses,gangrenous appendicitis, cholecystitis, FG, empyema, and HS	Stimulates pro-inflammatory TH1 immune response	[44]
Alistipes sp	Gram-negative, rod-shaped obligate anaerobe; bile-resistant, pigment-producing	Found in cases of acute appendicitis, brain abscesses, and perirectal abscesses	contribute to the production of beneficial metabolites	[45]
Bacteroides sp	Gram-negative, rod-shaped obligate anaerobe; variable motility	Higher abundance linked to IBD	Activates CD4+ T cells	[46]
Lactobacillus sp	Gram-positive, rod-shaped facultative anaerobe	Contributes to reducing IBD	SCFA synthesis exhibits anti-inflammatory and anti-cancer properties	[47]
Bifidobacterium sp	Gram-positive, branched, nonmotile obligate anaerobe	Lower levels observed in obesity	Produces SCFAs, enhances gut mucosal barrier, reduces intestinal LPS	[48]

Stress

Gut dysbiosis is often associated with stress, indicating that the gut microbiome also responds to chronic stress [49]. A study on germ-free mice showed the production of adrenocorticotropic hormone (ACTH) and corticosterone compared to the control when mild stress was given to the mice. This indicates that the gut microbiome responds to stress and is critical in hypothalamic pituitary adrenal (HPA) axis development [50]. The gut microbiome is also associated with behavioral and physiological changes in response to exposure to stress, like HPA axis dysregulation, social and behavioral changes, impaired cognition, and intestinal barrier function, causing intestinal permeability, which leads to leaking gut and increased inflammation [51, 52, 53]. Now, it is established that stress badly impacts the gut microbiome, damages the microbial community's ecology, and promotes dysbiosis [49]. Many clinical and animal-based studies have reported that stress negatively impacts gut health [49]. Various kinds of stresses like restraint conditions, maternal separation, crowding, heat stress, and noise were able to negatively change the gut microbiome in different animal model studies [54]. For instance, chronic restraint and maternal separation stress tend to lower the Lactobacillus level [55]. A survey of the animal model, which was kept under stress, showed improved cognition, behavioral, and biochemical results after administering Lactobacillus [56]. Under chronic stress conditions, a decrease in the Bacteriodetes abundance and an increase in the clostridiales family has been observed. Both of these correlate with changed pro-inflammatory cytokines levels [57]. All these studies indicate that stress directly impacts the gut microbiome, which may be associated with various diseases like dysbiosis. So, stress management techniques must be adopted in our daily life to maintain gut microbiome at optimal conditions.

Prebiotics

Prebiotics, from the family of dietary fibers, play a significant role in the gut microbiome. Prebiotics can be defined as substrates that can be utilized by host microorganisms that have health benefits for the host system [58]. The most common prebiotics are HMOs, FOS, galacto-oligosaccharides, and inulin. These prebiotics promote the growth of probiotic bacteria like Lactobacilli and Bifidobacteria. These probiotic microbes produce SCFA and many other bioactive compounds that have health benefits for the host system [59]. Many studies have already reported that prebiotics can influence the ability of gut microbes to produce SCFAs. The SCFAs activate the GPR43/41 receptors of the L cells, thus promoting the secretions of PYY and GLP-1. They also promote the secretions of the BLP-2. This peptide maintains the gut barrier functions by stimulating blood flow, stimulating proliferative epithelial cells of the intestine, and improving the integrity of tight junctions [60]. After all, the prebiotic and gut microbic interactions play a role in the reduction of intestinal permeability, and they also decrease food intake, prevent metabolic endotoxemia and hepatic steatosis, and improve sensitivity and secretions of insulin. All of these are known to reduce inflammation (Figure 2).

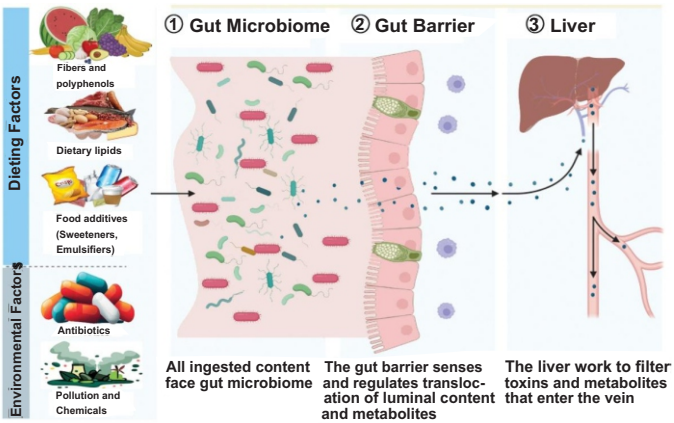

Figure 2: There Are Three Lines of Defense. (1) Gut Microbiome Influenced by Environmental Factors and Diet. (2) Then They Interact with Gut Barrier and The Gut Barrier Regulate the Translocation of Metabolites and Luminal Components. (3) The Liver Work to Filter The Toxins and Metabolites.

Polyphenols

Polyphenols abundantly found in plant-based diets, including tea, fruits, coffee, vegetables, and wine, are the

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most complex metabolic compounds. They are divided into two main groups: non-flavonoids and flavonoids. Flavonoids are divided into flavanols, anthocyanins, isoflavones, and flavanones. At the same time, non-flavonoids are divided into lignans, stilbenes, and phenolic acids [61]. Polyphenols are not present in blood circulation because most of them are found in food as polymers, glycosides, and esters that may not be absorbed in their native form, so most of them work locally. Polyphenols have been considered for their ability to prevent different diseases that may be caused by oxidative stress, and they are also known for their potent antioxidant potential. Gut microbes are known for their ability to produce various enzymes that enhance the bioactivity and bioavailability of polyphenols in the intestine. So, the polyphenols affect the gut microbiome composition. The polyphenols strengthen the growth of gut microbes like Bifidobacterium, Lactobacillus, A. muciniphila, Faecalibacterium, and Roseburia and inhibit the growth of pathogenic microorganisms [61, 62]. The gut microbiota and polyphenols interaction also play a role in the gut barrier function anticarcinogenic and anti-inflammatory effects. Phenyl propionic acid, a phenolic compound produced by the gut microbiome, has anti-inflammatory activity and plays a role in the gut barrier using the dependent mechanism of aryl hydrocarbon receptor (AhR) [63, 64]. The polyphenolic compounds 4-hydroxyphenylacetic acid and Hydroxyphenylacetic acid have been known for their antioxidant and anti-inflammatory potential and protect the body from obesity, cardiovascular disease, and many types of cancers [64]. Many other phenolic acid compounds, like gallic acid, ferulic acid, and caffeic acid, are produced by gut microbes using dietary polyphenols and have many health benefits [63].

Fatty Acids

The function and composition of the gut microbiome are also greatly influenced by the dietary fat that any individual is consuming. For example, saturated fatty acids, usually present in processed food and animal fat, increase pro-inflammatory microbes and decrease gut microbial diversity [65]. Polyunsaturated fatty acids (PUFAs), like omega-3, usually present in flaxseed and fish oil, are known to enhance the growth of A. mucinipila and Bifidobacterium, which play a role in health improvement [66]. Some gut microbes, like Clostridium, Lactobacillus, Enterobacter, and Bifidobacterium, can metabolize PUFAs into keto and hydroxy derivatives. The beneficial effect of HYA and CLA has been reported in the mice model of cancer, obesity, and colitis by activating PPARa, GPR140, PPARy, and GPR120 and using peristalsis via EP3 activation [67]. Dietary cholesterol is highly dependent on gut microbiome composition. Some cholesterol is absorbed in the upper portion of the intestine, and about 2g of cholestrol enters the colon daily. In the colon, cholesterol-degrading bacteria convert the cholesterol to coprostanol and then, to a lesser extent, form the coprostanone [68]. Some cholesterol-degrading microbes, such as Oscillibacter and Dysosmobacter, have been cultured and isolated, but overall, isolating cholesterol-degrading bacterial species is a complicated and challenging task. These microbes have shown activities to lower the cholestrol level in humans and have demonstrated the potential to convert cholesterol to comprostanone [69].

Artificial Sweeteners

Many non-calorie artificial sweeteners are used to enhance the quality and taste of packaged foods. Although food regulatory authorities approve of them, many of the artificial sweeteners are known to cause risks for various diseases. In research studies, aspartame, sucralose, and saccharin have been reported to cause greater glucose intolerance than glucose. Saccharin has more potential to cause glucose intolerance than other ones. Most of the non-caloric artificial sweeteners pass the digestive tract while remaining undigested. These undigested artificial sweeteners interact with the gut microbiome, changing their function and composition. Artificial sweeteners may also cause type 2 diabetes by upregulating the pathways involved in LPS biosynthesis, as revealed by metagenomic studies [70]. A positive correlation between metabolic indicators like blood glucose levels and hemoglobin A1c and artificial sweetener consumption in humans has been reported. Regular usage of artificial sweeteners and refined sugar badly impacts the gut microbiome composition. This altered composition may lead to metabolic disorders like diabetes, impaired glucose metabolism, reduced microbial diversity, and gut microbiota deviation [71].

Emulsifiers

The emulsifiers are typically used to improve the shelf life and texture of food, but they also have a terrible impact on gut barrier function and gut microbiota. Although they are widely used in the food industry, safety concerns about emulsifiers still need to be resolved. The emulsifiers like polysorbate 80 and carboxymethylcellulose have been reported to induce many metabolic diseases. They mutate the intestinal mucus layer and thus lead to leaking gut by increasing gut permeability. Some emulsifiers like carboxymethylcellulose disturber the gut microbial community by overgrowth of some bacterial species while many like polysorbate 80, play a role in microbial translocation [72]. These emulsifiers have also been reported to cause metabolic syndrome and inflammation in

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mice models. Furthermore, these emulsifiers damage the mucus layer, making direct contact between intestinal walls and bacterial cells and leading to pathogenic infections.

Alcohol

Alcohol is also known to cause changes in the gut microbiome. In alcohol-addicted individuals, the gut microorganisms play a role in alcoholic liver disease, and dysbiosis is commonly observed in alcoholic individuals. In patients suffering from alcoholic liver diseases, the abundance of Enterococcus and Bacteroidetes has been observed. A study conducted on mice models has reported overgrowth of some bacterial species, like Enterobacteriaceae class. The study further reported the occurrence of intestinal inflammation upon regular alcohol consumption for seven days [73]. The change in gut microbiome composition upon alcohol consumption does not seem to be influenced by ethanol: acetate production, but by the enzymes that the host produces also play a role. Some studies have suggested that probiotics may help improve liver-associated enzyme levels, which are affected by alcohol consumption [74, 75].

Cigarettes

Studies on e-cigarettes and cigarettes have reported that smoking contributes to low gut microbial diversity. They also reported that this leads to an imbalance between gut microbial species. The exact mechanisms that lead to low gut microbial diversity are unknown, but most likely many of the toxic chemicals in the cigarette might be responsible. Many of the compounds like aldehydes, benzenes, heavy metals, nitrosamines, and polycyclic aromatic hydrocarbons may change the pH of the gut, affect the production of organic acid, and might act as antimicrobial agents for the gut microbiome, and can be metabolized by gut microbiome to produce further toxic substances. These compounds might inhibit some microbes' growth and promote others' development, thus leading to a dysbiosis state [76]. Nicotine, a chemical compound found in cigarettes, damages the gut microbial community by reducing the growth of Firmicutes and Actinobacteria while promoting the growth of Bacteroidetes and Proteobacteria [77].

Environmental Pollutants

Environmental pollutants like pesticides, dyes, and heavy metals have been reported to affect the gut microbial composition, leading to harmful health effects and dysbiosis. For instance, exposure of mice to arsenic, cadmium, and lead heavy metals causes damage to the gut microbial structure and relative abundance by altering ratios of Bacteroidetes and Firmicutes [78]. In response to heavy metals, gut microbes offer physical barriers to the heavy metal absorption and secret enzymes that detoxify heavy metals and convert them to less toxic substances. Probiotics like Bifidobacterium and Lactobacillus, usually present in fermented food products, can detoxify heavy metals, limit their absorption, reduce the expression of metal transporters, and maintain gut barrier integrity [79]. Pesticides like fungicides, insecticides, and herbicides also affect the gut microbiome by promoting some microbes' growth while inhibiting others' growth. Still, sometimes they also show contrasting results [80]. More research should be conducted on the impact of pesticides on the gut microbiome to understand the exact role of these compounds on gut health.

Sleep and Circadian Rhythm

The gut-brain axis not only plays a role in mental health disorders but also contributes to the sleep cycle. The immune system, vagus nerve, serotogenic system, and microbial metabolites are all communicating vehicles between the brain and gut, which regulate the sleep cycle [81]. Recently, studies have reported a rhythm in the gut microbiome and its metabolites that might be controlled by feeding patterns and circadian cues, i.e., light/dark cycles [82, 83]. Host circadian rhythm patterns influence the gut microbiome, and gut microbes produce metabolites to modulate host rhythm [83]. An equilibrium disturbance like traveling has been associated with gut microbiome changes. These changes might be caused by sleep loss and lag, which affect the diurnal rhythms and result in the shift in function and composition of gut microbes [84]. The state of dysbiosis has been observed in individuals having disturbed sleeping cycles. Dietary supplements like vitamins, probiotic intake, attention to feeding habits can potentially improve gut microbiome, sleeping cycle, and circadian rhythm [83]. All these indicate that the sleep cycle and circadian rhythm influence the gut microbiome, which results in various health consequences.

CONCLUSIONS

The gut microbiome plays an essential role in disease prevention and promotes the individual's overall health. This review article focused on the multifaceted impact of lifestyle choices like physical activity, stress, diet, and environmental factors on gut microbial diversity and functionality. The gut microbiome helps fight against chronic disease and improves mental health via the gut-brain axis. The targeted interventions for gut microbiome can significantly enhance health outcomes. Beyond individual health benefits, the societal effects of gut microbiome modulation are profound. A healthy gut can help us eliminate the economic burdens of healthcare systems. Using a plant-based and fiber-containing diet can improve gut health, which can solve many health problems worldwide, especially in underdeveloped areas. The fiber-rich diet, physical activities and sustainable practices

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benefit physical fitness and boost the gut microbiome. Advanced research and emerging technologies provide an opportunity to address many gut issues and quick tests to diagnose gut problems. These technologies can help to provide personalized gut health interventions and enable precise and practical strategies to improve public health.

Authors Contribution

Conceptualization: NM
Methodology: AS, AA, SHAS, HJ, WA, ZN, RM, HRK
Formal analysis: AS, AA, SHAS, HJ, WA, ZN, RM, HRK
Writing, review and editing: NM, IL

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Conflicts of Interest

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REFERENCES

[1] Thursby E and Juge N. Introduction to the Human Gut Microbiota. Biochemical Journal.2017; 474(11): 1823-36. doi:10.1042/bcj20160510.

[2] Aoun A, Darwish F, Hamod N. The Influence of the Gut Microbiome on Obesity in Adults and the Role of Probiotics, Prebiotics, and Synbiotics for Weight Loss. Preventive Nutrition and Food Science.2020; 25(2): 113-23. doi:10.3746/pnf.2020.25.2.113.

[3] Sacks D, Baxter B, Campbell B, Carpenter J, Cognard C, Dippel D, *et al.* Multisociety Consensus Quality Improvement Revised Consensus Statement for Endovascular Therapy of Acute Ischemic Stroke. Journal of Vascular and Interventional Radiology. 2018; 29(4): 441-53. doi: 10.1016/j.jvir.2017.11.026.

[4] Valdes AM, Walter J, Segal E, Spector TD. Role of the Gut Microbiota in Nutrition and Health. BMJ (British Medical Journal). 2018; 361: k2179. doi:10 .113 6/ bmj .k 2179.

[5] Org E, Blum Y, Kasela S, Mehrabian M, Kuusisto J, Kangas AJ, *et al.* Relationships Between Gut Microbiota, Plasma Metabolites, and Metabolic Syndrome Traits in the METSIM Cohort.Genome Biology. 2017; 18: 70. doi:10.1186/s13059-017-1194-2.

[6] Liaqat I, Muhammad N, Mubin M, Arshad N, Iftikhar T, Sajjad S, *et al.* Antibacterial and Larvicidal Activity of Ethyl Acetate Extract of Actinomycetes from Soil Samples. Pakistan Journal of Zoology. 2023; 55(6): 2065-74. doi: 10.17582/journal.pjz/20200526130518.

[7] Belkaid Y, Hand TW. Role of the Microbiota in Immunity and Inflammation. Cell.2014; 157(1): 121-41. doi:10.1016/j.cell.2014.03.011.

[8] Noverr MC, Huffnagle GB. Does the Microbiota Regulate Immune Responses Outside the Gut? Trends in Microbiology.2004; 12(12): 562-8. doi: 10.10 16/j.tim.2004.10.008.

[9] David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, *et al.* Diet Rapidly and Reproducibly Alters the Human Gut Microbiome. Nature.2014; 505(7484): 559-63. doi:10.1 038/ nature 12820.

[10] Lee S, Sung J, Lee J, Ko G. Comparison of the Gut Microbiotas of Healthy Adult Twins Living in South Korea and the United States.Applied and Environmental Microbiology.2011;77(20):7433-7. doi:10.1128/AEM.05490-11.

[11] Deschasaux M, Bouter KE, Prodan A, Levin E, Groen AK, Herrema H, *et al.* Depicting the Composition of Gut Microbiota in a Population with Varied Ethnic Origins but Shared Geography. Nature Medicine. 2018; 24(10): 1526-31. doi:10.1038/s41591-018-0160-1.

[12] Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, *et al.* Environment Dominates Over Host Genetics in Shaping Human Gut Microbiota. Nature.2018;555(7695):210-5.doi:10.1038/nature2 5973.

[13] Vangay P, Johnson AJ, Ward TL, Al-Ghalith GA, Shields-Cutler RR, Hillmann BM, *et al.* US Immigration Westernizes the Human Gut Microbiome.Cell.2018; 175(4): 962-72. e10. doi: 10.1016/j.cell.2018.10.029.

[14] Mulasi-Pokhriyal U, Smith C, Franzen-Castle L. Investigating Dietary Acculturation and Intake Among US-Born and Thailand/Laos-Born Hmong American Children Aged 9-18 Years. Public Health Nutrition.2012;15(1):176-85.doi:10.1017/S136898001 1001649.

[15] Kates AE, Jarrett O, Skarlupka JH, Sethi A, Duster M, Watson L, *et al.* Household Pet Ownership and the Microbial Diversity of the Human Gut Microbiota. Frontiers in Cellular and Infection Microbiology. 2020; 10: 73. doi:10.3389/fcimb.2020.00073.

[16] Tun HM, Konya T, Takaro TK, Brook JR, Chari R, Field CJ, *et al.* Exposure to Household Furry Pets Influences the Gut Microbiota of Infants at 3-4 Months Following Various Birth Scenarios. Microbiome.2017; 5(1): 40. doi:10.1186/s40168-017-02 54-x.

[17] Donati Zeppa S, Agostini D, Gervasi M, Annibalini G, Amatori S, Ferrini F, *et al.* Mutual Interactions Among Exercise, Sport Supplements, and Microbiota. Nutrients. 2020; 12(1): 17. doi:10.3390/nu12010017.

[18] Hughes RL and Holscher HD. Fueling Gut Microbes: A Review of the Interaction Between Diet, Exercise, and the Gut Microbiota in Athletes. Advances in

DOI: https://doi.org/10.54393/fbt.v5i1.155

Nutrition.2021;12(6):2190-215.doi:10.1093/advances/nmab077.

[19] Keohane DM, Woods T, O'Connor P, Underwood S, Cronin O, Whiston R, *et al.* Four Men in a Boat: Ultra-Endurance Exercise Alters the Gut Microbiome. Journal of Science and Medicine in Sport.2019; 22(9): 1059-64. doi: 10.1016/j.jsams.2019.04.004.

[20] Scheiman J, Luber JM, Chavkin TA, MacDonald T, Tung A, Pham L-D, *et al.* Meta-Omics Analysis of Elite Athletes Identifies a Performance-Enhancing Microbe That Functions via Lactate Metabolism. Nature Medicine.2019; 25(7): 1104-9. doi:10.103 8/s41591-019-0485-4.

[21] Morita H, Kano C, Ishii C, Kagata N, Ishikawa T, Hirayama A, *et al.* Bacteroides uniformis and Its Preferred Substrate, Cyclodextrin, Enhance Endurance Exercise Performance in Mice and Human Males. Science Advances.2023;9(4):eadd2120.doi :10.1126/sciadv. add2120.

[22] Marttinen M, Ala-Jaakkola R, Laitila A, Lehtinen MJ. Gut Microbiota, Probiotics and Physical Performance in Athletes and Physically Active Individuals. Nutrients.2020;12(10):2936.doi:10.3390/nu12102936.

[23] Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The Effect of Diet on the Human Gut Microbiome: A Metagenomic Analysis in Humanized Gnotobiotic Mice. Science Translational Medicine. 2009; 1(6): 6ra14. doi:10.1126/scitranslmed.3000322.

[24] Wu GD, Chen J, Hoffmann C, Bittinger K, Chen Y-Y, Keilbaugh SA, *et al.* Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes. Science. 2011; 334(6052): 105-8. doi:10.1126/science.1208344.

[25] Ma C-L, Ma X-T, Wang J-J, Liu H, Chen Y-F, Yang Y. Physical Exercise Induces Hippocampal Neurogenesis and Prevents Cognitive Decline. Behavioural Brain Research.2017;317:332-9.doi:10.1016/j.bbr.2016.09.067.

[26] Dao MC, Everard A, Aron-Wisnewsky J, Sokolovska N, Prifti E, Verger EO, *et al.* Akkermansia muciniphila and Improved Metabolic Health During a Dietary Intervention in Obesity: Relationship with Gut Microbiome Richness and Ecology. Gut.2016; 65(3): 426-36. doi:10.1136/gutjnl-2014-308778.

[27] Lukovac S, Belzer C, Pellis L, Keijser BJ, de Vos WM, Montijn RC, *et al.* Differential Modulation by Akkermansia muciniphila and Faecalibacterium prausnitzii of Host Peripheral Lipid Metabolism and Histone Acetylation in Mouse Gut Organoids. mBio. 2014; 5(4): e01438-14. doi:10.1128/mBio.01438-14.

[28] Ma D, Wang AC, Parikh I, Green SJ, Hoffman JD, Chlipala G, *et al.* Ketogenic Diet Enhances Neurovascular Function with Altered Gut Microbiome in Young Healthy Mice. Scientific Reports.2018; 8(1): 6670. doi:10.1038/s41598-018-25190-5.

[29] Olson CA, Vuong HE, Yano JM, Liang QY, Nusbaum DJ, Hsiao EY. The Gut Microbiota Mediates the Anti-Seizure Effects of the Ketogenic Diet.Cell.2018; 173(7): 1728-41. e13. doi: 10.1016/j.cell.2018.04.027.

[30] Swidsinski A, Dörffel Y, Loening-Baucke V, Gille C, Göktas Ö, Reißhauer A, *et al.* Reduced Mass and Diversity of the Colonic Microbiome in Patients with Multiple Sclerosis and Their Improvement with Ketogenic Diet. Frontiers in Microbiology.2017;8:1141. doi:10.3389/fmicb.2017.01141.

[31] Shimizu H, Tosaki T, Kanai Y, Hishikawa Y, Ohta K, Seino S. Insulin-Induced Apoptosis and Involvement of the Forkhead Transcription Factor FKHR. Diabetes.1999;48(2):407-13.doi:10.2337/diabetes.48.2.407.

[32] Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, *et al.* Akt Promotes Cell Survival by Phosphorylating and Inhibiting a Forkhead Transcription Factor. Cell. 1999;96(6):857-68.doi:10.1016/s0092-8674(00)80595-4.

[33] van der Horst A, Burgering BM. Stressing the Role of FoxO Proteins in Lifespan and Disease. Nature Reviews Molecular Cell Biology.2007; 8(6): 440-50. doi:10.1038/nrm2190.

[34] Gross DN, van den Heuvel AP, Birnbaum MJ. The Role of FoxO in the Regulation of Metabolism. Oncogene. 2008; 27(16): 2320-36. doi:10.1038/sj.onc.1210950.

[35] Eijkelenboom A, Burgering BM. FOXOs: Signalling Integrators for Homeostasis Maintenance. Nature Reviews Molecular Cell Biology.2013; 14(2): 83-97. doi :10.1038/nrm3507.

[36] Webb AE, Brunet A. FOXO Transcription Factors: Key Regulators of Cellular Quality Control. Trends in Biochemical Sciences.2014;39(4):159-69.doi:10.1016/j.tibs.2014.02.003.

[37] Dansen TB, Smits LM, van Triest MH, de Keizer PL, Vries-Smits AM, Burgers PP, *et al.* Redox-Sensitive Cysteine Residues in the Forkhead Transcription Factor FoxO4 Regulate PKB-Mediated Phosphorylation. Proceedings of the National Academy of Sciences of the United States of America.2009; 106(27): 9240-5. doi:10.1073/pnas.0900423106.

[38] Hedrick SM. The Acquired Immune System: A Vantage from Beneath. Immunity.2004; 21(6): 607-10. doi:10.1016/j.immuni.2004.10.008.

[39] Ouyang W, Li MO. Foxo: In Command of T Lymphocyte Homeostasis and Tolerance.Trends in Immunology. 2011; 32(1): 26-33. doi:10.1016/j.it.2010.11.002.

DOI: https://doi.org/10.54393/fbt.v5i1.155

[40] Dejean AS, Beisner DR, Ch'en IL, Kerdiles YM, Babour A, Arden KC, *et al.* Transcription Factor Foxo3 Controls the Generation of Memory CD8+ T Cells by Regulating Proliferation in Response to IL-15. Proceedings of the National Academy of Sciences of the United States of America.2009; 106(48): 20121-6. doi:10.1073/pnas.0906357106.

[41] Kerdiles YM, Beisner DR, Tinoco R, Dejean AS, Castrillon DH, DePinho RA, *et al.* Foxo1 Links Homing and Survival of Naive T Cells by Regulating L-Selectin, CCR7 and Interleukin 7 Receptor. Nature Immunology.2009; 10(2): 176-84. doi:10.1038/ ni.1689.

[42] Ouyang W, Liao W, Luo CT, Yin N, Huse M, Kim MV, *et al.* Novel Foxo1-Dependent Transcriptional Programs Control T Regulatory Cell Function.Nature.2012; 491(7425): 554-9. doi:10.1038/nature11581.

[43] Kim MV, Ouyang W, Liao W, Zhang MQ, Li MO. The Transcription Factor Foxo1 Controls Central-Memory CD8+ T Cell Responses to Infection. Immunity.2013; 39(2): 286-97. doi:10.1016/j.immuni.2013.07.013.

[44] Scott-Browne JP, Lopez-Moyado IF, Trifari S, Wong V, Chavez L, Rao A, *et al.* Dynamic Changes in Chromatin Accessibility Occur in CD8+ T Cells Responding to Viral Infection.Immunity.2016;45(6):1327-40.doi:10 .1016/j.immuni.2016.10.028.

[45] Seoane J, Le HV, Shen L, Anderson SA, Massagué J. Integration of Smad and Forkhead Pathways in the Control of Neuroepithelial and Glioblastoma Cell Proliferation. Cell. 2004;117(2):211-23.doi:10.1016/s0 092-8674(04)00302-3.

[46] Essaghir A, Dif N, Marbehant CY, Coffer PJ, Demoulin JB. The Transcription of FOXO Genes Is Stimulated by FOXO3 and Repressed by Growth Factors. The Journal of Biological Chemistry.2009;284(16):103 34-42. doi:10.1074/jbc.M807114200.

[47] Iyer AK, Azad N, Wang L, Rojanasakul Y. Role of Reactive Oxygen Species and Nitric Oxide in the Mechanism of Apoptosis Induced by Aflatoxin B1 in HepG2 Cells. Free Radical Biology and Medicine. 2008;44(7):1224-31.doi:10.1016/j.freeradbiomed. 2007.11.018.

[48] Zhang Y, Gan B, Liu D, Paik JH. FoxO Family Members in Cancer. Cancer Biology & Therapy.2011; 12(4): 253-9. doi:10.4161/cbt.12.4.16014.

[49] Manning BD, Toker A. AKT/PKB Signaling: Navigating the Network.Cell.2017;169(3):381-405.doi:10.1016/ j.cell.2017.04.001.

[50] Greer EL, Brunet A. FOXO Transcription Factors at the Interface Between Longevity and Tumor Suppression.Oncogene.2005;24(50):7410-25.doi:10 .1038/sj.onc.1209086.

[51] Arden KC. Multiple Roles of FOXO Transcription Factors in Mammalian Cells Point to Multiple Roles in Cancer. Experimental Gerontology.2006; 41(8): 709-17. doi:10.1016/j.exger.2006.06.040.

[52] Martinez-Garcia V, Ayllon V, Garcia-Olmo DC, Torres-Ruiz R, Garcia-Olmo D, Kremer L, *et al.* The Emerging Role of FOXO Transcription Factors in Pancreatic Beta Cells. Journal of Molecular Endocrinology. 2016; 56(1): R35-50. doi:10.1530/JME-15-0114.

[53] Kenyon C. The Plasticity of Aging: Insights from Long-Lived Mutants. Cell.2005; 120(4): 449-60. doi: 10.1016/j.cell.2005.02.002.

[54] Lin K, Dorman JB, Rodan A, Kenyon C. daf-16: An HNF-3/Forkhead Family Member That Can Function to Double the Lifespan of Caenorhabditis Elegans. Science.1997;278(5341):1319-22.doi:10.1126/science .278.5341.1319.

[55] Hwangbo DS, Gershman B, Tu MP, Palmer M, Tatar M. Drosophila dFOXO Controls Lifespan and Regulates Insulin Signalling in Brain and Fat Body.Nature .2004;429(6991):562-6.doi:10.1038 /nature 02549.

[56] Yamamoto R, Tatar M. Insulin Receptor Substrate Chico Acts with the Transcription Factor FOXO to Extend Lifespan in Drosophila. Mechanisms of Ageing and Development.2011; 132(10): 468-79. doi: 10 .1016/j.mad.2011.10.003.

[57] Alic N, Hoddinott MP, Vinti G, Partridge L. Lifespan Extension by Increased Expression of the Drosophila Homolog of the Mammalian Forkhead Transcription Factor FOXO Is Dependent on Insulin Receptor Substrate chico. Aging Cell.2011; 10(4): 729-32. doi :10.1111/j.1474-9726.2011.00706.x.

[58] Martins R, Lithgow GJ, Link W. Long Live FOXO: Unraveling the Role of FOXO Proteins in Aging and Longevity. Aging Cell. 2016; 15(2): 196-207. doi: 10. 11 11/acel.12427.

[59] Weigel D, Jürgens G, Küttner F, Seifert E, Jäckle H. The Homeotic Gene Fork Head Encodes a Nuclear Protein and Is Expressed in the Terminal Regions of the Drosophila Embryo.Cell.1989; 57(4): 645-58.doi :10.1016/0092-8674(89)90134-2.

[60] Kaestner KH, Knochel W, Martinez DE. Unified Nomenclature for the Winged Helix/Forkhead Transcription Factors. Genes & Development.2000; 14(2): 142-6. doi:10.1101/gad.14.2.142.

[61] Hannenhalli S, Kaestner KH. The Evolution of Fox Genes and Their Role in Development and Disease. Nature Reviews Genetics.2009;10(4): 233-40.doi: 10.1038/nrg2523.

[62] Arden KC. FOXO Animal Models Reveal a Variety of Diverse Roles for FOXO Transcription Factors. Oncogene.2007; 27(16): 2345-50.doi: 10.1038/sj.

DOI: https://doi.org/10.54393/fbt.v5i1.155

onc.1210951.

[63] Mahmud DL, Plesner A, Nielsen MD, Flyvbjerg A, Kristiansen SB, Andersen CL, *et al*. Forkhead Box O Transcription Factors Are Essential Regulators of Proangiogenic Vascular Endothelial Growth Factor-A Signaling in Endothelial Cells and Neovascularization in Mice. Journal of Biological Chemistry.2014; 289(34): 23677-88. doi:10.1074/jbc.M114.570085.

[64] Gan B, Lim C, Chu G, Hua S, Ding Z, Collins M, *et al.* FoxOs Enforce a Progression Checkpoint to Restrict Differentiation of Tumor Cells. Cell.2010; 141(5): 749-60. doi:10.1016/j.cell.2010.03.041.

[65] Nakae J, Kitamura T, Silver DL, Accili D. The Forkhead Transcription Factor Foxo1 (Fkhr) Confers Insulin Sensitivity to Adipocytes. Journal of Clinical Investigation.2001; 108(9): 1359-67. doi:10.11 72/J Ci12852.

[66] Kitamura T, Kahn CR, Accili D. Insulin Receptor Knockout Mice. Annual Review of Physiology .2003;65:313-32.doi:10.1146/annurev.physiol.65.09 2101.142540.

[67] Paik JH, Kollipara R, Chu G, Ji H, Xiao Y, Ding Z, *et al.* FoxOs Are Lineage-Restricted Redundant Tumor Suppressors and Regulate Endothelial Cell Homeostasis. Cell.2007;128(2):309-23.doi: 10.1016/j . cell.2006 .12.029.

[68] Rached MT, Kode A, Xu L, Yoshikawa Y, Paik JH, DePinho RA, *et al*. FoxO Transcription Factors Maintain Osteoblast Differentiation and Protect Against Glucocorticoid-Induced Oxidative Stress. Cell Metabolism.2010;11(2):147-60.doi:10.1016/j.cmet .2010.01.002.

[69] Salih DA, Brunet A. FOXO Transcription Factors in the Maintenance of Cellular Homeostasis During Aging. Current Opinion in Cell Biology.2008;20(2):126-36. doi: 10.1016/j.ceb.2008.02.005.

[70] Gomis RR, Alarcón C, Nadal C, Van Poznak C, Massagué J. C/EBPβ at the Core of the TGFβ Cytostatic Response and Its Evasion in Metastatic Breast Cancer Cells. Cancer Cell. 2006; 10(3): 203-14. doi: 10.1016/j.ccr.2006.08.001.

[71] Singh A, Joyner AL. Sonic Hedgehog Signaling Development of the Nervous System. Development. 2019; 146(12): dev176736. doi:10.1242/dev.176736.

[72] Rhinn M, Dollé P. Retinoic Acid Signalling in Development. Development.2012;139(5):843-58.doi :10.1242/dev.065938.

[73] Zhang Y, Gan B, Liu D, Paik JH. FoxO Family Members in Cancer. Cancer Biology & Therapy. 2011; 12(4): 253-9. doi:10.4161/cbt.12.4.16014.

[74] Daitoku H, Sakamaki J, Fukamizu A. Regulation of FoxO Transcription Factors by Acetylation and Protein-Protein Interactions.Biochimica et Biophysica Acta.2011;1813(11):1954-60.doi10.1016/j .bbamcr.2010.09.002.

[75] Greer EL, Brunet A.FOXO Transcription Factors in Ageing and Cancer.Acta Physiologica.2008;192(1): 19-28. doi:10.1111/j.1748-1716.2007.01785.x.

[76] Huang H, Tindall DJ. Regulation of FoxO Protein Stability via Ubiquitination and Proteasome Degradation. Biochimica et Biophysica Acta.2011; 1813(11): 1961-4. doi:10.1016/j.bbamcr.2010.09.007.

[77] Furuyama T, Nakazawa T, Nakano I, Mori N. Identification of the Differential Distribution Patterns of mRNAs and Consensus Binding Sequences for Mouse DAF-16 Homologs. Biochemical and Biophysical Research Communications.2000;272(2):587-92.doi:10.1006/ bbrc.2000.2792.

[78] Accili D, Arden KC. FoxOs at the Crossroads of Cellular Metabolism,Differentiation, and Transformation. Cell.2004; 117(4): 421-6. doi:10.1016/s0092-8674 (04) 00452-0.

[79] Lee S, Dong HH. FoxO Integration of Insulin Signalling with Glucose and Lipid Metabolism.Journal of Endocrinology.2017;233(2):R67-79.doi:10.153 0/JOE-16-0495.

[80] Obsil T, Obsilova V. Structural Basis for DNA Recognition by FOXO Proteins. Biochimica et Biophysica Acta.2011; 1813(11): 1946-53. doi: 10.1016/j. bbamcr.2010.12.029.

[81] Li H, Jogl G. Structural and Functional Insight into Insulin Regulation of FOXO Transcription Factors. Current Biology.2009; 19(24): 2156-62. doi: 10.1016/j. cub.2009.10.080.

[82] Maiese K, Chong ZZ, Shang YC, Wang S. Targeting Disease Through Novel Pathways of Apoptosis and Autophagy. Expert Opinion on Therapeutic Targets. 2012; 16(2): 120-8. doi:10.1517/14728222.2011.643299.

[83] Huang H, Tindall DJ. Regulation of FOXO Protein Stability via Ubiquitination and Proteasome Degradation. Biochimica et Biophysica Acta.2011; 1813(11): 1961-4. doi: 10.1016/j.bbamcr.2010.09.007.

[84] Accili D, Arden KC. FoxOs at the Crossroads of Cellular Metabolism.Cell. 2004; 117(4): 421-6. doi:10.1016 /s0 0 92-8674(04)00452-0.

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Review Article

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Genetics of Cotton Fiber Color: Unveiling the Mechanisms and Exploring Gene-Based Approaches for Color Expression

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ABSTRACT

Cotton is a vital natural fiber, and its color significantly impacts its commercial value and marketability. Traditional cotton cultivation focuses on white fibers, but naturally colored cotton (NCC) is gaining attention due to its potential for sustainable and environmentally friendly textile production. This review explores the genetics and molecular mechanisms underlying cotton fiber color, focusing primarily on anthocyanins and gossypol pigments. We discuss the role of key genes involved in pigment biosynthesis and regulatory pathways. Additionally, we analyze the potential of gene-based color expression approaches, including traditional breeding and genetic engineering. We highlight the advantages and limitations of each approach and discuss future research directions for developing NCC varieties with improved fiber quality, yield, and color diversity.

INTRODUCTION

Cotton is one of the most important natural fibers in the world, and its color is a critical quality trait that influences its commercial value and marketability. Traditionally, cotton has been cultivated for its white fibers, but there is growing interest in developing colored cotton varieties for use in various textile applications. Gene-based color expression offers a promising approach to achieving this goal. Throughout the world, in the 1990s, polyester and other man-made fibers faced strong competition. This competition increased further in the early 2000s [1]. Cotton fibers or cotton lint are mostly used in fabrics today [2]. There is a unique way of fiber formation during cotton plant growth. Fiber is created when Smaller cells on the surface of the cotton seed stretch outward. Inner structure breakdown during fiber maturation, and the cell walls shrink, leaving behind a flat, twisted shape [3]. Cotton is a key material in the textile industry. Different dyes are added during production to color fabrics. However, the use of these dyes is causing pollution, which is harmful to human beings [4]. Naturally colored cotton has its color built into the fibers [5, 6]. Scientists are creating improved versions by breeding white cotton with colorful cotton types to produce different colors in cotton fibers naturally. These plants have better color retention, longer fibers, and stronger material than the original colorful cotton. These days, naturally colored cotton is becoming popular

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because of the demand for organic, eco-friendly products. Its biggest advantage of naturally colored cotton is that there is no need to add dyes, which is the most polluting step in fabric production, so it is cleaner and safer for both people and the environment [7, 8]. There are many benefits of naturally colored cotton; it serves as a crucial raw material for eco-friendly textiles [9]. It may cut down on chemical residue, pollution, and processing costs. it may also be less flammable and have a higher UV protection rating than regular white cotton [10, 11]. This colored cotton has been grown for many years, but it hasn't improved as fast as regular white cotton. The biggest reasons are that colored cotton plants produce fewer fibers than white cotton, and their colors look faded. These flaws are making them less practical for large-scale farming [9]. Scientists want to fix this by making the fibers stronger, longer and finer [12]. When the cotton bowl opens, the color in the cotton fibers appears. These natural colors come from pigments which are mixed with the cotton's cellulose. This color trait is passed to the next generation through genes. However, traditional breeding struggles to improve both the cotton's quality (like fiber strength) and its yield at the same time; if one gets better, the other might get worse. These days, scientists are using genetic approaches to create these cotton varieties.

Genetics of Cotton Fiber Color

The color of cotton fibers is determined by the accumulation of pigments, primarily anthocyanins and gossypol. These pigments are synthesized through a complex network of biochemical pathways that are regulated by genes. Several genes have been identified that play important roles in cotton fiber color. For example, the MYB113 gene is a transcription factor that regulates the expression of genes involved in anthocyanin biosynthesis. Targeted Mutations in the MYB113 gene can lead to the production of brown or green cotton fibers [13]. Natural compounds called proanthocyanidins (PAs) and related substances are responsible for the brown color in cotton fibers. Early research found that these flavonoid compounds are responsible for the brown shades [14]. Brown shades, for example, come from compounds made through the plant's natural processes, similar to how flowers or fruits get their colors [15-17]. Pathways by which colored cotton is produced are shown in Figure 1.

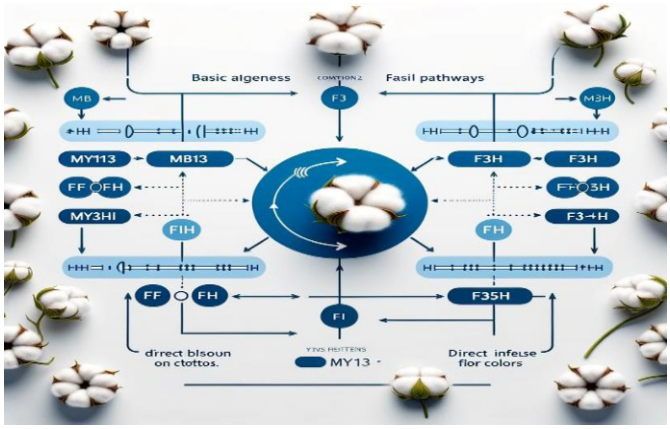

Figure 1: Genetic pathways in Cotton Fiber Coloration

Cotton gets natural brown or green shades from pigments in the fibers. There is a chemical pathway for the production of pigments. Proanthocyanins pigments are responsible for the brown color, and anthocyanins pigments are responsible for other colors. Gene directly controls the formation of these pigments. MYB, bHLH, and WD40 are important genes that switch to turn on the production of color-related compounds. Different enzymes such as F3H, F3'H, and F3'5'H add chemical groups to naringenin and create different forms of modified naringenin, which results in the production of pigments like pelargonidin (involved in red color formation), cyanidin (involved in magenta color formation), and delphinidin (involved in blue color formation) [18]. In the anthocyanin pathway, one of the important steps of pigments like pelargonidin, cyanidin, and delphinidin formation is the addition of a hydroxyl group to naringenin to create dihydrokaempferol, which gives colors to flowers, and is added by flavanone 3-hydroxylase enzyme., F3H's role is most important in color formation, while other enzymes like F3'5'H modify pigments later [19]. These enzymes play a role in the formation of anthocyanins, which are involved in red, blue, and purple shades in plants [20]. When the F3H enzyme is less active, it changes the color of cotton petals. F3'H and F3'5'H enzymes are involved in red and blue pigments and use the same starting materials in a color production process. Different colors are produced by the rise and fall of the level of these enzymes. For example, Brown cotton fibers have more active GhF3H genes linked to this color process as compared to white fibers [21]. In contrast to white cotton fiber, there is a greater level of GhCHS, GhC4H, GhF3`H, and GhF3`5`H enzymes in brown cotton fiber expression during flavonoid synthesis. It is studied the expression of the GhCHS, GhANR, and GhLAR genes affects the concentration of anthocyanidin and fiber color [22]. Finding the key genes, their roles in pigment synthesis, and the specific pigments they influence in cotton fiber coloration (Table 1).

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Table 1: Summarizing Key Genes Involved in Cotton Fiber Coloration

Gene	Role in Pigment Synthesis	Influenced Pigments	References
MYB113	Regulates anthocyanin biosynthesis	Anthocyanins	[23]
F3H	Catalyzes hydroxylation in the anthocyanin pathway	Dihydroflavonols, leading to pelargonidin, cyanidin, delphinidin	[24]
F3`H	Involved in flavonoid B-ring hydroxylation for cyanidin synthesis	Cyanidins (red hues)	[20]
F3`5`H	Facilitates hydroxylation for delphinidin synthesis	Delphinidins (blue hues)	[25]
GhMYB113	post-anthesis (DPA) fibers with tissue-specific promoter	brown mature fibers	[26]
GhPIF4	Regulate the flowering time of cotton	–	[27]
GhCHS	Colored fiber formation	–	[22]
GhANR, and GhLAR	affect anthocyanidin content and fiber color	–	[22]
GhF3H	anthocyanin biosynthesis and fiber color formation	–	[15]
GhDFR1	brown color formation of cotton fiber	–	[15]
GhANS	strongly associated with brown color	–	[29]

Traditional Breeding

This approach involves crossing cotton varieties with different fiber colors to introduce desirable color traits into new varieties. So far, varieties of colored cotton have been developed mostly by selection and recurrent crossing approaches from the germplasm [30]. Selection, the discrimination among variability, is the oldest method of plant improvement [31]. However, this approach is time-consuming and can be challenging due to the complex inheritance patterns of cotton fiber color. The use of statistical genetics and biometrical techniques such as principal component analysis (PCA) and correlation analysis can help in the selection and evaluation of breeding material, optimizing the breeding process, and identifying parental pairs for hybridization [32, 33]. Complex hybridization methods, such as double, complex, and double crossing, have been studied in cotton selection research and have shown promising results in collecting valuable traits from different genetic origins in a single genotype [34].

Genetic Engineering

This approach involves directly introducing genes into cotton plants that control the production of pigments. The science of genetics revolutionized the selection process, taking the guesswork out of it, facilitating it and making it more efficient [35]. Modern plant breeders follow standard methods to create variability, discriminate among the variability, and develop cultivars for release to farmers [36]. This approach can be more efficient than traditional breeding methods, but many ethical concerns and regulatory hurdles need to be addressed (Table 2).

Table 2: Traditional Breeding and Genetic Engineering

Aspect	Traditional Breeding	Genetic Engineering
Time Required	Time-consuming due to multiple breeding cycles	Faster, direct introduction of desired genes
Efficiency	Variable, depends on genetic variability and inheritance patterns	Highly efficient, precise targeting of traits
Challenges	Complex inheritance patterns, lower yield of colored fibers	Regulatory approvals, public acceptance, and possible unintended effects
Potential Outcomes	New varieties with desired color traits and possible yield improvements	Consistent color expression, potential for enhanced fiber qualities
Ethical and Regulatory Concerns	Generally accepted, no significant ethical concerns	Ethical concerns require regulatory approvals

Current Status and Future Prospects

Remarkable progress has been made in understanding the genetic basis of cotton fiber color throughout the world. Many key genes have been identified that play important roles in pigment synthesis in cotton, and transgenic cotton lines with altered fiber colors have been developed by using advanced genetic engineering techniques. Large-scale colored cotton production would be possible if we could overcome the following challenges. Improving the color Expression: Improvement in stability and color expression is needed as the color of transgenic cotton fibers can be variable and sensitive to environmental conditions. Ensuring the safety of colored cotton: Colored cotton fibers must be as safe as white cotton fibers and must not cause any health or environmental risks. Addressing regulatory hurdles and ethical concerns: Genetically edited cotton cultivars must pass regulatory, safety and gain regulatory approval before they can be adopted on a large scale. Many more colors in cotton can be produced once these issues are resolved, and in this way, consumers will be safe from the negative effects which are caused by artificial dyes. This could also lead to new opportunities for textile manufacturers, and it could reduce the extensive cost on use of using dyes.

CONCLUSIONS

If naturally colored cotton varieties are produced, it may be beneficial for both the textile industry and the environment. By understanding the genetics of cotton fiber colors, their formation and applying advanced breeding and genetic engineering such as CRISPR Cas9 and RNAi techniques, scientists can overcome the limitations of traditional methods and can produce a wider spectrum

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of sustainable colors. Naturally colored cotton has the potential to reduce environmental pollution which are associated with dyeing processes, in this way it may be the eco-friendly solution for the fashion industry. Not only can above mentioned benefits be obtained from them they also have other qualities as well, such as improved flammability resistance and UV protection. Once these issues are resolved and new genetic engineering techniques are used, gene-based color expression can transform the cotton industry so in the future, textiles will be eco-friendly.

Authors Contribution

Conceptualization: MNA
Methodology: MNA, MAQ, MR, RB, AA, NS
Formal analysis: RB, AA
Writing review and editing: MAQ, MR, NS

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

Conflicts of Interest

All the authors declare no conflict of interest.

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REFERENCES

[1] Barros MA, Silva CR, Lima LM, Farias FJ, Ramos GA, Santos RC. A Review On Evolution of Cotton in Brazil: GM, White, and Colored Cultivars. Journal of Natural Fibers. 2022 Jan; 19(1): 209-21. doi: 10.1080/15440478.2020.1738306.

[2] Buainain AM, Alves E, Silveira JM, Navarro Z. The Rural World in 21st Century Brazil. The Formation of a New Agrarian and Agricultural Pattern. Embrapa. 2014.

[3] Lee JA and Fang DD. Cotton as a World Crop: Origin, History, and Current Status. Cotton. 2015 Aug; 57: 1-23. doi: 10.2134/agronmonogr57.2013.0019.

[4] Weisburger JH. Comments on the History and Importance of Aromatic and Heterocyclic Amines in Public Health. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2002 Sep; 506: 9-20. doi: 10.1016/S0027-5107(02)00147-1.

[5] Nagarajan S, Purushothaman Y, Selvavinayagam M, Govindharaj P, Musthafa A. Studies on Colored Cotton: Biochemical and Genetic Aspects. In Cotton. 2022 Oct. doi: 10.5772/intechopen.104898.

[6] Yan Q, Wang Y, Li Q, Zhang Z, Ding H, Zhang Y *et al.* Up-regulation of Gh TT 2- 3A in Cotton Fibres During Secondary Wall Thickening Results in Brown Fibers with Improved Quality. Plant Biotechnology Journal. 2018 Oct; 16(10): 1735-47. doi: 10.1111/pbi.12910.

[7] Rathinamoorthy R and Parthiban M. Colored Cotton: Novel Eco-Friendly Textile Material for the Future. Handbook of Ecomaterials. 2019; 3: 1499-519. doi: 10.1007/978-3-319-68255-6_91.

[8] Günaydin GK, Avinc O, Palamutcu S, Yavas A, Soydan AS. Naturally Colored Organic Cotton and Naturally Colored Cotton Fiber Production. Organic Cotton: Is it a Sustainable Solution? 2019: 81-99. doi: 10.1007/978-981-10-8782-0_4.

[9] Lv YP, Zhao G, Xie YF, Owusu AG, Wu Y, Gao JS. Transcriptome and Metabolome Profiling Unveil Pigment Formation Variations in Brown Cotton Lines (Gossypium Hirsutum L.). International Journal of Molecular Sciences. 2023 Mar; 24(6): 5249. doi: 10.3390/ijms24065249,

[10] Crews PC and Hustvedt G. The Ultraviolet Protection Factor of Naturally-Pigmented Cotton. 2005.

[11] Kibria G, Repon MR, Hossain MF, Islam T, Jalil MA, Aljabri MD *et al.* UV-Blocking Cotton Fabric Design for Comfortable Summer Wears: Factors, Durability and Nanomaterials. Cellulose. 2022 Sep; 29(14): 7555-85. doi: 10.1007/s10570-022-04710-7.

[12] Naoumkina M, Thyssen GN, Fang DD, Jenkins JN, McCarty JC, Florane CB. Genetic and Transcriptomic Dissection of the Fiber Length Trait from A Cotton (Gossypium Hirsutum L.) MAGIC Population. BMC Genomics. 2019 Dec; 20: 1-4. doi: 10.1186/s12864-019-5427-5.

[13] Peng Z, Gao Q, Luo C, Gong W, Tang S, Zhang X *et al.* Flavonoid Biosynthetic and Starch and Sucrose Metabolic Pathways Are Involved in the Pigmentation of Naturally Brown-Colored Cotton Fibers. Industrial Crops and Products. 2020 Dec; 158: 113045. doi: 10.1016/j.indcrop.2020.113045.

[14] Shi S, Tang R, Hao X, Tang S, Chen W, Jiang C *et al.* Integrative Transcriptomic and Metabolic Analyses Reveal That Flavonoid Biosynthesis Is the Key Pathway Regulating Pigment Deposition in Naturally Brown Cotton Fibers. Plants. 2024 Jul; 13(15): 2028. doi: 10.3390/plants13152028.

[15] Zheng H, Duan B, Yuan B, Chen Z, Yu D, Ke L *et al.* Flavanone and Flavonoid Hydroxylase Genes Regulate Fiber Color Formation in Naturally Colored Cotton. The Crop Journal. 2023 Jun; 11(3): 766-73. doi: 10.1016/j.cj.2022.10.004.

[16] Mei J, Niu Q, Xu K, Huang Y, Bai S, Zhu J *et al.* GhmiR858 Inhibits the Accumulation of Proanthocyanidins by Targeting Ghtt2l in Cotton (Gossypium Hirsutum). Journal of Agricultural and Food Chemistry. 2023 Oct; 71(41): 15341-51. doi: 10.1021/acs.jafc.3c03884.

DOI: https://doi.org/10.54393/fbt.v5i1.166

[17] Chen W, Zhang M, Zhang G, Li P, Ma F. Differential Regulation of Anthocyanin Synthesis in Apple Peel Under Different Sunlight Intensities. International Journal of Molecular Sciences. 2019 Dec; 20(23): 6060. doi: 10.3390/ijms20236060.

[18] Wang R, Ren C, Dong S, Chen C, Xian B, Wu Q *et al.* Integrated Metabolomics and Transcriptome Analysis of Flavonoid Biosynthesis in Safflower (Carthamus Tinctorius L.) with Different Colors. Frontiers in Plant Science. 2021 Jul; 12: 712038. doi: 10.3389/fpls.2021.712038.

[19] Yang H, An W, Gu Y, Peng J, Jiang Y, Li J *et al.* Integrative Metabolomic and Transcriptomic Analysis Reveals the Mechanism of Specific Color Formation in Phoebe Zhennan Heartwood. International Journal of Molecular Sciences. 2022 Nov; 23(21): 13569. doi: 10.3390/ijms232113569.

[20] Liang CY, Rengasamy KP, Huang LM, Hsu CC, Jeng MF, Chen WH et al. Assessment of Violet-Blue Color Formation in Phalaenopsis Orchids. BioMed Central Plant Biology. 2020 Dec; 20: 1-6. doi: 10.1186/s12870-020-02402-7.

[21] Khan AQ, Li Z, Ahmed MM, Wang P, Zhang X, Tu L. Eriodictyol Can Modulate Cellular Auxin Gradients to Efficiently Promote in Vitro Cotton Fibre Development. BioMed Central Plant Biology. 2019 Dec; 19: 1-5. doi: 10.1186/s12870-019-2054-x.

[22] Gao J, Shen LI, Yuan J, Zheng H, Su Q, Yang W *et al.* Functional Analysis of GhCHS, GhANR and GhLAR in Colored Fiber Formation of Gossypium Hirsutum L. BioMed Central Plant Biology. 2019 Dec; 19: 1-8. doi: 10.1186/s12870-019-2065-7.

[23] Zhou L, He Y, Li J, Liu Y, Chen H. CBFs Function in Anthocyanin Biosynthesis by Interacting with MYB113 in Eggplant (Solanum Melongena L.). Plant and Cell Physiology. 2020 Feb; 61(2): 416-26. doi: 10.1093/pcp/pcz209.

[24] Dai M, Kang X, Wang Y, Huang S, Guo Y, Wang R *et al.* Functional Characterization of Flavanone 3-Hydroxylase (F3H) and Its Role in Anthocyanin and Flavonoid Biosynthesis in Mulberry. Molecules. 2022 May; 27(10): 3341. doi: 10.3390/molecules27103341.

[25] Qi Y, Lou Q, Quan Y, Liu Y, Wang Y. Flower-Specific Expression of the Phalaenopsis Flavonoid 3`, 5`-Hydoxylase Modifies Flower Color Pigmentation in Petunia and Lilium. Plant Cell, Tissue and Organ Culture. 2013 Nov; 115: 263-73. doi: 10.1007/s11240-013-0359-2.

[26] Wang N, Zhang B, Yao T, Shen C, Wen T, Zhang R et al. Re Enhances Anthocyanin and Proanthocyanidin Accumulation to Produce Red Foliated Cotton and Brown Fiber. Plant Physiology. 2022 Jul; 189(3): 1466-81. doi: 10.1093/plphys/kiac118.

[27] Liu LY, Jia MZ, Wang SN, Han S, Jiang J. Identification and Characterization of Cotton Phytochrome-Interacting Factors in Temperature-Dependent Flowering. Journal of Experimental Botany. 2023 Jun; 74(12): 3765-80. doi: 10.1093/jxb/erad119.

[28] Zu QL, Qu YY, Ni ZY, Zheng K, Chen Q, Chen QJ. The Chalcone Isomerase Family in Cotton: Whole-Genome Bioinformatic and Expression Analyses of the Gossypium Barbadense L. Response to Fusarium Wilt Infection. Genes. 2019 Dec; 10(12): 1006. doi: 10.3390/genes10121006.

[29] Malik W, Anjum N, Usman Khan M, Abid MA, Ashraf J, Zhang R *et al.* Genomics of Naturally Colored Cotton: A Way Forward to Initiate Precision Breeding. In Cotton Precision Breeding. Cham: Springer International Publishing. 2021 Jun: 173-195. doi: 10.1007/978-3-030-64504-5_8.

[30] Majeed S, Chaudhary MT, Mubarik MS, Rana IA, Shaban M, Tan DK *et al.* Genetics of Biochemical Attributes Regulating Morpho-Physiology of Upland Cotton Under High Temperature Conditions. Journal of Cotton Research. 2024 Jan; 7(1): 3. doi: 10.1186/s42397-023-00164-9.

[31] Younas A, Riaz N, Rashid M, Fiaz S, Tufail A, Noreen Z *et al.* Modification in Conventional Methods and Modern Plant Breeding Techniques to Enhance Genetic Gain for Future Food Security. Crop Biofortification: Biotechnological Approaches for Achieving Nutritional Security Under Changing Climate. 2025 Feb: 377-94. doi: 10.1002/9781394273270.ch22.

[32] Çakmak F, Çinar VM, Balci Ş, Ünay A. Yield and Fiber Quality Balance in Upland Cotton (Gossypium Hirsutum L.) Breeding. Bangladesh Journal of Botany. 2023 Jun; 52(2): 283-90. doi: 10.3329/bjb.v52i2.67025.

[33] Madartov B, Abdiev F, Mavlonova N, Shukurov K. Using the Dominance Coefficient in Assessing Cotton Families. In E3S Web of Conferences. 2023; 381(01014). doi: 10.1051/e3sconf/202338101014.

[34] Kholmurodova G, Barotova A, Namazov S, Yuldasheva R, Jumashev M. Creation of Selected Items with High Fiber Yield and Length Based On Cotton Composite Hybrids. In E3S Web of Conferences. 2023; 371: 01039. EDP Sciences. doi: 10.1051/e3sconf/202337101039.

[35] Razzaq A, Saleem F, Kanwal M, Mustafa G, Yousaf S, Imran Arshad HM *et al.* Modern Trends in Plant Genome Editing: An Inclusive Review of the CRISPR/Cas9 Toolbox. International Journal of Molecular Sciences. 2019 Aug; 20(16): 4045. doi:

DOI: https://doi.org/10.54393/fbt.v5i1.166

10.3390/ijms20164045.

[36] Raza A, Mubarik MS, Sharif R, Habib M, Jabeen W, Zhang C *et al.* Developing Drought-Smart, Ready-to-Grow Future Crops. The Plant Genome. 2023 Mar; 16(1): e20279. doi: 10.1002/tpg2.20279.

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Original Article

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Investigating the Usage of Random Forest Method on Next-Generation Sequencing Data to Predict MSH2 and MSH6 Associated Mutations

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ABSTRACT

Colorectal cancer (CRC) is one of the most prevalent cancers and the second leading cause of cancer-related deaths globally. Germline mutations in CRC are associated with the MSH2 and MSH6 genes, which prevent infection for the DNA MMR pathway. **Objectives:** To enhance CRC-related prediction of mutations using the Random Forest algorithm on NGS data of MSH2 and MSH6 gene. Given the tremendous amount of genetic information obtained from NGS, a model for the early diagnosis and individual treatment of CRC is necessary. **Methods:** The raw sequencing data of MSH2 and MSH6 genes were meticulously downloaded from the NCBI's SRA database. The three datasets of 1000, 2000, and 3000 sequences were carefully analyzed to assess genomic features, including ORF count, nucleotide content, AT/CG ratio, G-quadruplex signal, and mutation rates, to understand their correlation with colorectal cancer. The data were then divided into a training set (80%) and a test set (20%) for model training and testing in Python, employing the Biopython package for mutation analysis and feature extraction. The model was rigorously evaluated using accuracy, confusion matrix, and classification report, instilling confidence in the research process for accurate CRC mutation prediction. **Results:** The Random Forest model yielded high accuracy of 96.25%, 98.37%, and 99. 5% for the datasets of 1000, 2000, and 3000 sequences, respectively. The confusion matrix showed that the model was very accurate in identifying true negatives, especially in the large data set. **Conclusions:** The study successfully applied the Random Forest algorithm to predict CRC using NGS data of MSH2 and MSH6 gene mutations. The model's potential to revolutionize CRC research is both exciting and optimistic.

INTRODUCTION

Colorectal cancer (also known as CRC) is one of the most widespread cancers globally and has incidence rates that are on the rise in both developed and developing nations. Colorectal cancer is the second most common cause of death, and a rapid increase has been observed from the year 2000 to 2019 [1]. Colorectal cancer rates in several studies from different regions of Pakistan varied between 4 and 6. 8% [2]. It is believed that genetic mutations, family history, and inherited syndromes all contribute to its hereditary nature. However, lifestyle factors such as food high in red and processed meats, laziness, obesity, excessive alcohol consumption, and smoking increase the risk of developing this condition [3]. Complications associated with colorectal cancer include bowel obstruction, haemorrhage, metastasis to other organs, such as the liver and lungs, and systemic manifestations, such as anaemia and fatigue [4]. MSH6 and MSH2 genes are necessary for the DNA mismatch repair (MMR) system. This system maintains genomic stability by identifying and repairing base mismatches during DNA replication [5]. Microsatellite instability, or MSI, is distinguished by the accumulation of insertion or deletion errors at microsatellite DNA sequences. These errors are brought on by mutations in the genes that control microsatellites.

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Due to the genome's instability, an environment conducive to neoplastic transformation is created [6]. Hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome, is more likely to occur in people who have inherited mutations in either the MSH6 or MSH2 gene [7]. Next-generation sequencing (NGS) development was a key find in molecular biology, allowing knowledgeable scientists to understand broader aspects of the human genome quickly. This high-speed method can produce clear and in-depth images, decoding millions of DNA sequences simultaneously [8]. Machine learning and AI are taking on increased importance in the ever-changing healthcare industry, which is possible due to their flexibility in function [9]. Significantly, the prospects of ML with next-generation sequencing (NGS) for cancer prediction have advanced their way into human consciousness [10]. Random Forest (RF) is an ensemble learning approach that ensures dependable predictions from big genetic datasets by providing high accuracy and well-handling overfitting [11]. Its principal function is to build several trees during the training period. For classification problems, it uses the mode of the classes, and for regression problems, it uses mean perdition [12]. Applying the Random Forest model to sequence data from next-generation sequencing (NGS) on colorectal cancer may significantly raise prediction accuracy. The method advances the development of personalized treatment procedures by using NGS's massive genetic data to make diagnoses that are much more determined [13].

This research aimed to predict CRC related mutations prediction based on different DNA features in MSH2 and MSH6 genes.

METHODS

The current study focused on the Next Generation Sequences of Homo Sapiens based on the MSH2 (Accession Number: SRR25243226 and SRR25243227) and MSH6 genes (Accession Number: SRR1518357) from the NGS reads from NCBI's SRA repository in FASTA format [14]. These sequences were grouped into three sets with thousand, two thousand, and three thousand sequences, respectively. Reference sequences for MSH2 (Accession Number: NG_007110.2) and MSH6 (Accession Number: NG_007111.1) normal genes were retrieved from the NCBI database that was used to determine the mutation rates in NGS reads. Features including ORF count, average nucleotide (Cytosine, Guanine, Thymine, and Adenine), AT/CG ratio and its content, presence of G-quadruplex, and mutation rates were extracted from the collected sequences in this phase by comparing NGS reads with normal sequences.These eleven features were then stored in a .csv file. Matrices selection was applied to input the construction of the classification process. All the analyses were done on VS Code, but Google Colabs was used to compute the mutation rate. The total mutation rate was calculated per NGS read through Python library-assisted comparisons between NGS data features and reference sequences. The methodology of feature extraction was based on previously published work by Kurian and Jyothi. A random forest classifier was used to evaluate the dataset's classification performance. Initially, the necessary libraries, such as the Random Forest Classifier and train_test_split, were imported from Scikit-learn for selection and functions for accuracy. The confusion matrix and classification report from Scikit-learn were exported along with pandas for data operation. The dataset was loaded and split into features and target variables, with irrelevant columns removed. Python and biopython package were used for feature extraction and computing the mutation rate in this study. The data were then divided into training and testing sets with an 80:20 split using train_test_split. A Random Forest model with two estimators was built and fitted to the training set. The test set was used as input, and the model's predictions and accuracy were determined. A confusion matrix and classification report were also generated to analyze the model's results and give a clear view of the model's performance. The data of mutation from the NGS reads related to MSH2 and MSH6 genes were analyzed and correlated with colorectal cancer prediction, employing the Random Forest model to assess the statistical significance of these mutations (Figure 1).

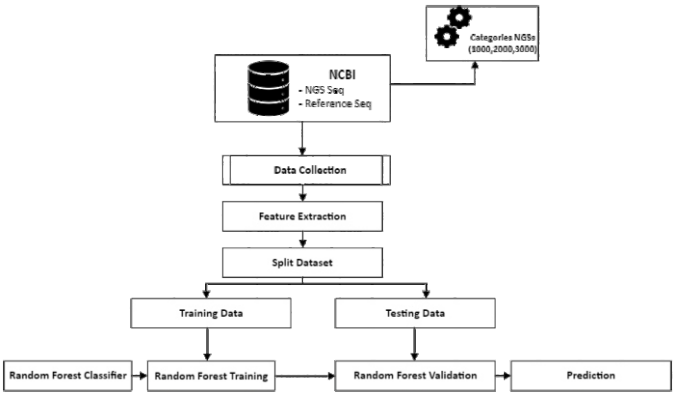

Figure 1: Architectural Diagram of Methodology for CRC Prognosis.

RESULTS

The study adopted the Random Forest (RF) ML algorithm for analysis. Random Forest (RF) is one of the most used techniques of the ensemble learning methodology, offering reliable outcomes from large genetic datasets as it offers high precision and manages overfitting. Thus, the

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presented software tool can help obtain accurate and fast results from next-generation sequencing data analysis. Total datasets were divided into training sets, and testing sets with a ratio of 80:20 for the progression of ML classification. Different tendencies surfaced in this study that compared ML models trained on colorectal cancer NGS reads based on datasets of varying sizes. The same datasets are used for all four models. Confusion matrix is characterized as 2 × 2 matrix which signifies the total number of classes to be classified. The sum of sets for testing for each class was derived by using row summation from the confusion matrix. Figure 2 indicates the confusion matrix results of the Random Forest algorithm. Random Forest (RF) successfully identified genuine negatives with high accuracy, especially in more enormous datasets, but struggled to classify real positives, especially in smaller datasets. The mutation rates in the MSH2 and MSH6 genes were found to be significantly higher in colorectal cancer (CRC)-positive samples compared to normal sequences.

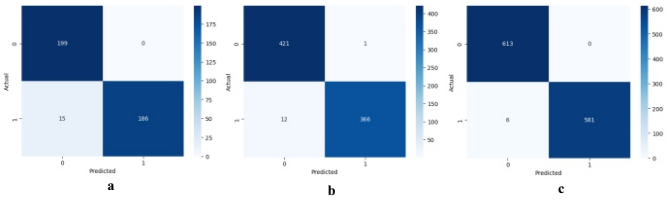

Figure 2: Graphical Explanation of Confusion Matrix generated by VS Code after classification. (a): On the first dataset of 1000 reads, the model correctly classified 199 positive instances and 186 negative instances. The model was misclassified 15 times, predicting negative while it predicted positive, and there were no misclassifications where the model predicted positive while it predicted negative, (b): On the second dataset of 2000 reads, the model appropriately classified 421 positive instances and 366 negative instances. The model was misclassified 12 times, predicting negative while it predicted positive, and there was only 1 misclassifications where the model predicted positive while it predicted negative, and (c): On the third dataset of 3000 reads, the model correctly classified 613 positive instances and 581 negative instances.The model was misclassified 6 times, predicting negative while it predicted positive, and there were no misclassifications where the model predicted positive while it predicted negative.

One way to get the classification accuracy rate is to multiply the total testing data size by 100 and divide the result by the number of adequately indicated classes. Table 1 presents the results of classification accuracy rates of RF. Machine learning models showed clear accuracy patterns when evaluated on varying datasets of NGS reads. With a modest improvement with increasing dataset size, despite dealing with datasets of varied sizes, Random Forest attained excellent accuracy, ranging from 96.25 per cent for the smallest dataset to 99.5 per cent for the biggest.

Table 1: Classification Accuracy Rate

ML Model	Dataset Size		
	1000	2000	3000
Random Forest (RF)	96.25	98.37	99.5

Random Forest performs well on smaller datasets (1000 samples) with average recall, accuracy, and F1-score values close to 0.96. RF performs admirably, and in the most extensive dataset, it achieves practically perfect results. Table 2 indicates the System Generated Classification report of Random Forest. Precision refers to the proportion of accurate positive predictions among all positive predictions. The proportion of true positive predictions among all actual positive is called recall. F1-score is used as the harmonic average of precision and recall, and thus, a balance between precision and recall. The support is the number of the actual occurrences of the class in the dataset. Table 2 shows the accuracy of the Random Forest model in classifying MSH2 and MSH6 gene mutations in CRC for different dataset sizes. The model's performance increases with the increase in the dataset size from 1000 to 3000 sequences, and the precision, recall, and F1-score for both MSH2 and MSH6 are closer to the maximum values. In the largest dataset of 3000 sequence reads, the precision and recall for MSH2 were 0.99 and 1.00, respectively, and MSH6 had a perfect score of 1.00 in all the measures.

Table 2: System Generated Classification Report of Random Forest

Variables	Dataset Size	1000				2000				3000			
Machine Learning Model	Class Label	Precision Value	Recall Value	F1- Score Value	Support Value	Precision Value	Recall Value	F1- Score Value	Support Value	Precision Value	Recall Value	F1- Score Value	Support Value
Random Forest (RF)	MSH2	0.93	1.00	0.96	199	0.97	1.00	0.98	422	0.99	1.00	1.00	613
	MSH6	1.00	0.93	0.96	201	1.00	0.97	0.98	378	1.00	0.99	0.99	587
	Avg/Total	0.97	0.96	0.96	400	0.98	0.98	0.98	800	1.00	0.99	0.99	1200

Precision is the ratio of true positive predictions over all predicted positive predictions. The recall is the fraction of all actual positives that are true positives. F1-score is the harmonic mean of precision and recall, thus balancing out the two. This is referred to as support and denotes the number of real instances of each class in the dataset, or how many instances of each class have occurred in the dataset.

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DISCUSSION

The current study shows that it is possible to use machine learning, specifically Random Forest algorithm, to predict CRC based on the genomic changes in the MSH2 and MSH6 genes. The current research approach based on the NGS data is a reliable method for finding mutations that may cause CRC and thus, makes a significant contribution to developing the concept of precision medicine. Combining NGS with machine learning can help in early diagnosis, essential for enhancing patient prognosis and developing individualized treatment plans. The results of the current study suggested that the Random Forest model achieved classification accuracy rates of 96.25%, 98.37%, and 99.5% for the datasets of 1000, 2000, and 3000 sequences, respectively. These results suggest that Random Forest is a very efficient method in terms of relevant mutation detection, and the accuracy of the method increases with the growth of data, which underlines the significance of large datasets in improving CRC prediction.These results prove that the proposed model is effective and can be applied to analyze big genomic data, which can be used for the early diagnosis and personalized treatment of colorectal cancer because accuracy more than 90% is considered best as mentioned in previous studies [16, 17]. Another study focusses on using Random Forest (RF) algorithms to analyze NGS data for prediction of cancer-associated genomic alterations [18]. Although current study aims to predict colorectal cancer using MSH2 and MSH6 genes, the former literature investigated multiple tumor types, such as colorectal, melanoma, lung, and glioma, based on a 27-gene panel. Both studies use RF because of its ability to handle large data and minimize overfitting. The classification accuracy rates of our RF model were 96. 25%, 98.37%, and 99.5% for the datasets of 1000, 2000, and 3000 sequences, respectively, which proves that the proposed method's performance increases with the dataset's size proposed method's performance increases with the dataset size. It was claimed that the accuracy of the RF model was 99.77% with an ROC-AUC of 0. 99 indicates good predictability across a wider range of genomic changes [18]. Although the two studies have different emphases and dataset compositions, both show that RF is a useful tool for genomic data analysis and is highly accurate in predicting cancer-related mutations. Another study on the Lung Cancer Research obtained an accuracy of 87% in biomarkers for NSCLC and SCLC using RNA-Seq data. The biomarkers for NSCLC include BRAF, KRAS, NRAS, and EGFR, while those for SCLC include ATF6, ATF3, PGDFA, PGDFD, PGDFC, and PIP5K1C. On the other hand, the present study on CRC using MSH2 and MSH6 gene mutations and NGS data showed accuracy rates of 96%. 25%, 98. 37%, and 99. 5% for the datasets of 1000, 2000, and 3000 sequences, respectively. Accuracy in the Random Forest method refers to the percentage of correct predictions (both true positives and true negatives) made by the model relative to the total predictions made. An accuracy of 96.25% means that 96.25% of the total predictions for the dataset were correct [19]. This implies that the Random Forest model in our study was more efficient, probably because of the genomic interest and the feature engineering from NGS data as opposed to the general and intricate RNA-Seq data used in the lung cancer study. The Random Forest algorithm is a very efficient and reliable tool in genomic data analysis for disease prognosis and personalized medicine. It can handle big and intricate data, does not overtrain, is suitable for genetic data, and has high prediction power [20]. The high accuracy of the Random Forest model in classifying mutations in MSH2 and MSH6 genes in colorectal cancer can be helpful in early diagnosis and treatment planning. Random Forest consists of multiple decision trees that enhance the overall prediction, making it a very efficient method for predicting the relationships existing in genomic data. Consequently, it holds an essential position in forming precision medicine and individualized therapy [21]. The current study's research suggests some directions for enhancing future research in the academic field. Including other omics data, like transcriptomics and proteomics, could complement the current knowledge of CRC and enhance the prediction models. Further, the methodology could be used to predict other types of cancer by analyzing other genetic mutations and thus increase the applicability of this study. Clinical confirmation through performing clinical trials is crucial to confirm the model's predictions in clinical scenarios, which is significant for the application of research outcomes in CRC diagnosis. In addition, creating systems that can sequence and predict in real-time may bring about a meaningful change in the diagnostic process since accurate interventions can be made at the right time, enhancing the patient's quality of life. One limitation of this study is the diversity of the datasets used. While the study utilized datasets of different sizes, the diversity of the sequences might still be limited. Additionally, the study primarily focuses on mutations in the MSH2 and MSH6 genes, leaving out other genetic and epigenetic factors contributing to CRC, which might limit the model's comprehensiveness. Furthermore, the computational demands of processing and analyzing large NGS datasets can be significant, posing a challenge in optimizing the model for resource efficiency without compromising accuracy.

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CONCLUSION

The study effectively proves that using Random Forest algorithms for the NGS data of MSH2 and MSH6 genes can accurately predict mutations due to an accuracy of more than 90% in three different types of datasets. The Random Forest model obtained classification accuracy rates of 96. 25%, 98.37%, and 99.5% for the datasets of 1000, 2000, and 3000 sequences, respectively. These results demonstrate the efficiency and applicability of the model in dealing with big genomic data and thus can be used for early diagnosis and individualized treatment of colorectal cancer.

Authors Contribution

Conceptualization: OU, MH
Methodology: OU, MH, AA
Formal analysis: NK
Writing review and editing: OU, MH, NK, SF, AS, MZ
All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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REFERENCES

[1] Pan H, Zhao Z, Deng Y, Zheng Z, Huang Y, Huang S, *et al.*The Global, Regional, And National Early-Onset Colorectal Cancer Burden and Trends From 1990 To 2019: Results from The Global Burden of Disease Study 2019. Bmc Public Health.2022;22(1):1896.doi:10.1186/s12889-022-14274-7.

[2] Idrees R, Fatima S, Abdul-Ghafar J, Raheem A, Ahmad Z.Cancer Prevalence in Pakistan: Meta-Analysis of Various Published Studies to Determine Variation in Cancer Figures Resulting from Marked Population Heterogeneity In Different Parts Of The Country. World Journal of Surgical Oncology.2018; 16: 1-11. doi:10.1186/s12957-018-1429-z.

[3] Kastrinos F, Samadder NJ, Burt RW. Use Of Family History and Genetic Testing to Determine Risk of Colorectal Cancer.Gastroenterology.2020;158(2):389-403. doi:10.1053/j.gastro.2019.11.029.

[4] Biller LH and Schrag D. Diagnosis and Treatment of Metastatic Colorectal Cancer: A Review. Jama.2021; 325(7): 669-85. doi:10.1001/jama.2021.0106.

[5] Pećina-Šlaus N, Kafka A, Salamon I, Bukovac A. Mismatch Repair Pathway, Genome Stability and Cancer. Frontiers In Molecular Biosciences.2020; 7: 122. doi:10.3389/fmolb.2020.00122.

[6] Li K, Luo H, Huang L, Luo H, Zhu X. Microsatellite Instability: A Review of What the Oncologist Should Know. Cancer Cell International.2020;20:1-13.doi :10.1186/s12935-019-1091-8.

[7] Nolano A, Medugno A, Trombetti S, Liccardo R, De Rosa M, Izzo P, *et al.* Hereditary Colorectal Cancer: State of The Art in Lynch Syndrome. Cancers.2022; 15(1): 75. doi:10.3390/cancers15010075.

[8] Ahuja SK, Shrimankar DD, Durge AR. A Study and Analysis of Disease Identification Using Genomic Sequence Processing Models: An Empirical Review. Current Genomics.2023;24(4):207.doi:10.2174/0113892029695232311010514 55.

[9] Qayyum MU, Sherani AMK, Khan M, Hussain HK. Revolutionizing Healthcare:The Transformative Impact of Artificial Intelligence in Medicine.Bin: Bulletin Of Informatics. 2023; 1(2): 71-83.

[10] Iqbal MJ, Javed Z, Sadia H, Qureshi IA, Irshad A, Ahmed R, *et al.* Clinical Applications of Artificial Intelligence and Machine Learning in Cancer Diagnosis: Looking into The Future.Cancer Cell International.2021; 21(1): 270. doi:10.1186/s12935-021-01981-1.

[11] Pachouly J, Ahirrao S, Kotecha K, Selvachandran G, Abraham A. A Systematic Literature Review on Software Defect Prediction Using Artificial Intelligence: Datasets, Data Validation Methods, Approaches, And Tools. Engineering Applications for Artificial Intelligence.2022; 111: 104773. doi:10.10 16/j.engappai.2022.104773.

[12] Singh R and Pal S. Machine Learning Algorithms and Ensemble Technique to Improve Prediction of Students Performance. International Journal of Advanced Trends in Computer Science and Engineering.2020;9(3).doi:10.30534/ijatcse/2020/221932020.

[13] Rubio-Mangas D, García-Arranz M, Torres-Rodriguez Y, León-Arellano M, Suela J, García-Olmo D. Differential Presence of Exons (DPE): Sequencing Liquid Biopsy by Nges. A New Method for Clustering Colorectal Cancer Patients. BMC Cancer.2023;23(1): 1-14. doi:10.1186/s12885-022-10459-w.

[14] Eldem V and Balcı MA. Mining NCBI Sequence Read Archive Database: An Untapped Source of Organelle Genomes for Taxonomic and Comparative Genomics Research.Diversity.2024;16(2):104.doi:10.3390/d16020104.

[15] Kurian B and Jyothi V. Breast Cancer Prediction Using an Optimal Machine Learning Technique for Next Generation Sequences. Concurrent Engineering.2021;29(1):49-57. doi:10.1177/1063293X21991808.

[16] Risal S, Zhu W, Guillen P, Sun L. Improving Phase Prediction Accuracy for High Entropy Alloys with

DOI: https://doi.org/10.54393/fbt.v5i1.131

Machine Learning. Computational Materials Science. 2021;192:110389.doi:10.1016/j.commatsci.2021.110389.

[17] Ibrahim I and Abdulazeez A.The Role of Machine Learning Algorithms for Diagnosing Diseases. Journal Of Applied Science and Technology Trends. 2021; 2(01): 10-9. doi:10.38094/jastt20179.

[18] Pellegrino E, Jacques C, Beaufils N, Nanni I, Carlioz A, Metellus P, *et al*. Machine Learning Random Forest for Predicting Oncosomatic Variant Ngs Analysis. Scientific Reports.2021;11(1):21820.doi:10.1038/s41598-021-01253-y.

[19] Lavanya C, Pooja S, Kashyap AH, Rahaman A, Niranjan S, Niranjan V. Novel Biomarker Prediction for Lung Cancer Using Random Forest Classifiers.Cancer Informatics.2023;22.doi:10.1177/11769351231167992.

[20] Qi Y. Random Forest for Bioinformatics.Ensemble Machine Learning: Methods And Applications.2012 307-23. doi:10.1007/978-1-4419-9326-7_11.

[21] Sun Z, Wang G, Li P, Wang H, Zhang M, Liang X. An Improved Random Forest Based on The Classification Accuracy and Correlation Measurement of Decision Trees. Expert Systems with Applications.2024;237:121549.doi:10.1016/j.eswa.2023.121549.

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Effects of Episodic Administration of Gonadotropin-Releasing Hormone on Luteinizing Hormone Concentrations and Libido in Pubertal Male Kundhi Buffalo Calves

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ABSTRACT

This study investigated the effects of gonadotropin-releasing hormone (GnRH) administration on luteinizing hormone (LH) concentrations and libido in pubertal male Kundhi buffalo calves. **Objective:** To determine the effects of GnRH administration on LH concentrations and libido in pubertal male Kundhi Buffalo calves. **Methods:** Eight calves, aged 16 months, were divided into two groups: Group A (treatment n=4), administration of GnRH analog (25ug lecirelin) and Group B (control n=4), there is not any administration. Each group consisting of four calves under a semi-intensive management system, the calves were fed a diet including 2 kg/day/calf of cotton seed cake, along with wheat bran, wheat straw, and green fodder, at the Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences. Results revealed a significant temporal change ($P < 0.000$) in the mean circulating concentration of LH over time after the intramuscular administration of GnRH analog (25ug lecirelin, Fatro®, International) three times. **Results:** The LH pulse was highest after the second GnRH dose (29.46±1.828), with significant differences in libido parameters, including mounting activity, erection, and sniffing, between the treatment (Group A) and control (Group B) groups. Statistical analysis revealed significant effects of episodic GnRH administration on both LH concentrations and libido. **Conclusion:** The episodic administration of GnRH significantly increased LH concentrations and improved libido in pubertal male Kundhi buffalo calves.

INTRODUCTION

The Kundhi breed emerges as the second most significant buffalo breed in Pakistan, primarily concentrated in the southern Sindh region. Its distribution spans from Kashmore to Shah Bandar along the Indus River, reaching urban centers such as Hyderabad, Karachi, Larkana, Nawabshah, Mirpurkhas, and extending into Quetta and the surrounding regions of Balochistan [1]. Attaining sexual maturity and optimal reproductive behavior in male Kundhi buffalo calves pose challenges, and limited research has delved into the effects of GnRH administration on Luteinizing Hormone (LH) concentrations and libido in this particular population. Examining the impact of episodic GnRH administration on LH concentrations and libido in pubertal male Kundhi buffalo calves is imperative for enhancing reproductive efficiency and optimizing livestock production, especially in regions where buffalo farming predominates, such as Pakistan. Sexual puberty in male buffalo calves represents the initiation of reproductive activities, which are oriented by a responsive fetal hypothalamus-pituitary-gonadal axis controlled hormonal cascade. LH is a key gonadotropin, and its secretion depends on the neuropeptide GnRH. LH, in its turn stimulates testosterone production impacting the development of secondary sexual characteristics and reproductive competence [2]. The general principles of GnRH action are understood and also LH elevation during

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puberty but regarding effect at episodic administration of GnRH on LH in pubertal male Kundhi buffalo calves is a missing link. Kundhi buffalo is endowed with distinctive features and economical importance, therefore it serves as a good model to understand the reproductive physiology intricacies. These results will provide new insights into the role of episodic GnRH administration on LH concentration during pubertal male Kundhi buffalo calves and likely to contribute reproductive biology in this important livestock. This can help to both better breeding allotment and thus improve agricultural productivity (Herbison, 2018). GnRH plays a critical role in the Hypothalamic-Pituitary-Gonadal (HPG) axis, controlling LH and FSH biosynthesis and secretion. Pulsatile release of GnRH is essential to maintain normal reproductive function that drives the pulsatile secretion of LH and FSH from the pituitary gland [3]. In males' bulls, failure of the libido may be due to low testosterone levels which leads to not only slower response but also less mounts a successful ejaculation, smaller ejaculate volume and reduced sperm per ejaculate. Knowledge of hormonal and physiological complexities, which underpin puberty and reproductive performance will aid in exploiting best field practices to harvest the full potential productivity efficiency from buffalo heifers into breeding system [4]. Conclusion Role of GnRH is well established in reproductive physiology among the species but here it may be proved and need to work more on certain effects like its stimulatory action upon libido in male pubertal Kundhi Buffalo calves. This research could be significant regarding the hormonal influence on sexual behaviors in this breed, which might help to improve breeding strategies and productivity of Kundhi Buffalo.

The objective of this study was to evaluate the effects of episodic administration of gonadotropin-releasing hormone (GnRH) on luteinizing hormone (LH) concentrations and to assess its impact on the libido of male pubertal Kundhi buffalo calves. By analyzing the hormonal response to GnRH administration, the study aims to determine variations in LH secretion patterns, which play a crucial role in reproductive physiology. Additionally, the research seeks to observe changes in sexual behavior and libido in response to GnRH treatment, providing insights into its potential role in enhancing reproductive performance in Kundhi buffalo calves.

METHODS

Management of Experimental Animals

Eight Kundhi buffalo calves of age 16 months were used and divided into two groups i.e group A treatment and group B control group and each group had four number of calves. These animals were kept under semi-intensive management system and were fed, and water was provided ad libitum to all experimental animals throughout the experimental phase at the Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences. Vaccination and drenching were given as per farm schedule. The calves in the treatment group (group A) received an intramuscular administration of 25ug lecirelin (GnRH analog) Fatro®, International. Administered the first dose on day 1, the second dose 48 hours following the first dose and the third dose after one month of second dose. Control Group (Group B): (n=4)– No administration.

Blood Sampling

Blood samples were drawn from the jugular vein then immediately transferred into the Sodium Citrate vacutainer tube with ± 3 mL blood. The blood samples were collected on day-0 from Group-B respectively, in order to obtain baseline LH measurements. On day 1, blood samples were collected from Group-A, this time, GnRH 25 µg lecirelin (GnRH analog) (Fatro®, International) was injected through IM immediately. Following giving injection, four times blood samples were collected for 2 hours at the interval of 30 minutes from animals individually. To increase the number of observations, the experiment was repeated after 48 hours and then after one-month interval.

Measurement of LH concentration and Libido Observation

The serum of all collected samples was analyzed for the measurement of LH levels (concentration) commercially. The all collected Samples were sent to Asian Institute of Medical Sciences Diagnostic and Research Lab, Tandojam. The concentration of luteinizing hormone was determined by using method cobas-411 Roche immunoassay analyzer. Libido was observed twice a day, morning, and evening immediately after administration of GnRH injection. The sexual parameters were monitored i.e mounting, erection (protrusion), sniffing, close contact (neck to neck), mounting with erection and mounting without erection.

The study was conducted over three months at the Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences. Date collected was tabulated in Excel sheet and analyzed for statistical difference between group and with group using Excel state version statistics 8.1. Data analysis was performed using ANOVA LSD test and Descriptive statistics.

RESULTS

In present study eight Kundhi buffalo calves were used and aimed to assess the effect of episodic administration of GnRH on luteinizing hormone level (concentrations) and to observe the influence of GnRH on the libido of male pubertal Kundhi Buffalo Calves.

Luteinizing Hormone Level (Concentration)

The figure 0.1 shows that after episodic administration of GnRH, the level of luteinizing hormone in control group was

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0.922 ng/ml, on day-0 the level was 1.095 ng/ml. Whereas, after 48 hours the level was 1.385 ng/ml, after one month, the level was 1.057 ng/ml. The highest level was found after 48 hrs. The significant differences lie in the incremental increases in LH levels at each time point, with the highest level (1.385 ng/ml) observed after 48 hours, indicating a peak response to the GnRH administration. The decrease in LH level after one month suggests a return to near-baseline levels.

The Comparisons Between The Control and The 1st Dose Of GnRH

The figure 0.2 shows that after episodic administration of GnRH, the level of luteinizing hormone was 0.922 ng/ml in the control group, whereas it was 1.ng/ml on day 0 after 1st dose, increased by 0.17 ng/ml or 18.5% from control. GnRH showed numerically the highest values after the first dose.

The Comparisons Between The Control and The 2nd Dose Of GnRH

The figure 0.3 show that after episodic administration of GnRH, the level of luteinizing hormone was 0.922 ng/ml in the control group, whereas it was 1.385 ng/ml in 2nd dose of GnRH after 48hrs increased by 0.465 ng/ml or 42.4% from control and 0.295 ng/ml or 27.0% from Day 0), the 2nd dose of GnRH highest values of concentration was observed.

The Comparisons Between The Control and The 3rd Dose Of GnRH

The Figure 0.4 shows that after episodic administration of GnRH, the level of luteinizing hormone was 0.922 ng/ml in the control group, whereas it was 1.057 ng/ml 3rd dose after one month, increased by 0.13 ng/ml or 14.1% from control, but decreased by 0.335 ng/ml or 24.2% from 48 hours the significate difference values were observed after 3rd dose of GnRH.

The Comparisons Between The 1st and The 2nd Dose Of GnRH

The Figure 0.5 show that after episodic administration of GnRH, the level of luteinizing hormone was 0.922 ng/ml in the 1st dose, whereas it was 1.095 ng/ml in 2nd dose, after 48 hours +0.295 ng/ml 27% increase after the 2nd dose of GnRH showed numerically the highest values.

The Comparisons Between The 1st, 2nd and 3rd Dose Of GnRH

The Figure 0.6 show that after episodic administration of GnRH, the level of luteinizing hormone 1.095 ng/ml in 1st dose on day-0 whereas, after 48 hours it was 1.385 ng/ml, +0.295 ng/ml (27% increase) and after one month the level was 1.057 ng/ml, -0.335 ng/ml (24% decrease from 2nd dose) and Day 0 vs. 1 month: -0.04 ng/ml (4% decrease). The highest level of 1.385 ng/ml was found after 48 hours.

Libido Observation

The mean of libido was observed from group A and B as shown in Table 1.1. The comparison of libido parameters between treatment group A and control group B reveals significant differences, with group A showing numerically higher values across all measured parameters. The specific findings are: The behavioral parameters observed in the study indicate that Group A exhibited significantly higher frequencies of mounting (2.82±0.054 vs. 1.42±0.046), mounting with erection (2.37±0.043 vs. 1.16±0.030), erection (2.07±0.057 vs. 1.05±0.018), close contact neck to neck (2.63±0.051 vs. 1.40±0.046), and sniffing (2.92±0.055 vs. 1.38±0.045) compared to Group B. The result shows that treatment group A consistently exhibited higher mean values across all libido parameters measured compared to control group B. These differences were significant and suggest that the GnRH treatment administered to group A effectively enhanced various aspects of sexual behavior. The consistency in the higher mean values for mounting, mounting with erection, erection, close contact (neck to neck), and sniffing highlights the potential efficacy of the treatment in increase male libido and sexual behaviors. Table 1 presents the standard values of libido (Mean ± % SEM) in Group A (GnRH treatment) and Group B (control), highlighting significant differences in reproductive behaviors.

Table 1: Standard Values of Libido

Observation	Treatment Group A (Mean ± SD)	Control Group B (Mean ± SD)
Mounting	2.82 ± 0.054[a]	1.42 ± 0.046[b]
Mounting with Erection	2.37 ± 0.043[a]	1.16 ± 0.030[b]
Erection (Protrusion)	2.07 ± 0.057[a]	1.05 ± 0.018[b]
Close Contact Neck To Neck	2.63 ± 0.051a	1.40 ± 0.046[b]
Sniffing	2.92 ± 0.055[a]	1.38 ± 0.045[b]

[a.b]Different Superscripts within the column show significant difference(P<0.5)

Fgure 1 shows a significant difference between the control and all three doses of GnRH.

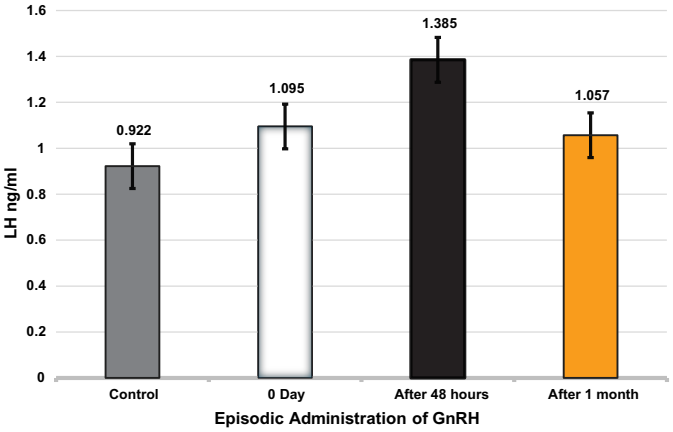

Figure 1: Significant difference between control and 1st, 2nd, and 3rd doses of GnRH

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Figure 2 demonstrates a significant difference between the control and the first dose of GnRH,

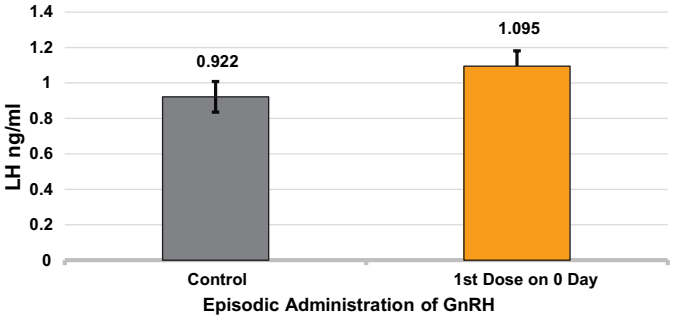

Figure 2: Significant difference between control and first dose of GnRH

Figure 3 highlights a significant difference between the control and the second dose

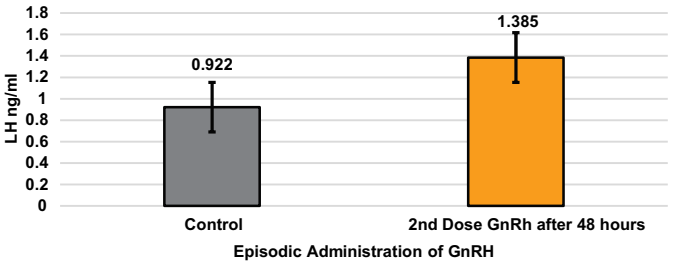

Figure 3: Significant difference between control and second dose of GnRH

Figure 4 indicates a significant difference between the control and the third dose of GnRH

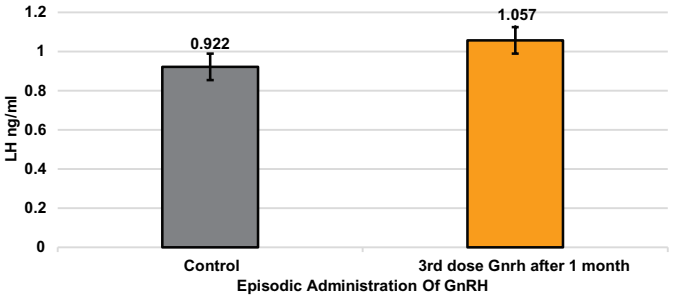

Figure 4: Significant difference between control and third dose of GnRh

Figure 5 illustrates a significant difference between the first and second doses of GnRH.

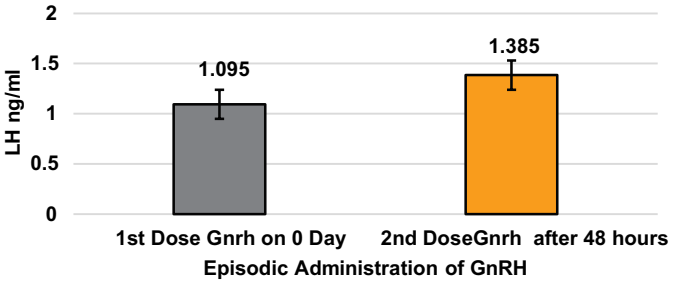

Figure 5: Significant difference between first and second dose of GnRH

Figure 6 shows a significant difference among the first, second, and third doses of GnRH

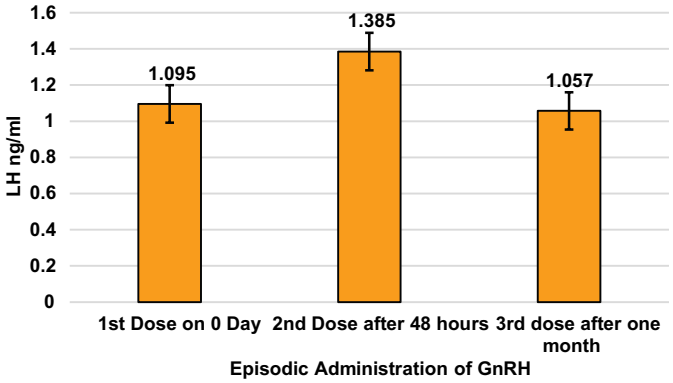

Figure 6: Effect of Episodic GnRH Administration on Luteinizing Hormone (LH) Levels in Buffalo Calves

DISCUSSION

In the present study the effects of episodic administration of GnRH on Luteinizing Hormone (LH) concentrations and libido in pubertal male Kundhi buffalo calves were investigated. In the treatment group, a notable increase in LH levels was observed compared to baseline levels and the control group. Specifically, the initial administration of GnRH resulted in a surge in LH concentration, peaking at 1.385 ng/ml after 48 hours, suggesting a strong hormonal response to exogenous GnRH stimulation. Subsequent doses maintained elevated LH levels, although with fluctuations observed over time. The findings of this study show that administration of GnRH significantly increased the level of LH and libido in the animals of the treatment group compared to the control group. The results of present study are consistent with Kumar BB *et al.*, in 2016, it has been shown that in bull calves GnRH stimulated the secretion of LH [5]. Also, in other male animals like male rats studied by Funabashi T *et al.*, in 2002 who given a subcutaneous GnRH injection (450 ng/100 g body weight) which resulted in enhanced libido, FSH, LH, and testosterone concentrations, testicular volume and testicular weight and scrotal circumferences were also increased [6]. Similar reports were documented in bubaline species that GnRH analogue administration has significantly improved the endocrinological profiles, libido and semen quality profiles, by El-Khawaga AR *et al.*, in 2011 [7]. These results are consistent with previous studies indicating the stimulatory effect of GnRH on LH secretion in male Ganjam Goat Nayak J *et al.*, in 2022 [8]. The highest LH level observed 48 hours after the first dose of GnRH suggests a temporal response to GnRH administration, which has implications for optimizing reproductive management in buffalo breeding programs. Our findings revealed the significant variations in LH concentrations following episodic administration of GnRH. The hormonal and behavioral responses have important implications for

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understanding the process of puberty and reproductive performance in male buffalo calves. GnRH, originating from the hypothalamus, is at the top of a cascade that triggers LH secretion and in many ways serves as a master signal for reproductive function, so our results may also collectively suggest exogenous GnRH can be an effective means to stimulate LH release and thereby boost libido among pubertal males. This preprint explored how cholinergic and GABAergic co-transmission regulates GnRH neuron activity and luteinizing hormone secretion [9]. In another study, weekly injection of GnRH significantly raised the gonadal androgen concentration and scrotal circumference in bovine species Ali S *et al.*, in 2012 [10]. The injection of exogenous GnRH resulted in a significantly increased testosterone concentration in the bovine species, as reported by Kumar BB *et al.*, in 2016 [5]. These findings suggest that GnRH administration may be a useful strategy for improving reproductive efficiency in buffalo breeding programs. GnRH treatment has been suggested to influence male sexual behavior through the elevation of testosterone levels in the bloodstream McDonnell SM, in 1992 [11]. Research indicates that in male rats, a rapid rise in testosterone levels, induced by GnRH treatment, promptly reduces the time taken to initiate mounting behavior (James and Nyby, 2002). In addition to hormonal changes, libido-related behaviors in response to GnRH administration were observed. Mounting behavior, a key indicator of sexual arousal and activity, exhibited significant differences between the treatment and control groups. Calves receiving GnRH demonstrated higher levels of mounting compared to the control group, indicating an enhancement of sexual behavior following hormonal intervention. This article provided a historical overview of methods and advancements in measuring GnRH levels in pituitary portal blood, highlighted their significance in understanding reproductive neuroendocrinology [12]. These effects may contribute to accelerated sexual maturation and improved reproductive efficiency in male buffalo calves, potentially facilitating breeding programs and livestock management practices, Kumar BB *et al.*, in 2016 [5]. It is important to acknowledge the limitations of current study, including the small sample size and the short-term nature of the intervention. Further studies with longer observation periods to fully elucidate the effects of episodic GnRH administration on reproductive physiology and behavior in male buffalo calves may be done. Additionally, investigating the long-term implications of GnRH intervention on fertility and reproductive performance would provide valuable insights into its potential applications in livestock breeding programs as stated by Kumar *et al.*, 2016; Singh AK, in 2016 [5, 13]. This paper provides a detailed physiological and pharmacological overview of gonadotropin-releasing hormone (GnRH), exploring its mechanisms of action and potential therapeutic applications [14]. In another study investigated the effects of episodic GnRH administration in buffalo bulls, showing that it stimulates testosterone secretion and testicular growth without negatively impacting semen quality [15]. These findings align with Herbison (2018), who underscored the critical role of the GnRH pulse generator in modulating reproductive function [16]. The broader implications of GnRH in animal reproduction have been extensively reviewed. Singh, (2022) discussed subclinical mastitis in dairy animals, drawing attention to hormonal influences on immune function and fertility [13]. Goodman RL *et al.*, (2022) provided insights into how testosterone rapidly affects copulatory behavior, reinforcing the significance of hormonal regulation in male reproductive strategies [17]. Hassanein *et al.*, (2024) detailed the structural and biosynthetic aspects of GnRH and its role in estrous synchronization, a crucial aspect of reproductive management [18]. Additionally, Gupta and Barański *et al.*, explored the effects of GnRH analogues on LH and testosterone concentrations in buffalo bulls, particularly during the non-breeding season [19, 20]. At the molecular level, Stamatiades and Kaiser (2018) elucidated the signaling pathways and gene expression changes induced by pulsatile GnRH administration, providing a mechanistic understanding of its regulatory role in reproductive endocrinology [21]. These insights collectively support the potential of GnRH therapies in optimizing reproductive efficiency, particularly in managed breeding programs for buffalo and other livestock species.

CONCLUSION

In conclusion, episodic GnRH administration positively influences LH secretion and libido in pubertal male Kundhi buffalo calves, offering potential benefits for reproductive management. This approach may enhance breeding efficiency and fertility in buffalo farming. However, further research is needed to refine protocols and explore its therapeutic applications, particularly for infertile or sub-fertile animals.

Authors Contribution

Conceptualization: FHJ
Methodology: PK
Formal analysis: FHJ
Writing, review and editing: HS, FR, RA, SA

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

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Conflicts of Interest

All the authors declare no conflict of interest.

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REFERENCES

[1] Kachiwal AB, Sheikh BA, Sheikh SA, Qureshi TA, Memon MA. Ultrasonographic biometry of the ovaries of pregnant Kundhi buffaloes. Journal of Buffalo Science. 2012 Jul; 1(2). doi: 10.6000/1927-520X.2012.01.02.10.

[2] Duittoz AH, Tillet Y, Le Bourhis D, Schibler L. The timing of puberty (oocyte quality and management). Animal Reproduction(AR). 2018 Jul; 13(3): 313-33. doi: 10.21451/1984-3143-AR874.

[3] Ieda N, Minabe S, Ikegami K, Watanabe Y, Sugimoto Y, Sugimoto A. GnRH (1-5), a metabolite of gonadotropin-releasing hormone, enhances luteinizing hormone release via activation of kisspeptin neurons in female rats. Endocrine Journal. 2020 Jan; 67(4): 409-18. doi: 10.1507/endocrj.EJ19-0444.

[4] Al-Qarawi AA. Infertility in the dromedary bull: a review of causes, relations and implications. Animal Reproduction Science. 2005 Jun; 87(1-2): 73-92. doi: 10.1016/j.anireprosci.2004.11.003.

[5] Kumar BB, Pandita S, Prakash BS, Mallick S, Mili B. Responsiveness of prepubertal crossbred bull calves to exogenous GnRH and its impact on reproductive hormones under tropical conditions. SpringerPlus. 2016 Mar; 5: 288. doi: 10.1186/s40064-016-1956-4.

[6] Funabashi T, Mitsushima D, Nakamura TJ, Uemura T, Hirahara F, Shinohara K. Gonadotropin-releasing hormone (GnRH) surge generator in female rats. Progress in Brain Research. 2002 Jan; 141: 165-73. doi: 10.1016/S0079-6123(02)41091-6.

[7] El-Khawaga AR, Kandiel MM, Sosa GA, Abou El-Roos ME, Abdel-Ghaffar AE, El Azab AE. Effect of GnRH analogue on libido and semen characteristics of puberal buffalo bulls. Benha Vet Med J Spec Issue I. 2011 May: 28-34.

[8] Nayak J, Nahak AK, Mishra PC, Karna DK, Mishra C, Ponraj P. Exogenous gonadotropin releasing hormone (GnRH) modulates scrotal and testicular biometrics, libido, endocrinological and hematological profiles in Ganjam goat under humid tropical coastal ecosystem of Odisha. Asian Pacific Journal of Reproduction. 2022 Nov; 11(6): 285-91. doi: 10.4103/2305-0500.361224.

[9] Vastagh C, Farkas I, Csillag V, Watanabe M, Kalló I, Liposits Z. Cholinergic control of GnRH neuron physiology and luteinizing hormone secretion: involvement of ACh/GABA co-transmission. bioRxiv. 2023 Aug: 2023-08. doi: 10.1101/2023.08.22.554236.

[10] Ali S, Ullah N, Akhter S. GnRH and diet effects testosterone and scrotal circumference of bulls: Study conducted on Nili-Ravi buffalo bulls. LAP Lambert Academic Publishing; 2012 Jun: 52.

[11] McDonnell SM. Ejaculation: physiology and dysfunction. Veterinary Clinics of North America: Equine Practice. 1992 Apr; 8(1): 57-70. doi: 10.1016/S0749-0739(17)30466-2.

[12] Moenter SM, Evans NP. Gonadotropin-releasing hormone (GnRH) measurements in pituitary portal blood: a history. Journal of neuroendocrinology. 2022 May; 34(5): e13065. doi: 10.1111/jne.13065.

[13] Singh AK. A comprehensive review on subclinical mastitis in dairy animals: Pathogenesis, factors associated, prevalence, economic losses and management strategies. Cabi Reviews. 2022 Dec. doi: 10.1079/cabireviews202217057.

[14] Casati L, Ciceri S, Maggi R, Bottai D. Physiological and pharmacological overview of the gonadotropin releasing hormone. Biochemical pharmacology. 2023 Jun; 212: 115553. doi: 10.1016/j.bcp.2023.115553.

[15] Malecki IA, Szulc T, Czubaszek M, Gilun P, Bartlewski PM. Episodic administration of GnRH to buffalo bulls stimulates testosterone secretion and testicular growth without affecting semen quality. Animal Reproduction Science. 2020; 212: 106266.

[16] Herbison AE. The gonadotropin-releasing hormone pulse generator. Endocrinology. 2018 Nov; 159(11): 3723-36. doi: 10.1210/en.2018-00653.

[17] Goodman RL, Herbison AE, Lehman MN, Navarro VM. Neuroendocrine control of gonadotropin-releasing hormone: pulsatile and surge modes of secretion. Journal of neuroendocrinology. 2022 May; 34(5): e13094. doi: 10.1111/jne.13094.

[18] Hassanein EM, Szelényi Z, Szenci O. Gonadotropin-releasing hormone (GnRH) and its agonists in bovine reproduction I: structure, biosynthesis, physiological effects, and its role in estrous synchronization. Animals. 2024 May; 14(10): 1473. doi: 10.3390/ani14101473.

[19] Gupta VK. Doctor of Philosophy in Veterinary Gynaecology and Obstetrics. [Doctoral dissertation]. National Dairy Research Institute. 2023.

[20] Barański W, Nowicki A, Crowe MA, Tobolski D, Zduńczyk S. Effect of repeated low doses of gonadotropin-releasing hormone on the secretion of

DOI: https://doi.org/10.54393/fbt.v5i1.158

luteinizing hormone and follicle-stimulating hormone, and ovarian function in dairy cows suffering from anovulation type I. Animal Reproduction Science. 2024 Nov; 270: 107602. doi: 10.1016/j.anireprosci.2024.107602.

[21] Stamatiades GA and Kaiser UB. Gonadotropin regulation by pulsatile GnRH: Signaling and gene expression. Molecular and Cellular Endocrinology. 2018 Mar; 463: 131-41. doi: 10.1016/j.mce.2017.10.015.

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Utilization of Size Exclusion Chromatography for the Recovery of Microbial Pectinases

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ABSTRACT

Size exclusion chromatography (SEC) is an effective analytical technique employed for the purification of biomolecules. In size exclusion chromatography (SEC), biomolecules are sorted according to their size. **Objectives:** To investigate the purification of pectinases from a microbiological source by size exclusion chromatography (SEC). To evaluate the amount of pectinase and total proteins in the collected fractions through measurement and qualitative analysis. **Methods:** Utilizing Sephadex G-25 as the stationary phase and a 0.05 M sodium phosphate buffer with increasing concentrations of NaCl as the mobile phase. Using a 3,5-dinitro-salicylic acid (DNS) assay and a pectin-containing agar plate assay, the existence of pectinases in the fractions that were taken was verified quantitatively and subjectively. **Results:** The increasing order of salt concentration was 0.15, 0.5, 0.8, 1 and 1.6 M NaCl concentration. At 0.15 and 0.5 M salt concentrations, desired proteins were strongly combined to the stationary phase of the Sequential Injection Chromatography (SIC) column and eluted at the last fraction while at 0.8, 1 and 1.6 M sodium chloride concentration pectinases were eluted in the early fractions as compared to the buffers containing a lower concentration of sodium chloride. **Conclusions:** It was concluded that the suitable NaCl concentration for the purification of pectinase enzyme through SEC was 0.8 M because at these concentrations pectinases can be separated very short time and at a low cost.

INTRODUCTION

Pectinases are the enzymes which can break down pectin. Pectin is a compound in the plant cell wall. It can lower the viscosity of the juice. The first commercial application of pectinases was to prepare wines and fruit juice in 1930 [1]. Pectinases are derived from different kinds of microorganisms [2, 3]. Pectinases are also reported to be produced in combination with other industrially significant enzymes by the same microbial isolate [4, 5]. Pectin enzymes can be classified into three groups mention the name of groups. This enzyme's activity on protein results in the formation of extremely polymerized soluble pectin [6, 7]. Approximately 70 million dollars were spent on pectinases worldwide [8], which accounted for only 5% of all enzyme sales worldwide. Aspergillus niger is typically used to make industrial pectinase enzymes (polygalacturonase, pectin esterase, and pectinlyase strains). Unexpected reactions may arise from the proteolytic or hydrolytic adverse effects of crude enzymes [9-11]. Commercial pectinases are used to extract and clarify sparkling clear juices (apple, pear, and grape juices), cloudy juices (citrus, prune, tomato, and nectar juices), and unicellular products by selectively hydrolyzing the middle lamella polysaccharides to preserve plant cell integrity [12]. But they do not have to be commercialized. Pectinases are among the most significant industrial enzymes [13].

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This study aims to inoculate microorganisms to produce the enzyme pectinase. To use gel filtration chromatography at various salt concentrations to separate the fermentation broth's supernatant from the microbial culture. To evaluate the amount of pectinase and total proteins in the collected fractions through measurement and qualitative.

METHODS

An experimental design was held from August 2024 to January 2025. Balance, Falcon tubes, PH meter, pipettes, tips, Eppendorf tubes, centrifuge, burette, vortex, beaker, magnetic stirrer, test tubes, water bath, racks, spectrophotometer, and cuvette. Sigma-Aldrich provided the isopropanol, ethanol, methanol, Tris, or sodium dodecyl sulphate. Merck was the supplier of ammonium sulphate. We bought sodium hydroxide from Riedel-de Haen in Seezle. Sodium chloride and yeast extract were bought. The supplier of glycine was Phyto Technology Laboratory Services, located in Shawnee, Kansas, in the United States. Hydrochloric acid was purchased from Scharlau , and Tris-HCl. The presence of pectinases was confirmed on nutrient agar plates. The agar plates were prepared using the composition NaNO3 (0.2%) KH2PO4 (0.1%) KCL (0.05%) MgSO4 (0.05%) Trypton (0.5%) pectin (0.5%) After the screening, 50 ml liquid broth medium having composition peptone(1%)yeast extract(0.5%)Nacl(1%)were inoculated by a loop full of the colony from the agar plate. The media were autoclaved at 121C °C for 20 minutes. The composition of the medium for pectinase production includes NaNO3 (0.2%), KH2PO4 (0.1%), KCL (0.05%), MgSO4 (0.05%), tryptone (0.5%) citrus peel powder (2.5%). After preparation of the media, it was autoclaved at 121°C for 20 minutes. The freshly prepared inoculum quantity of 0.3 mL/100 mL broth was added to the medium. The media was then incubated for at least 48 hours and then checked for the presence of pectinases. The cultured broth was then centrifuged at 7500 rpm for 10 minutes until a clear supernatant was obtained. Pectinase isolation was accomplished in several steps. The supernatant of a crude culture was applied to a Sephadex G-25 disposable column that was preequilibrated with 0.05 M sodium phosphate buffer. The protein elution and binding were performed with the same buffer. To check the effect of sodium chloride concentration on pectinase elution, sodium chloride concentration was increased stepwise. The salt concentration was further increased to 0.15, 0.5, 0.8, 1 and 1.6 M of NaCl. Twenty different fractions in each run were collected and further checked for pectinase activity and total protein concentrations. The unbound enzymes were eluted first. The combinations were incubated in separate test tubes (a and b) at 70°C in a shaking water bath for 10 minutes. After incubation, the test tubes were cooled under tap water, and only the control combination received 0.3 ml of crude enzyme. After adding the DNS solution to test tubes a and b, they were incubated in boiling water for 10 minutes. After incubation, the test tubes were cooled under tap water and transferred to 1.5 ml Eppendorf tubes for 5 minutes of centrifugation. Calculate pectinase units by comparing the difference in optical density (OD) at 575 nm between test and control combinations using the formula: Pectinase unit = optical density × 22/3. Additionally, total protein was computed as (optical density- 0.0247) / 0.0487. In the chromatographic experiment, gel filtration in group separation mode removed tiny molecules from bigger ones, such as salts or labels. Gel filtration chromatography beads were placed into a commercial 1.0 ml column and equilibrated with 10 column volumes of sodium phosphate buffer. Packing Column is a very perilous stage in gel filtration chromatography. A well-packed column is vital for high-resolution fractionation in gel filtration chromatography. For column packing Sephadex G-25 as supplied, fill up to one column volume and equilibrate with the help of 10 column volumes of (0.15 M sodium phosphate) buffer. In the chromatographic experiment, a mobile phase consisting of 0.05 M sodium phosphate buffer and varying concentrations of NaCl (0.15, 0.5, 0.8, 1, and 1.6 M) was utilized as both a binding and elution buffer. Key considerations included the buffer composition, pH, ionic strength, and the presence of denaturing agents or detergents, which could induce conformational changes in the target molecules. The protein sample was purified and free from particulate matter through centrifugation. After pre-equilibration, 300 ml of the protein sample was loaded onto the column using a pipette, and non-bound materials were eluted with 10 column volumes of buffer, while compounds of interest were collected in fractions using a simple step elution procedure. Following protein loading, different fractions of up to 1 ml were collected via a stepwise increase of sodium phosphate buffer for subsequent analysis of protease activity. To regenerate the gel filtration column, it was washed with 0.2 M sodium hydroxide (NaOH) and non-ionic detergents, then stored at 4°C in the dark with an antimicrobial agent (0.02-0.05% sodium hydroxide or 20% ethanol). The protein amount was determined using the Bradford assay, which measures the capability of proteins to bind to Coomassie Brilliant Blue, forming a complex with a significantly higher extinction coefficient than that of free dye. At 595 nm, absorbency was determined with a UV-visible spectrophotometer. 100 mg of Coomassie Brilliant Blue G250 was dissolved in 50 milliliters of 94% ethanol and 100 milliliters of 84% phosphoric acid to create the Bradford reagent. Then diluted to a final volume of 1 liter. To assess pectinase activity in the collected fractions, a DNS assay was performed according to standard procedures

RESULTS

The presence of pectinase enzyme in cultured broth was

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confirmed on nutrient agar plates using the well diffusion method. The clear zones around the wells indicated the presence of pectinases in the broth. Results found that by increasing the supernatant, the diameter of the zone elevated significantly, as shown in figure 1.

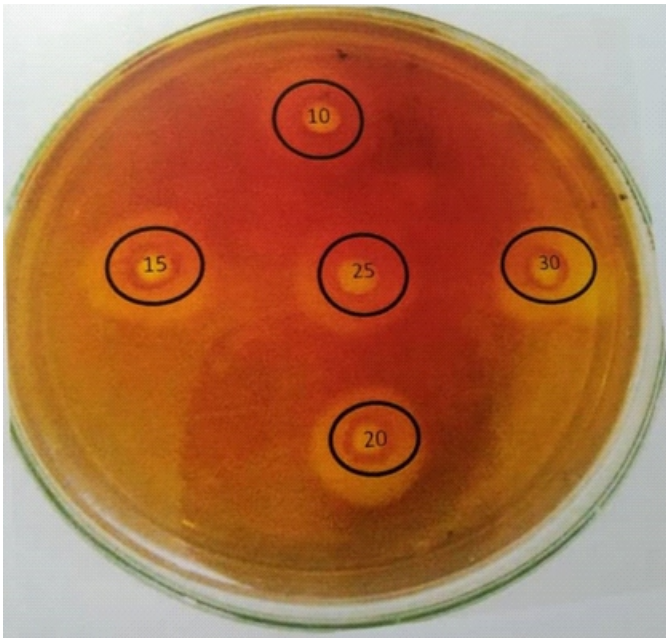

Figure 1: Plate assay for Pectinases: Zone Diameter After 48 Hours of Incubation

Results show that the fractions collected at the beginning have lower pectinase activity as well as lower concentrations of total protein. However, in the latter fractions, the pectinase enzyme activity was higher, as well as total protein. It was observed that a gradual increase occurred, and the highest activity (0.75) of pectinase enzyme was found in fraction no.19. The Pectinase enzyme unit and total proteins in the collected fractions using a gel G-25 column are presented in table 1.

Table 1: Standard Values of Libido

Fractions	Test	Control	OD	Pectinase Unit	Total Protein
1	0.06	0.04	0.02	0.14	0.00
2	0.08	0.08	0.01	0.04	0.00
3	0.06	0.02	0.04	0.27	0.25
4	0.01	0.09	0.00	0.00	0.00
5	0.04	0.06	0.00	0.30	0.00
6	0.05	0.00	0.05	0.38	0.56
7	0.06	0.05	0.01	0.08	0.00
8	0.06	0.02	0.04	0.29	0.29
9	0.01	0.02	0.00	0.00	0.00
10	0.04	0.07	0.00	0.00	0.00
11	0.08	0.02	0.06	0.42	0.66
12	0.07	0.02	0.05	0.40	0.60
13	0.05	0.02	0.03	0.25	0.19
14	0.03	0.02	0.01	0.06	0.00
15	0.09	0.02	0.07	0.48	0.83
16	0.07	0.02	0.05	0.37	0.54
17	0.03	0.02	0.01	0.10	0.00
18	0.08	0.02	0.06	0.41	0.64
19	0.13	0.02	0.10	0.75	1.59
20	0.10	0.02	0.08	0.56	1.05

Results illustrate the pectinase activity of the collected fraction through gel filtration sephadex G-25 column using 0.05M phosphate buffer along with 0.15 M NaCl, as binding buffer, as well as elution buffer elution pattern from G-25 column with size exclusion chromatography utilizing 0.05 M phosphate buffer along with 0.15 M NaCl. Fractions collected after elution were then checked for pectinase activity as well as for total protein. Black bars represent pectinases activity while gray bars represent total proteins, as shown in table 2.

Table 2: Table Pectinase Activity and Total Protein Concentration

Fraction No.	Pectinase Activity (U)	Total Protein (mg/ml)
1	0.08	0.30
2	0.18	0.55
3	0.00	0.15
4	0.04	0.30
5	0.00	0.55
6	0.07	0.90
7	0.00	0.20
8	0.10	0.60
9	0.00	0.40
10	0.06	0.55
11	0.24	0.90
12	0.00	0.60
13	0.40	1.00
14	0.12	0.75
15	0.34	0.60
16	0.20	0.90
17	0.06	0.55
18	0.00	0.30
19	0.70	1.50
20	0.50	1.20

The finding illustrates the pectinase activity of the collected fraction through gel filtration Sephadex G-25 column using 0.05M phosphate buffer along with 0.5 M NaCl, as binding buffer as well as elution buffer, shows purification of pectinases through the chromatographic process of the G-25 column. Supernatant of the crude culture was applied for elution from a G-25 size exclusion chromatographic column by using 0.05 M Sodium phosphate buffer along with 0.5 M NaCl. Twenty different fractions were checked for pectinase activity as well as for total proteins. Binding and elution of the target protein were performed with the same buffer. Black bars represent pectinase enzyme activity, while gray colored bars represent total protein concentration in the collected fractions, and the results are shown in table 3.

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Table 3: Pectinase Activity and Total Protein Concentration

Fraction No.	Pectinase Activity (U)	Total Protein (mg/ml)
1	0.00	0.00
2	0.00	0.00
3	0.00	0.00
4	0.00	0.00
5	0.15	0.10
6	0.25	0.20
7	0.35	0.30
8	0.00	0.00
9	0.10	0.05
10	2.30	1.00
11	0.00	0.00
12	1.10	0.60
13	1.20	0.65
14	0.60	0.40
15	1.20	0.65
16	0.30	0.20
17	0.10	0.05
18	0.20	0.15
19	2.80	1.30
20	2.00	1.10

Results illustrate the pectinase activity of the collected fraction through gel filtration sephadex G-25 column using 0.05M phosphate buffer along with 0.8 M NaCl, as binding buffer as well as elution buffer, represents the elution pattern through sephadex G-25 column size exclusion chromatography using 0.05 M phosphate buffer along with 0.8 M NaCl. The black bars illustrate pectinases activity while gray bars reveal total protein contents and findings shown in table 4.

Table 4: Estimated Pectinase Activity and Total Protein Concentration

Fraction No.	Pectinase Activity (U)	Total Protein (mg/ml)
1	0.00	0.10
2	0.00	0.00
3	0.00	0.00
4	0.90	0.45
5	1.10	0.60
6	1.40	0.70
7	2.30	0.90
8	1.00	0.40
9	1.40	0.60
10	0.70	0.30
11	1.00	0.50
12	0.80	0.40
13	0.60	0.30
14	0.90	0.45
15	0.50	0.25
16	0.70	0.35
17	0.20	0.10
18	0.60	0.30
19	0.10	0.05
20	0.00	0.10

Study illustrated the pectinase activity of the collected fraction through gel filtration Sephadex G-25 column using 0.05M phosphate buffer along with 1.0 M NaCl as binding buffer as well as elution buffer, showing elution pattern through size exclusion chromatography with G-25 column using 0.05 M phosphate buffer along with 1.0 M NaCl at PH 8.0. The samples were loaded on a size-exclusion chromatography Sephadex G-25 column. The fractions were applied to a gel filtration chromatography column. Twenty different fractions were collected and analyzed for pectinase activity and total protein content, and the results are shown in table 5.

Table 5: Pectinase Activity and Total Protein Concentration in Fractions

Fraction No.	Pectinase Activity (U)	Total Protein (mg/ml)
1	1	0.0
2	1	0.1
3	1	0.2
4	2	0.3
5	2	0.4
6	3	0.5
7	3	0.6
8	3	0.7
9	4	0.8
10	4	0.9
11	5	1.0
12	5	1.1
13	6	1.2
14	6	1.3
15	7	1.9
16	6	1.5
17	5	1.4
18	4	1.2
19	3	1.0
20	1	0.0

Illustrating the pectinase activity of the collected fraction through gel filtration sephadex G-25 column using 0.05M phosphate buffer along with 1.6 M NaCl, as binding buffer as well as elution buffer, shows purification mechanism from the sephadex G-25 column using 0.05 M phosphate buffer along with 1.6 M NaCl. Twenty different fractions were collected and then checked for pectinase activity and total proteins. Black bars represent pectinases activity while gray bars indicate total proteins, and results are shown in table 6.

Table 6: Pectinase Activity and Total Protein Concentration in Fractions

Fraction No.	Pectinase Activity (U)	Total Protein (mg/ml)
1	1	0.0
2	1	0.1

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3	1	0.2
4	2	0.3
5	2	0.4
6	3	0.5
7	3	0.6
8	3	0.7
9	4	0.8
10	4	0.9
11	7	1.2
12	5	1.1
13	6	1.3
14	5	1.4
15	4	1.5
16	5	1.6
17	7	1.7
18	4	1.4
19	3	1.2
20	1	0.9

DISCUSSION

The selected bacterial strain for pectinase production was confirmed with the help of pectin-containing agar plates [14, 15]. The pectinase function in the supernatant is shown by agar plates with clear zones surrounding the wells [16]. The pectinase enzyme in the supernatant has broken down the substrate pectin, visible by the clear zone surrounding the wells [17]. The outcome demonstrates that the area of clear zones surrounding the wells grows with the volume of supernatant, indicating a rise in the pectinase enzyme's efficiency. The hydrolysis zone produced on the pectin agar plates could be related to the results found in the literature [18]. In this investigation, gel filtration chromatography was used to purify pectinases from fermented cultured broth. The stationary phase was a gel filtration disposable column containing Sephadex G-25, while the mobile phase was 0.05 M phosphate buffer with increasing NaCl concentrations (0.15, 0.05, 0.8, 1.0, 1.6 M). Contact between the size exclusion stationary phase and proteins of interest separated them. The mobile phase pH and ionic strength were controlled to elute target proteins from chromatographic columns. Zones were not seen in total protein. Pectinase activity was measured by the DNS test and given as Soxhlet units, whereas total protein was measured by the Bradford assay [19]. Pectinase enzyme was found to be strongly bound to the stationary phase, and hence, pectinase was eluted in the last fraction, no.19. By increasing sodium chloride concentration up to 0.5 M, almost the same elution pattern of pectinase enzyme was observed. To examine how sodium chloride concentration affected pectinase enzyme elution, it was raised to 0.8 M. Pectinase activity at 0.8 M sodium chloride, with fraction 7 having the greatest activity. In our study, fraction 15 had the greatest pectinase enzyme activity and total protein content with 1.0 M NaCl. The sodium chloride concentration was raised to 1.6 M to further test its influence on pectinase enzyme elution. Greatest pectinase enzyme activity and total protein content. NaCl compounds in the buffer determine their ionic strength, which may explain this behaviour. A thick dielectric double layer and reduced protein-protein interaction (aggregation) depend on ionic strength. Sodium chloride affected protein elution volume. Salt in the mobile phase delineated protein peaks and inhibited protein-matrix interactions, improving molecular size determination [20].

CONCLUSION

It was concluded that this study can be further pursued for further purification of microbial pectinases by utilizing the same conditions for the pilot-scale purification of pectinases.

Authors Contribution

Conceptualization: KU[1], OK
Methodology: MB, TS, KU[2], HB, M[2]
Formal analysis: AU
Writing review and editing: SM, M[1]

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

Conflicts of Interest

All the authors declare no conflict of interest.

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REFERENCES

[1] Singh B, Soni SK, Mathur P, Garg N. Microbial Multi-Enzyme Viz., Pectinase, Cellulase and Amylase Production Using Fruit and Vegetable Waste as Substrate a Review. Applied Microbiology. 2024 Aug; 4(3): 1232-46.

[2] Afzia N, Shill N, Kalita BJ, Sit N. Optimization of Conditions for Production of Pectinase in Solid-State Fermentation with Aspergillus Flavus Using Dried Assam Lemon Peel Powder as Substrate. Measurement: Food. 2024 Jun; 14: 100166.

[3] Ahmed NN, Abd El Rahman HM. Optimizing the Production of Pectinase of Orange Peel Waste by Penicillium Chrysogenum MF318506 Using Response Surface Methodology in Submerged Fermentation. Journal of Microbiology, Biotechnology and Food Sciences. 2021 Aug; 11(1): e3931-.

[4] Esawy MA, Gamal AA, Kamel Z. Optimization of Aspergillus Niger NRC1ami Pectinase Using Citrus Peel Pectin, Purification, and Thermodynamic

DOI: https://doi.org/10.54393/fbt.v5i1.163

Characterization of the Free and Modified Enzyme. Waste and Biomass Valorization. 2022 Dec; 13(12): 4823-37.

[5] Amin F, Bhatti HN, Bilal M. Recent Advances in the Production Strategies of Microbial Pectinases—A Review. International Journal of Biological Macromolecules. 2019 Feb; 122: 1017-26.

[6] Pang Y, Peng Z, Ding K. An in-depth review: Unraveling the Extraction, Structure, Bio-Functionalities, Target Molecules, and Applications of Pectic Polysaccharides. Carbohydrate Polymers. 2024 Jul: 122457.

[7] Zheng L, Xu Y, Li Q, Zhu B. Pectinolytic Lyases: A Comprehensive Review of Sources, Category, Property, Structure, and Catalytic Mechanism of Pectate Lyases and Pectin Lyases. Bio-Resources and Bioprocessing. 2021 Dec; 8: 1-3.

[8] Haile S and Ayele A. Pectinase from Microorganisms and Its Industrial Applications. The Scientific World Journal. 2022; 2022(1): 1881305.

[9] Pang CY, Wang H, Pang Y, Xu C, Jiao Y, Qin YM *et al.* Comparative Proteomics Indicates That Biosynthesis of Pectic Precursors Is Important for Cotton Fiber and Arabidopsis Root Hair Elongation. Molecular and Cellular Proteomics. 2010 Sep; 9(9): 2019-33.

[10] Ashaolu TJ, Lee CC, Ashaolu JO, Tarhan O, Pourjafar H, Jafari SM. Pepsin: An Excellent Proteolytic Enzyme for The Production of Bioactive Peptides. Food Reviews International. 2024 Oct; 40(7): 1875-912.

[11] Sujitha P and Shanthi C. Importance of Enzyme Specificity and Stability for the Application of Proteases in Greener Industrial Processing-A Review. Journal of Cleaner Production.2023 Nov; 425: 138915.

[12] Shrestha S, Rahman MS, Qin W. New Insights in Pectinase Production Development and Industrial Applications. Applied Microbiology and Biotechnology. 2021 Dec; 105: 9069-87.

[13] Yasmin I, Saeed M, Pasha I, Zia MA. Development of Whey Protein Concentrate-Pectin-Alginate Based Delivery System to Improve Survival of B. Longum BL-05 In Simulated Gastrointestinal Conditions. Probiotics and Antimicrobial Proteins. 2019 Jun; 11: 413-26.

[14] Haile S, Masi C, Tafesse M. Isolation and Characterization of Pectinase-Producing Bacteria (Serratia Marcescens) From Avocado Peel Waste for Juice Clarification. Biomed Central Microbiology. 2022 May; 22(1): 145.

[15] Alzahrani OM, Sohail M, Mahmoud SF, Alswat AS, El-Halmouch Y. Preparation of a Pectinase-Enriched Multi-enzyme under Solid State Fermentation of Sugarcane Bagasse. Fermentation. 2023 Jan; 9(2): 141.

[16] Oumer OJ and Abate D. Screening and Molecular Identification of Pectinase-Producing Microbes from Coffee Pulp. BioMed Research International. 2018; 2018(1): 2961767.

[17] KC S, Upadhyaya J, Joshi DR, Lekhak B, Kumar Chaudhary D, Raj Pant B *et al.* Production, Characterization, and Industrial Application of Pectinase Enzyme Isolated from Fungal Strains. Fermentation. 2020 Jun; 6(2): 59.

[18] De Alencar Guimarães NC, Glienke NN, Galeano RM, Ruller R, Zanoelo FF, Masui DC *et al.* Polygalacturonase from Aspergillus Japonicus (Pgaj): Enzyme Production Using Low-Cost Carbon Source, Biochemical Properties and Application in Clarification of Fruit Juices. Bio-Catalysis and Agricultural Biotechnology. 2022 Jan; 39: 102233.

[19] Jiang Y, Ye J, Zhou L, Chen M, Wei Y, Jiang S *et al.* Cell Wall Metabolism During the Growth of Peach Fruit: Association of Hardness with Cell Wall Pectins. Scientia Horticulturae. 2024 Apr; 330: 113058.

[20] Sumiyoshi M, Inamura T, Nakamura A, Aohara T, Ishii T, Satoh S *et al.* UDP-Arabinopyranose Mutase 3 Is Required for Pollen Wall Morphogenesis in Rice (Oryza Sativa). Plant and Cell Physiology. 2015 Feb; 56(2): 232-41.

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Effect of β-Galacto-Oligosaccharides on Growth Performance in Physiologically Stressed Rabbits

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ABSTRACT

Growth performance analysis under this study evaluated the dietary impact of β-galacto-oligosaccharides (β-GOS). Scientists have established that prebiotic β-GOS promotes health in the gut tissue, thus leading to enhanced productivity alongside improved general health during dexamethasone-induced stress conditions. **Objectives:** To investigate whether the addition of β-GOS to rabbit diets would positively affect their physiology-linked weight gain as well as their feed efficiency and developmental metrics. **Methods:** A 12-week experiment with 40 rabbits belonging to 3 ± 0.98 months of age and weighing 1.48 ± 0.41 kg allocated 8 animals to each dietary treatment group. Among all monitored aspects, live body weight and food intake, and feed conversion efficiency (FCR) were ranked as significant items. Rabbits received feed consumption measurements each day and underwent body weight assessment every week. **Results:** Feeding rabbits β-GOS under stress greatly improved their health and growth performance. Compared to Groups A-D, Group E (D-S15 + 0.3% β-GOS) had the greatest FCR and the largest gain in live body weight. The group that consumed the most feed was Group A (Negative Control). Group E. Conclusions showed the most efficient feed conversion. **Conclusions:** It was concluded that supplementing stressed rabbits with β-GOS can successfully increase their growth, feed conversion efficiency, and general health, providing a useful dietary strategy to alleviate growing deficits caused by stress.

INTRODUCTION

Rabbits are regarded as a prime candidate for meat production due to their brief life span, high prolificacy, brief gestational duration and efficient feed conversion capacity on both grain and forage-based diets [1]. These monogastric hindgut fermenters exhibit a distinctive digestive physiology that enables them to acquire proteins and vitamins via caecotrophy. However, the consumption of rabbits has experienced a worldwide decrease due to concerns regarding consumer approval and the extended duration needed for cooking [2]. Prebiotics and probiotics have emerged as promising strategies for mitigating digestive tract diseases in livestock and enhancing the productivity of the animals. These dietary supplements effectively inhibit carcass contamination while augmenting immune responses among animals. Prebiotics, non-digestible dietary components, selectively promote the growth of beneficial microorganisms in the colon. Several functional oligosaccharides, including galacto-oligosaccharide, mannan-oligosaccharide, chito-oligosaccharide, and fructo-oligosaccharide, have been shown to enhance growth performance and improve the well-being of rabbits [3]. β-Galacto-Oligosaccharide is widely acclaimed for its lacto-bifidogenic property, which enhances gastrointestinal health structure & immune function across both humans and animals [4-6]. However, β-GOS failed to improve broilers' performance under

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thermoneutral conditions originating from Bifidobacterium galactosidase, suggesting variable effects between species [7]. Insufficient information exists regarding the effects of β-GOS on rabbit growth performance in instances of heat stress [8].

This study aims to evaluate the effect of dietary β-galacto-oligosaccharide on the growth performance of rabbits undergoing physiological stress.

METHODS

Experimental design was used in this study. The rabbits were housed at Animal House, Sindh Agriculture University in Tandojam for a comprehensive study conducted over twelve weeks. A total of forty rabbits, consisting of both male and female individuals aged 3 + 0.98 months old, with an average weight of 1.48 ± 0.41 kg. The rabbits were randomly allocated into five distinct groups, with eight rabbits assigned to each group [9-11]: namely, Group-A=Negative Control (No stress; 0% β-GOS) Group-B=Positive Control-Dexa-stressed 15 mg/kg (D-S 15+0% β-GOS), Group-C (D-S 15+0.1% β-GOS) Group-D (D-S 15+0.2% β-GOS) Group-E (D-S 15 + 0.3% β-GOS). The rabbits were allowed adlib for water via the nipple system. Weekly feed consumption and body weight were recorded, while daily assessments of feed consumption, body weight increase, and feed conversion ratio (FCR) were computed by Abdelatty *et al.*, [12].A basal diet comprising ingredients was administered as control, along with dexamethasone and varying percentages of β-GOS.The feed formula consists of 30% corn, 25% soybean meal, 9% wheat bran, 30% rice husk, 3% fishmeal, 2% di-calcium phosphate, 0.5% salt and 0.45% vitamin-mineral premix per kilogram. Methionine is present at a concentration of .03 % (per kg) while Lysine is present at a concentration of .02 % (per kg). The proximate composition includes metabolizable energy at a rate of 2744 kcal/kg, dry matter 870.47 g/kg and crude protein 180.16 g/kg.Additionally, the feed contains crude fiber (120.64 g/kg), ether extract (110.62 g/kg) and ash (120.05g/kg).Additionally, the antioxidant and carrier limestone CaCO3 [13].All male rabbits (n=8 per treatment) were utilized for the evaluation of growth performance.The weight of the rabbits was documented every week throughout the entirety of the 12-week experiment.The initial weight of each rabbit was recorded upon their arrival, and subsequently measured every week to track their weight gain progress within each group.Fresh food was generously given to the rabbit, and any instances of rejection were noted, measured, and then deducted from the total amount delivered.The final quantity of food consumed was then recorded using the following formula. Motor calculation of feed intake requires subtracting group-level feed denial (g/group/d) from total feed offering (gm).This operation yields the direct product of feed intake (g/b/d).The researchers estimated feed conversion ratio through precise measurements of personal weight growth and eaten feed weight during the 12-week evaluation period using the simple calculation: total consumed feed/total gained weight.The weekly feed intake calculation was performed using this data to compute the FCR ratio through the equation: Feed intake / Body Weight growth. The FCR calculation for each rabbit proceeded through DMI/ADG ratio assessment after researchers measured and recorded beginning and ending body weights and ADG and DMI data. A fully randomized design (CRD) was used to analyze the data collected through the statistical program for social sciences (SPSS version 20.0) by presenting mean ± standard error of the mean (SEM).Tukey's Test evaluated group differences using $p<0.05$ as the significance threshold.

RESULTS

Data indicate that significantly maximum ($p<0.05$) live body weight (2400 ± 61g) was noted in group E (basal diet + D-S 15 + 0.3% β-GOS) as compared to Group-B (Negative control No stress, 0% β-GOS), group C (basal diet + D-S 15 + 0.1% β-GOS) and Group-D (basal diet + D-S 15 + 0.2 β-GOS) with average live body weight (2200 ± 48g, 2300 ± 32g and 2350 ± 40g), respectively. Significantly minimum ($p<0.05$) live body weight (2100 ± 40g) was recorded from group A=Positive Control-Dexa- stressed 15 mg/kg (D-S 15+0% β-GOS). Statistical analysis of the live body weight data between groups revealed essential differences based on the $p<0.05$ criterion. Five separate groups exhibited significant differences from each other according to the Tukey HSD test results. The research findings indicated that β-galacto-oligosaccharides reacted according to the body weight levels of the rabbits. The results regarding β-galacto-oligosaccharides supplement impacts on rabbit live body weight, presented in Figure 1.

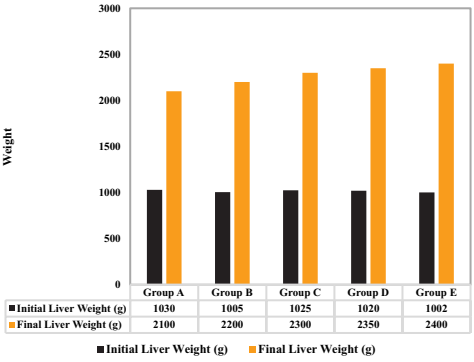

Figure 1: Effect of β-Galacto-Oligosaccharides Supplementation on Body Weight (G) of Growing Rabbits

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Data indicates that significantly maximum ($p<0.05$) feed intake (220 ± 4g) was noted in Group A=Positive Control-Dexa-stressed 15 mg/kg (D-S 15+0% β-GOS), as compared to Group B (Negative control No stress, 0% β-GOS), Group C (basal diet + D-S 15 + 0.1% β-GOS) and Group D (basal diet + D-S 15 + 0.2 β-GOS) with average feed intake (215 ± 6, 210 ± 3g and 200 ± 4g), respectively. Significantly minimum ($p<0.05$) feed intake (190±3g) was recorded from Group E (basal diet + D-S 15 + 0.3% β-GOS). All groups showed distinct ($p<0.05$) differences in their feed consumption based on statistical evaluation of gathered data.The data analysis demonstrated four distinct groups as separated by Tukey's HSD test to each possess a different level of significance when compared to other groups This indicated that the effect of β-galacto-oligosaccharides was dose-dependent on the feed intake of the rabbits. Results on the effects of β-galacto-oligosaccharides supplementation on feed intake of rabbits are presented in Figure 2.

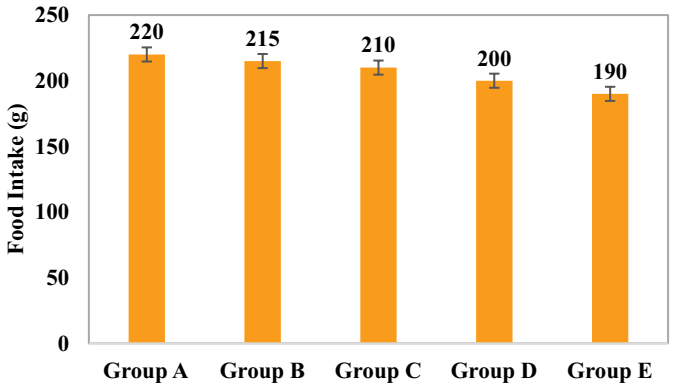

Figure 2: Effect of β-Galacto-Oligosaccharides Supplementation on Feed Intake (G) of Growing Rabbits

Data indicates that significantly maximum ($p<0.05$) body weight gain (80 ± 0.5 g) was noted in Group E (basal diet + D-S 15 + 0.3% β-GOS) as compared to Group B (Negative control No stress, 0% β-GOS), Group C (basal diet + D-S 15 + 0.1% β- GOS) and Group D (basal diet + D-S 15 + 0.2 β-GOS) with average body weight gain (60 + 0.8, 70 + 0.2 and 75 ± 0.5g), respectively. Significantly minimum ($p<0.5$) body weight gain (50 ± 0.4g) was recorded from Group A = Positive Control-Dexa-stressed 15 mg/kg (D-S 15+0% β-GOS). Statistical analysis of data revealed a significant ($p<0.05$) difference in body weight gain among all groups. According to Tukey's HSD test, there were five distinct groups which were significantly different from each other. This indicated that the effect of β-galacto-oligosaccharides was dose-dependent on live body weight gain of the rabbits. Results on the effects of β-galacto-oligosaccharides supplementation on body weight gain of rabbits are mentioned in Figure 3.

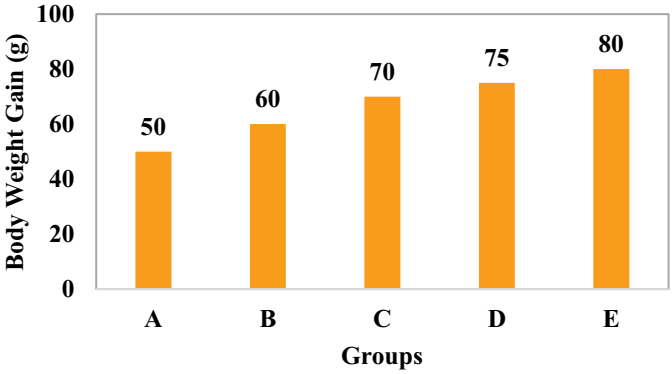

Figure 3: Effect of **B-**Galacto-Oligosaccharides Supplementation on Body Weight Gain (G) of Growing Rabbits

Data indicates that significantly minimum ($p<0.05$) FCR (2.38 ± 0.2) was noted in group E (basal diet + D-S 15 + 0.3% β-GOS) as compared to Group B (Negative control No stress, 0% β-GOS), Group C (basal diet + D-S 15 + 0.1% β-GOS) and Group D (basal diet + D-S 15 + 0.2 β-GOS) with average live body weight average FCR (3.58 + 0.02, 3.00 ± 0.3 and 2.67 ± 0.2), respectively. Significantly maximum ($p<0.05$) FCR (4.4 ± 0.01) was recorded from Group A=Positive Control-Dexa-stressed 15 mg/kg (D-S 15+0% β-GOS). Statistical analysis of data revealed a significant ($p<0.05$) difference in FCR among all groups. According to Tukey's HSD test, there were five distinct groups which were significantly different from each other. This indicated that the effect of β-galacto-oligosaccharides was dose-dependent on the FCR of the rabbits. Results on the effects of β-galacto-oligosaccharides supplementation on FCR of rabbits are mentioned in Figure 4.

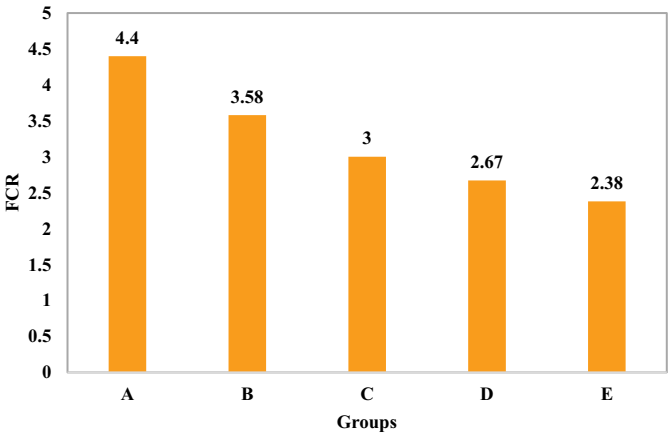

Figure 4: Effect of **B-**Galacto-Oligosaccharides Supplementation on the Feed Conversion Ratio of Growing Rabbits

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DISCUSSION

Studies have shown that incorporating prebiotics in the diet of broiler chickens can enhance growth by using non-antibiotic supplements, leading to improvements in growth parameters without any adverse effects on consumers [14]. Moreover, the discoveries regarding the beneficial impacts of prebiotics on growth performance were observed [15]. Beta-Galacto-oligosaccharide (Beta-GOS) promotes the proliferation of beneficial microorganisms in the gastrointestinal tract, particularly bifidobacteria and lactobacilli, consequently enhancing gut health and immune response [16]. Improved gastrointestinal health can result in enhanced breakdown and absorption of nutrients, thereby leading to increased utilization of feed and, subsequently, enhanced growth performance. Research has indicated that incorporating β-GOS into the diet can enhance intestinal health, boost the immune system, and stimulate growth performance in poultry [17], pigs [18], and fish [19]. The inclusion of dexamethasone in the rabbit's diet led to a reduced amount of weight loss when compared to the control group. Although previous studies have shown comparable effects on body weight in different animal species, our results differed from these findings. These variations may be attributed to individual differences and inconsistencies in the experimental approaches. Numerous variables, such as environment, diet, and heredity, affect the complex system of weight control [20, 21].Our results are in line with other studies that indicate stress has a detrimental effect on rabbits' ability to develop [22, 23]. Compared to the healthy control group, the rabbits in the positive control group gained less weight, fed less effectively, and had a higher feed conversion ratio.The study found that supplementing with β-GOS improved growth performance. When contrasted to the untreated stressed group, our research showed that the rabbits who got β-GOS supplementation had significantly higher body weight growth and greater utilization of feed.A notable improvement in the feed conversion ratio suggests that feed resources are being used more efficiently. As seen by Group B's slower growth rate than all other groups, our study's astounding findings exposed the detrimental impacts of stress on rabbits' development capabilities. This discovery aligns with prior research suggesting that stress can result in diminished weight gain, decreased feed intake, and reduced feed efficiency [22, 23].Rabbits in Groups C-E with β-GOS had higher body weights than Groups A-B with basal diet or basal diet with dexamethasone.Study findings are consistent with past research, showing prebiotics improve weight gain in animal models [24].Rabbits given Biotronic showed improved growth, consistent with the study [25] on prebiotics and animal growth.Prebiotic supplementation can lead to increased body weight in animals due to various mechanisms. Prebiotics act as substrates for beneficial gut bacteria, promoting their growth and metabolic functions, resulting in increased fermentation and production of short-chain fatty acids. The energy usage efficiency improved due to Biotronic consumption leading to weight gain in rabbits. The use of prebiotics improves nutrient absorption that accelerates both growth and body weight increase. The scientific research on prebiotics reveals that they strengthen animal immunity while improving nutrient uptake in animals and reducing stress effects on growing efficiency. This research shows Biotronic alongside β-galacto-oligosaccharide cause rabbits to gain more weight over 12 weeks. Biotronic acts to enhance immune response alongside energy management and nutritional uptake, thus supporting constant growth [26]. During a 12-week experiment, the rabbits fed with the standard diet (Group A-B) ate greater daily amounts than those consuming Biotronic prebiotic (Group C-E). Animal appetite and feed intake increase when prebiotics, including Biotronic, are added to the diet. The production of short-chain fatty acids increases through prebiotic support of gut bacterial growth. The research on rabbit populations indicates that Biotronic supplementation drives animals to consume more food because of its effects on both appetite and nutritional absorption. The supplements improve consumption by enhancing food taste as well as digestive performance. Professional studies verify that prebiotics enhance food consumption in animals, including pigs, through their effects on intestinal microorganisms and digestive processes. Research studies show that rabbits who receive prebiotic supplements in Biotronic eat more food because of hunger stimulation and better flavor absorption, and nutrient utilization [27]. Rabbits measure feed efficiency through the analysis of feed conversion ratio (FCR). The FCR measurements for Group C-E subjects revealed better numbers than those recorded for Group A-B subjects.The feed conversion ratio improves according to these results. Oral consumption of prebiotics helps rabbits absorb their diet better and leads to a reduction in feed conversion ratio. Prebiotic supplements operate as a dual-purpose agent for both immune system enhancement and the treatment of stress-triggered efficiency breakdowns. Research demonstrates that prebiotic consumption leads to better FCR according to [28]. The results of this study established that prebiotic dietary supplements produce favorable impacts on growth indicators. These substances both reduce the feed conversion ratio and enhance overall physical health. The New literature [29] established that prebiotics improve rabbit development as reported by [30]. To fully understand how these supplements affect the immune system and gut flora, further study is needed.

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CONCLUSION

It was concluded that supplementing stressed rabbits with β-GOS can successfully increase their growth, feed conversion efficiency, and general health, providing a useful dietary strategy to alleviate growing deficits caused by stress.

Authors Contribution

Conceptualization: JAS
Methodology: JAS, GAM, TJS
Formal analysis: ABK, SAS, GAM
Writing review and editing: JAS

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

Conflicts of Interest

All the authors declare no conflict of interest.

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REFERENCES

[1] Siddiqui SA, Gerini F, Ikram A, Saeed F, Feng X, Chen Y. Rabbit Meat—Production, Consumption and Consumers' Attitudes and Behavior.Sustainability. 2023 Jan; 15(3): 2008. doi: 10.3390/su15032008.

[2] Wu L. Worldwide Rabbit Meat Trade. In Veterinary Care of Farm Rabbits: A Complete Practice Guide to Rabbit Medicine and Production.Cham:Springer International Publishing.2024May:67-92.doi:10.1007/978-3-031-44542-2_4.

[3] Shaikh JA, Kachiwal AB, Soomro SA, Mughal GA. Evaluation of the Influence of Β-Galacto-Oligosaccharides On Hepatic Functions and Blood Metabolites in Physiologically Stressed Rabbits. International Journal of Stress Management.2024;31(4):127-160.

[4] Khan FA, Kachiwal AB, Khaliq J, Soomro SA, Ahmad T, Khan MB *et al.* Effect of Supplementation of B-Galacto-Oligosaccharide And Probiotic Mixture On Growth Performance Of Physiologically Stressed Rabbits.Journal of Advanced Zoology.2024Dec;45(6): 311-321. doi:10.53555/jaz.v45i6.5023.

[5] Abd El-Aziz A, Aboghanima M, Kamel S, El-Deeb E, Mahrous UE. Influence of Two Dietary Prebiotic Oligosaccharides Supplementation On Productive Performance and Carcass Traits with Special Attention to Their Biochemical Alterations in Two Rabbit Breeds.Damanhour Journal of Veterinary Sciences.2020Jan;3(1):1-7.doi:10.21608/djvs.2019.19337.1005.

[6] Hu Y, Aljumaah MR, Azcarate-Peril MA. Galacto-Oligosaccharides and the Elderly Gut:Implications for Immune Restoration and Health.Advances in Nutrition.2024Aug;15(8):100263.doi:10.1016/j.advnut.2024.100263.

[7] Mei Z, Yuan J, Li D. Biological Activity of Galacto-Oligosaccharides: A review.Frontiers in Microbiology.2022Sep;13:993052.doi:10.3389/fmicb.2022.993052.

[8] Ahmad S, Yousaf MS, Tahir SK, Rashid MA, Majeed KA, Naseem M *et al.* Effects of Co-Supplementation of β-Galacto-Oligosaccharides and Methionine on Breast Meat Quality, Meat Oxidative Stability and Selected Meat Quality Genes in Broilers.Pakistan Veterinary Journal.2023Jul;43(3).doi:10.29261/pakvetj/2023.043.

[9] Charan J and Kantharia N. How to calculate Sample Size in Animal Studies? Journal of Pharmacology and Pharmacotherapeutics.2013Dec;4(4):303-6.doi:10.4103/0976-500X.119726.

[10] Ko MJ and Lim CY. General Considerations for Sample Size Estimation in Animal Study.Korean Journal of Anesthesiology.2021Feb;74(1):23-9.doi:10.4097/kja.20662.

[11] Abd El-Aziz AH, Abo Ghanima MM, Alsanie WF, Gaber A, Alsenosy AE, Easa AA *et al.* Fructooligosaccharide Supplementation Boosts Growth Performance, Antioxidant Status, and Cecal Microbiota Differently in Two Rabbit Breeds.Animals.2022Jun;12(12):1528.doi:10.3390/ani12121528.

[12] Abdelatty AM, Mohamed SA, Moustafa MM, Al-Mokaddem AK, Baker MR, Elolimy AA *et al.* Nutrigenomic Effect of Conjugated Linoleic Acid On Growth and Meat Quality Indices of Growing Rabbit. PLoS One.2019Oct;14(10):e0222404.doi:10.1371/journal.pone.0222404.

[13] Memon S, Kachiwal AB, Malhi MC, Mughal GA. Effects of Dietary Prebiotics, Probiotics and Symbiotics On Growth Performance, Carcass Characteristics, Body Condition Score and Nutrients Digestibility of Rabbits.Jammu Kashmir Journal of Agriculture. 2024 Apr;4(1): 1-3.

[14] Khabirov A, Khaziakhmetov F, Kuznetsov V, Tagirov H, Rebezov M, Andreyeva A *et al.* Effect of Normosil Probiotic Supplementation on the Growth Performance and Blood Parameters of Broiler Chickens. Indian Journal of Pharmaceutical Education and Research. 2021; 55(1).

[15] Wang X, Farnell YZ, Peebles ED, Kiess AS, Wamsley KG, Zhai W. Effects of Prebiotics, Probiotics, and Their Combination On Growth Performance, Small Intestine Morphology, and Resident Lactobacillus of Male Broilers.Poultry Science.2016Jun;95(6):1332-40.doi:10.3382/ps/pew030.

DOI: https://doi.org/10.54393/fbt.v5i1.110

[16] Lee A, Mansbridge SC, Liang L, Connerton IF, Mellits KH. Galacto-Oligosaccharides Increase the Abundance of Beneficial Probiotic Bacteria and Improve Gut Architecture and Goblet Cell Expression in Poorly Performing Piglets, But Not Performance. Animals.2023Jan;13(2):230.doi:10.3390/ani13020230.

[17] Ahmad S, Yousaf MS, Tahir SK, Mustafa G, Majeed KA, Rashid MA *et al.* Effects of Co-Supplementation of β-Galacto-oligosaccharides and Methionine on Production Performance, Blood Metabolites, and Gut Histomorphometry in Broilers.Pakistan Veterinary Journal.2022Apr;42(2).doi:10.29261/pakvetj/2022.031.

[18] Stanley J. Characterisation of Bifidobacterium Induced from Prebiotic (Galactooligosaccharide) Supplemented Pigs (Doctoral dissertation, University of Nottingham). 2021.

[19] Keser O. Prebiotic Oligosaccharides As Feed Additives In Fish Farming.2024 Dec: 29.

[20] Shaikh JA, Kachiwal AB, Soomro SA, Mughal GA. Evaluation of Physiological Stress Induced by Dexamethasone On Growth Performance, Bone Morphometry and Meat Quality Parameters in Rabbits. Global Scientific Journals.2024 Jan; 12(1).

[21] MF MA and Abbas BA. Effect of Long-Term Treatment with Dexamethasone on the Liver and Kidney Histopathology, As Well as Blood Biochemistry in Male Rabbits (Lepus Cuniculus).Archives of Razi Institute.2022 Febb;77(1):333.

[22] Abdelnour SA, El-Saadony MT, Saghir SA, Abd El-Hack ME, Al-Shargi OY, Al-Gabri N *et al.* Mitigating Negative Impacts of Heat Stress in Growing Rabbits Via Dietary Prodigiosin Supplementation.Livestock Science.2020Oct;240:104220.doi:10.1016/j.livsci.2020.104220.

[23] Jaén-Téllez JA, Sánchez-Guerrero MJ, Valera M, González-Redondo P. Influence of Stress Assessed Through Infrared Thermography and Environmental Parameters on the Performance of Fattening Rabbits.Animals.2021Jun;11(6):1747.doi:10.3390/ani11061747.

[24] Smith J. Impact of B-galacto-oligosaccharide Supplementation on Nutrient Utilization and Feed Conversion Efficiency in Rabbits. Animal Nutrition. 2020;42(3):234-45.

[25] Nwachukwu CU, Aliyu KI, Ewuola EO. Growth Indices, Intestinal Histomorphology, And Blood Profile of Rabbits Fed Probiotics and Prebiotics-Supplemented Diets.Translational Animal Science. 2021Jul; 5(3):txab096.doi:10.1093/tas/txab096.

[26] Wilson TD, Reinhard DA, Westgate EC, Gilbert DT, Ellerbeck N, Hahn C *et al.* Just Think: The Challenges of the Disengaged Mind.Science.2014Jul;345(6192): 75-7.doi:10.1126/science.1250830.

[27] Garcia MA. Effects of Dexamethasone On White Blood Cell Count in Rats. Journal of Experimental Medicine. 2018;205(3): 527-32.

[28] Piles M, Mora M, Kyriazakis I, Tusell L, Pascual M, Sánchez JP. Novel Phenotypes of Feeding and Social Behaviour and Their Relationship with Individual Rabbit Growth and Feed Efficiency. Animal.2024Mar; 18(3): 101090. doi: 10.1016/j.animal.2024.101090.

[29] Rafiq K, Tofazzal Hossain M, Ahmed R, Hasan MM, Islam R, Hossen MI *et al.* Role of Different Growth Enhancers as an Alternative to In-Feed Antibiotics in the Poultry Industry. Frontiers in Veterinary Science. 2022 Feb; 8: 794588. doi: 10.3389/fvets.2021.794588.

[30] Yuzviak MO, Lesyk YV, Salyha YT.Prospects for the Use of Minerals in Rabbit Nutrition.Publishing House "Baltija Publishing".2023Jun.doi:10.30525/978-9934-26-316-3-10.

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Original Article

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Development of Plant-Based Milk by Combining Sesame Seeds, Pumpkin Seeds, Mango-Flavored Sesame, and Dates Flavored Sesame

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ABSTRACT

Despite the increased demand for plant-based milk alternatives, there is a lack of sustainable and nutritious options that combine the nutritional benefits of sesame and pumpkin seeds. **Objectives:** To develop plant-based milk by combining sesame seeds and pumpkin seeds, assess the sensory and nutritional properties by using a hedonic scale and proximate analysis and do a cost analysis of this plant-based milk. **Methods:** A cross-sectional study took place at the lab of UVAS Lahore, Pakistan.Three samples were developed per 100ml (sesame and pumpkin seeds milk, mango flavored sesame and pumpkin seeds milk and dates flavored sesame and pumpkin seeds milk).Sensory evaluation was done using a hedonic scale. Proximate analysis was also performed to calculate dry matter, crude protein, fat and ash content of the three samples. **Results:** Date flavor was the most liked sample (40%) rating in appearance, 50% in odor, 50% in taste and 60% in texture. Proximate analysis of the first sample (sesame and pumpkin seeds milk) showed that it contained about 19.17% dry matter, 7.04% crude protein, 2.20% fat and 0.90% ash content. Proximate analysis of the second sample (mango flavor) showed about 13.00% dry matter, 3.60% crude protein, 4.00% fat and 0.46% ash content. Proximate analysis of the third sample (dates flavor) achieved 15.67% dry matter, 3.74% crude protein, 5.00% fat and 0.38% ash content. **Conclusions:** It was concluded that it is easier to make this plant-based dish using ingredients which are easily available from the local store.

INTRODUCTION

The consumers have developed a great interest in consuming those products that are made from vegetable raw materials. Among the population, there is a great increase in the number of allergic diseases. These allergies affect both children and adults equally. Cow's milk protein or lactose allergy is one of the most prevalent allergies which due to some enzymatic changes, the body is not able to absorb lactose. These alternatives were being developed because the cow's milk contains up to 80% casein in its protein composition, while the plant-based milk alternatives do not contain lactose and casein [1]. Sesame seed is unusual in its composition, containing significant levels of crude protein, moisture, crude fat, carbs, crude fiber, and mineral components. It is observed that sesame seeds contain 4.53% of moisture, 22.41% crude protein, 41.20% crude fat, 3.42% crude fiber and 4.27% ash content. Sesame seeds are known not only for their traditional use but also for their pharmaceutical, nutraceutical and industrial roles [2]. Sesame milk contains nutraceutical properties such as anti-oxidative, hypocholesterolemia, anti-carcinogenic, anti-tumor and antiviral activities [3]. Sesame milk can overcome the restrictions of soy milk use, such as flatulence, allergy to soy proteins, and off-flavors [4]. Cold plasma processing is a non-thermal technique which can be used to reduce the Ige-mediated allergies caused by sesame milk [5]. Pumpkin belongs to the Cucurbita species and family Cucurbitaceae. There are 3 species of pumpkin, i-e.

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cucurbita maxima, cucurbita pepo, and cucurbita moschata [6]. It is a diploid plant that usually contains 20 pairs of chromosomes. The use of pumpkin seeds is different, as in domestic purposes, uncooked, cooked or roasted depending on the area and personal choices of the people [7].Pumpkin seeds consist of many beneficial nutrients and nutraceuticals such as curcubitans, unsaturated fatty acids, amino acids, phyto-sterols and tocopherols.They also contain zinc, iron, calcium, potassium, copper, phosphorus, magnesium and manganese. The calories in pumpkin seeds vary, but they usually include 574 calories of energy per 100 grams, 49 grams of fat, 6.6 grams of fiber, and 30 grams of protein. Pumpkin seeds are a fantastic addition to a healthy diet due to their high protein content.Pumpkin seeds also possess cyto-protective effects (the ability to protect the cells from metabolic attacks). Along with pumpkin seeds also have antimicrobial and wound healing effects [8].Pumpkin seeds have antioxidant properties.Thus, they are considered a valuable ingredient to be added to food products. Lack of beneficial nutrients is a major concern in the world. To overcome this problem, food additives can be used, originating from plant sources, and pumpkin seeds are one of them. By adding pumpkin seed flour, the nutritional quality of the product can be enhanced or enriched [9]. By producing biscuits made with ingredients like pumpkin seed meal, konjac and maltitol, it is proven that biscuits can be made healthier, more nutritious and can be beneficial for cardiac, diabetic and obese patients [10]. Pumpkin seed oil (PSO) is extremely beneficial not only to diabetics, but also to hypertension and dyslipidemia [11]. Heat treatment is the most crucial step to enhance the stability, improve the physicochemical properties of food products such as pumpkin seeds [12]. Pumpkin seeds prevent stomach, lung, or colon cancers [13]. In Korea, pumpkin seeds are used for the treatment of depression [14]. The use of pumpkin seeds daily reduces the chance of Parkinson's and Alzheimer's diseases [15]. In previous studies, there was a lack of a plant-based milk which was made from combining two or more seeds.

This study aims to develop a plant-based milk by combining sesame seeds and pumpkin seeds, and also produces two more flavors using mango and dates. The sensory and nutritional properties were also evaluated by using the hedonic scale and proximate analysis. Along with that, a cost analysis of this plant-based milk was also performed.

METHODS

This was a cross-sectional study in which a product was developed and given to the expert panel, then compared for differences based on sensory evaluation. This study took place at the lab of the University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. All the ingredients were bought from the local market.For the preparation of the product, a blender, mesh filter, measuring cups and bottles were used.A product was developed using two types of seeds.Along with that, two more flavors were produced using mango and dates.Four samples were produced. These samples were collected in 100 ml bottles. Quantities of ingrédients were sesame seeds (125g), pumpkin seeds (14g), mango (30g), and dates (72g).For the first sample (sesame and pumpkin seed milk), about 41.6 grams of sesame seeds, 4.6 grams of pumpkin seeds were taken. These seeds were roasted for 1 minute. Then blended with the help of a blender by adding 2.5 cups of water. After blending it, it was filtered out with the help of a mesh filter. Milk of thin consistency was obtained. For the preparation of the second sample using mango (Sindri), all the above steps were repeated. Mango pulp was extracted using a strainer. About 2.5 tablespoons of mango pulp were added to 1/2 cup of milk. Then the remaining pulp was strained using a mesh filter. For a thin consistency, water was added.For the preparation of the third sample, dates were used. All the steps of the first sample were repeated. After that, about 3 dates were added to 1/2 cup of milk. Then it was blended using a blender.It was strained. To achieve a thin consistency, a few drops of water were added. After the development of the samples, sensory evaluation (appearance, texture, smell, taste) was performed by the expert panel of 10 panelists. The hedonic scale was used for sensory evaluation. Hedonic scale used ratings from 1 to 9. 1 denotes extremely dislike, and 9 denotes extremely like. To calculate the dry matter, fat, protein, and ash content of the three samples, proximate analysis was conducted. For dry matter analysis, a dish was weighed before putting the sample on it (W1).All the samples were put into their respective dishes and weighed before drying (W2). After weighing the samples, it was dried in the oven at 102 degrees Celsius until the dry matter was obtained. Then the weight of the samples was recorded again, and dry matter was analyzed (W3). Dry matter = $W2 - W3 \div W2 - W1 * 100$. For fat analysis, the Gerber machine method was used.The Gerber machine uses centrifugal force to extract the fat content of the given sample. For this purpose, sulphuric acid of about 10 ml was taken and pipetted into the butyrometer. Along with that, a quantity of 10 ml of the sample was added to the sulphuric acid. Following this, about 1 ml of amyl alcohol was added to the mixture. It was shaken until the milk was completely absorbed by the acid. After this, the butyrometer was placed in a water bath for about five minutes at 65 degrees, and then it was centrifuged in a Gerber machine for five minutes. This step

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was repeated. The final reading of the fat content was recorded. SNF content (%) = Total solids (%) - Fat (%) (3). For the determination of ash content, a dish was weighed first (W1), then the sample was put into the dish after weighing it (W2), and the sample was heated in a furnace at about 500 degrees until a grey colour of ash appeared. The final readings were calculated for the ash content. % Ash = W3 - W1 ÷ W2 - W1 * 100. All the data were analyzed by using SPSS version 26.0 computer software. The data were expressed in the form of pie charts, and bar charts were made with the help of MS EXCEL.

RESULTS

In the sensory evaluation of Sample A, out of all sensory characteristics, the appearance of sesame and pumpkin milk was liked by the majority (60%) (Figure 1).

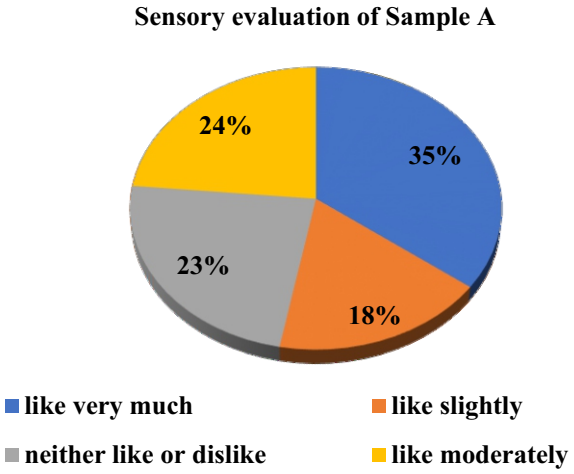

Figure 1: Sensory Evaluation of Sample D

For mango flavored sesame milk (Sample B), all the sensory attributes got an average rating of 30% from the panelists (Figure 2).

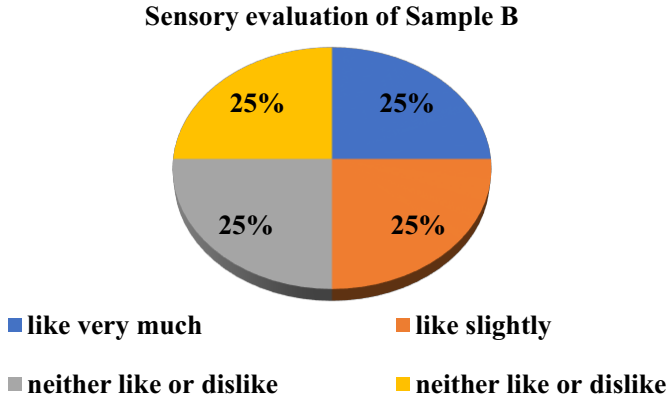

Figure 2: Sensory Evaluation of Sample B

For the dates flavour of the sesame and pumpkin seeds milk (Sample C), texture was the sensory quality that was liked by 60% of the panelists.But out of three samples, Sample C was the most liked one (Figure 3).

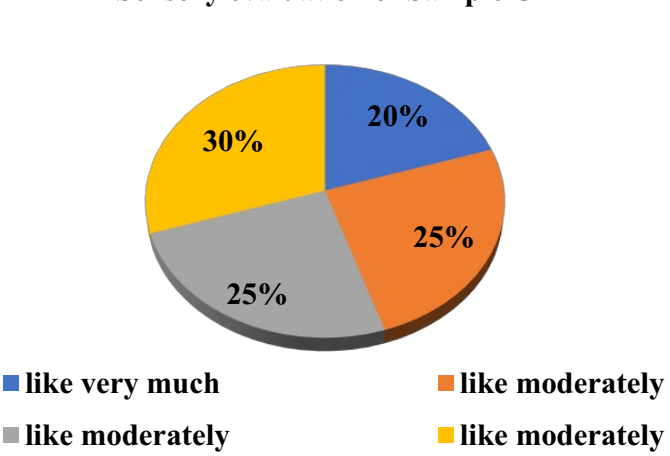

Figure 3: Sensory Evaluation of Sample C

Proximate analysis of the sesame and pumpkin seeds milk (Sample A) showed that it contained about 19.17% dry matter, 7.04% crude protein, 2.20% fat and 0.90% ash content. Proximate analysis of the mango flavour (Sample B) showed about 13.00% dry matter, 3.60% crude protein, 4.00% fat and 0.46% ash content. Proximate analysis of the date's flavor (Sample C) achieved 15.67% dry matter, 3.74% crude protein, 5.00% fat and 0.38% ash content. According to proximate analysis of the three samples, Sample A (sesame and pumpkin seeds milk) contains the highest amount of dry matter, crude protein, ash content and the lowest amount of fat as compared to Sample B and C (Figure 4).

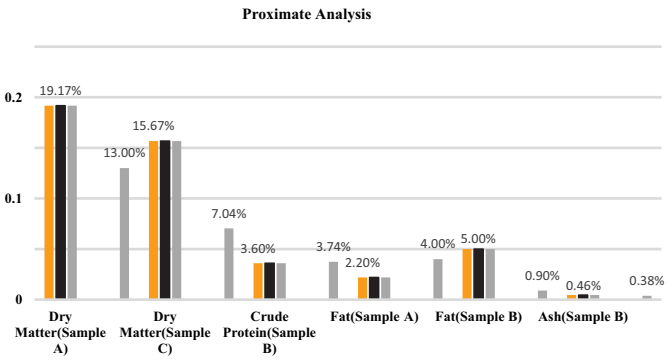

Figure 4: Proximate Analysis of the A, B and C Samples

Cost analysis of the sesame and pumpkin seeds (Sample A) showed that it costs Rs 58.6 per 100ml. Cost analysis of the mango flavor (Sample B) showed that it costs Rs 64.6 per 100ml. Cost analysis of the dates flavor (Sample C) showed that it costs Rs116.2 per 100ml. After assessing the cost analysis of the three samples, it was found that Sample A was the cheapest one, with the price of 58.6 rupees per 100 ml (Table 1).

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Table 1: Cost Analysis of Sample A, Sample B and Sample C

Sesame and Pumpkin Seed Milk per 100 ml			
Ingredients	**Quantity Used (g)**	**Price in Rupees**	**Price in Rupees per Quantity Used**
Sesame Seeds	41.6	300 per 250 g	40
Pumpkin Seeds	4.6	1000 per 250 g	18.6
Total	46.2	1300 g	58.6
Mango Flavored Sesame And Pumpkin Seed Milk per 100 Ml			
Sesame Seeds	41.6	300 per 250 g	40
Pumpkin Seeds	4.6	1000 per 250 g	18.6
Mango	30	500 per 500 g	6
Total	76.2	1800 g	64.6
Dates Flavored Sesame and Pumpkin Seed Milk per 100 ml			
Sesame Seeds	41.6	300 per 250 g	40
Pumpkin Seeds	4.6	1000 per 250 g	18.6
Dates	72	800 per 500 g	57.6
Total	118.2	2100 g	116.2

In terms of calories, Sample A contains about 258.8 kilocalories(Table 2).

Table 2: Calorie Content in Sesame and Pumpkin Seed Milk (100ml)

Variables	Quantity
Carbohydrate	11.9g
Protein	8.2g
Fat	21.6g
Total Calories	258.8 kcal

Sample B contains about 276.8 kilocalories(Table 3).

Table 3: Calorie Content in Mango Flavored Sesame and Pumpkin Seed Milk(100ml)

Variables	Quantity
Carbohydrate	16.4 g
Protein	8.2 g
Fat	21.7 g
Total Calories	276.8 kcal

Sample C contains 461.8 kilocalories. Hence, sample C contains the highest number of calories compared to the other two samples(Table 4).

Table 4: Calorie Content in Dates Flavored Sesame and Pumpkin Seed Milk(100ml)

Variables	Quantity
Carbohydrate	65.9 g
Protein	10 g
Fat	21.8 g
Total Calories	461.8 kcal

DISCUSSION

In this research, an experimental study was conducted. A plant-based milk was produced from the combination of sesame seeds and pumpkin seeds. The purpose of this research was to develop a product which is an effective alternative or substitute for cow's milk. In this study, three samples were produced. The first sample was made of sesame and pumpkin seed milk.The second sample was mango flavored sesame and pumpkin seeds. And third sample was dates flavored with sesame and pumpkin seeds.These samples were produced to do a comparison among them in terms of sensory evaluation, proximate analysis and cost analysis. A study was done by Posokina *et al.*, which compared sesame milk with cow's Milk. This alternative was being developed because the cow's milk contains up to 80% casein in its protein composition, while sesame milk does not contain lactose or casein. Sesame contains more than 20 % protein, 50 % fat, a significant amount of dietary fiber (13.3 %) as well as a high content of minerals such as calcium, phosphorus, iron and zinc and vitamins such as A, E and folic acid.The results proved that the sesame milk is not inferior to the cow's milk and even surpasses it in some cases.In conclusion, the functional drink from sesame seeds contributes to the improvement of the body systems, and its main advantage is that it does not contain milk sugar.Thus, it can be recommended to those with lactose intolerance [1].Comparing this study with the current research, it was found that the past study only compared sesame milk with cow's milk.No plant-based product was developed using sesame and pumpkin seeds [16, 17].Also, the current research developed two more flavors to enhance the palatability of the milk. In past studies, no other flavors were developed. After producing these samples, sensory evaluation was done using hedonic scale. Sesame and pumpkin seeds milk got an appearance rating of 60%. But it only scored 30% in taste due to its strong flavor [18, 19]. Mango flavored sesame and pumpkin seeds milk got an average of 30% in terms of appearance, odor, taste and texture [20].That was because it was tasteless.It was not liked by the panelist. However, dates flavored sesame and pumpkin seeds milk got the highest ratings in terms of appearance (40%), odor (50%), taste (50%) and texture (60%) without a single dislike.Hence, it was proved that dates flavored sesame and pumpkin seeds milk is the most accepted one. Proximate analysis of the three samples was done.Sample A showed that it contained about 19.17% dry matter, 7.04% crude protein, 2.20% fat and 0.90% ash content. Sample B showed about 13.00% dry matter, 3.60% crude protein, 4.00% fat and 0.46% ash content. Sample C achieved 15.67% dry matter, 3.74% crude protein, 5.00% fat and 0.38% ash content. These results showed that sesame and pumpkin seeds milk (Sample A) has the highest value of dry matter, crude protein and ash as compared to Samples B and C. Thus, Sample A is the most nutritious one. A study was done by El-Bialy *et al.*, to provide a nutritious and palatable replacement milk for lactose and casein sensitivity. Sésame Milk has higher amounts of potassium, copper, zinc, manganese and selenium.Sesame milk can overcome the restrictions of soymilk use, such as flatulence,

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allergy to soy proteins, and off flavours. In the chemical composition, sésame Milk contains 13.38% protein, 26.52% fat, 1.19% ash, 58.91% carbohydrate, and 87.97% moisture [4].Compared to current research, it was found that sesame and pumpkin seed milk contained about 19.17% dry matter, 7.04% crude protein, 2.20% fat and 0.90% ash content. All these values are less because the product is made from two seeds.The carbohydrate content and moisture content were calculated, which are about 58.91% and 87.97% in the above study. Whereas in this research, the carbohydrate content and moisture content were not calculated. According to this study, the sesame milk had good sensor judging value in terms of taste, flavor, colour, texture, etc. But in the current research, the sensory values were not as acceptable as the dates flavored sesame and pumpkin seeds milk. Cost analysis of all samples was done to determine which sample cost the least.It was evident that the sesame and pumpkin seeds milk cost only Rs. 58.6, making it the cheapest sample out of all three. As compared to past studies, cost analysis of the plant-based milk was not performed.

CONCLUSION

It was concluded that with an increasing trend of producing plant-based products, there is a high demand for dairy alternatives.Several plant-based milks have been produced, but there was a lack of a hybrid product. This research project developed a hybrid plant-based milk by combining sesame and pumpkin seeds. This study provided insight on how to make a plant-based milk, how much it costs and what the sensory attributes are acceptable and which attributes need more work to be better. This study successfully met all three objectives (development of plant-based milk, sensory and nutritional analysis, and cost analysis). However, there were some limitations to it; for example, shelf life was not taken into consideration. Future researches need to further work on it.

Authors Contribution

Conceptualization: TA
Methodology: AK, TA
Formal analysis: SND, AK, MS
Writing review and editing: SA, CB

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

Conflicts of Interest

All the authors declare no conflict of interest.

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REFERENCES

[1] Posokina NE, Alabina NM, Davydova AY. Development of Functional Beverages from Plant Raw Materials—Replacements for Dairy Products.Food systems .2019Jul;2(2):44-7.doi:10.21323/2618-9771-2019-2-2-44-47.

[2] Abbas S, Sharif MK, Sibt-e-Abbas M, Fikre Teferra T, Sultan MT, Anwar MJ. Nutritional and Therapeutic Potential of Sesame Seeds.Journal of Food Quality. 2022; 2022(1): 6163753. doi: 10.1155/2022/6163753.

[3] Thakur N, Gupta N, Sood M, Bandral JD, Singh J, Bhat A *et al.* Plant-Based Milk Alternatives:An Emerging Segment:A.The Pharma Innovation Journal.2022; SP-11(9):2752-2758.

[4] El-Bialy EF, Abd-Elkader MH, Yousef NS. Non-Dairy Alternative Milk for People with Lactose and Casein Intolerance.Journal of Food and Dairy Sciences. 2020Dec;11(12):347-53.doi:10.21608/jfds.2020.160395.

[5] Manoharan D, Rajan A, Stephen J, Radhakrishnan M. Evaluating the Influence of Cold Plasma Bubbling on Protein Structure and Allergenicity in Sesame Milk. Allergologia Et Immunopathologia.2023Mar;51(SP1): 1-3.doi:10.15586/aei.v51iSP1.783.

[6] Boujemaa I, El Bernoussi S, Harhar H, Tabyaoui M. The Influence of the Species on the Quality, Chemical Composition and Antioxidant Activity of Pumpkin Seed Oil.Oil Seeds and Fats, Crops and Lipids.2020; 27:40.doi:10.1051/ocl/2020031.

[7] Syed QA, Akram M, Shukat R. Nutritional and Therapeutic Importance of the Pumpkin Seeds. Seed.2019;21(2):15798-803.doi:10.26717/BJSTR.2019.21.003586.

[8] Batool M, Ranjha MM, Roobab U, Manzoor MF, Farooq U, Nadeem HR *et al.* Nutritional Value, Phytochemical Potential, and Therapeutic Benefits of Pumpkin (Cucurbita sp.).Plants.2022May;11(11):1394.doi:10.3390/plants11111394.

[9] Shevchenko A, Drobot V, Galenko O. Use of Pumpkin Seed Flour in Preparation of Bakery Products.2022; 11(1): 90-101.doi:10.24263/2304-974X-2022-11-1-10.

[10] Gao D, Helikh A, Duan Z, Liu Y, Shang F. Development of Pumpkin Seed Meal Biscuits.Eastern European Journal of Enterprise Technologies.2022Apr;2(11):116.doi:10.15587/1729-4061.2022.254940.

[11] Majid AK, Ahmed Z, Khan R. Effect of Pumpkin Seed Oil on Cholesterol Fractions and Systolic/Diastolic Blood Pressure.Food Science and Technology.2020 Aug; 40: 769-77. doi: 10.1590/fst.03720.

[12] Yu M, Peng M, Chen R, Chen J. Effect of Thermal Pretreatment on the Physicochemical Properties and Stability of Pumpkin Seed Milk.Foods.2023Mar;

DOI: https://doi.org/10.54393/fbt.v5i1.162

12(5): 1056. doi: 10.3390/foods12051056.
[13] Dowidar M, Ahmed A, Mohamed H. The Critical Nutraceutical Role of Pumpkin Seeds in Human and Animal Health: An Updated Review.Zagazig Veterinary Journal.2020Jun;48(2):199-212.doi:10.21608/zvjz.2020.22530.1097.
[14] Ceclu L, Mocanu DG, Nistor OV. Pumpkin–Health Benefits. Journal of Agroalimentary Processes and Technologies. 2020; 26(3): 241-6.
[15] Singh A and Kumar V. Pumpkin Seeds as Nutraceutical and Functional Food Ingredient for Future:A review.Grain and Oil Science and Technology.2024Mar;7(1):12-29.doi:10.1016/j.gaost.2023.12.002.
[16] Kuru C, Tontul I. Optimization of Plant-Based Milk Formulation Using Hazelnut, Sunflower Seed and Pumpkin Seed by Mixture Design. Turkish Journal of Agriculture-Food Science and Technology.2020 Nov; 8(11):2441-8.doi:10.24925/turjaf.v8i11.2441-2448.3726.
[17] Sá AG, da Silva DC, Pacheco MT, Moreno YM, Carciofi BA. Oilseed By-Products as Plant-Based Protein Sources: Amino Acid Profile and Digestibility.Future Foods.2021Jun;3:100023.doi:10.1016/j.fufo.2021.100023.
[18] Kudva KM. Formulation and Quality Evaluation of Sesame-Pumpkin Burfi.2022.
[19] Plamada D, Teleky BE, Nemes SA, Mitrea L, Szabo K, Călinoiu LF *et al.* Plant-Based Dairy Alternatives—A Future Direction to the Milky Way.Foods.2023May; 12(9):1883.doi: 10.3390/foods12091883.
[20] Kirker CL, Newman M. Mango: A Global History. Reaktion Books. 2024 Aug.