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Preface

We would like to present, with great pleasure, the inaugural volume-10, Issue-5, May 2024, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas.

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Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

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Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.

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Detection of Coronavirus (CCoV) in Dogs by Transmission Electron Microscopy Techniques

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***Abstract*—** *Coronaviruses are known for their ability to cause gastrointestinal, respiratory, and central nervous system diseases in various species of avian, mammalian, and human hosts. In dogs, it is one of the most important viral agents causing gastroenteritis. Canine coronavirus is an emerging infectious disease affecting animals of all ages. Canine Enteric Coronavirus (CCoV) belongs to the Coronaviridae family and the Nidovirales order, exerting a significant impact on veterinary activities in kennels and animal shelters due to the rapid spread of the virus, causing economic losses due to mortality and/or morbidity, especially in commercial production kennels. Pathogenic variants can cause severe disease, characterized by loss of appetite, vomiting, profuse bloody and watery diarrhea with a putrid odor, abundant serosanguineous fluid in the abdominal cavity, accompanied by fever, anorexia, vomiting, prostration, severe dehydration, and death in young animals. From 1994 to 2016, 643 samples of feces, rectal swabs, and organ fragments from dogs were submitted for viral agent research. The samples were processed for transmission electron microscopy by negative staining and immunoelectron microscopy techniques. Transmission electron microscopy examination by negative staining technique revealed the presence of pleomorphic, rounded, or spherical enveloped coronavirus particles containing typical radial projections in the form of a solar crown, with an average diameter of 140 nm in 287 (44.63%) out of 643 samples. In the immunoelectron microscopy technique, the antigen-antibody interaction was characterized by the aggregation of viral particles in 287 (44.63%) of the fecal samples, fecal swabs, intestinal mucosa, and intestinal fragments analyzed.*

***Keywords*— Coronavirus, Dogs, Gastroenteritis, Transmission Electron Microscopy.**

I. INTRODUCTION

Coronaviruses are known for their ability to cause gastrointestinal, respiratory, and central nervous system diseases in various species of avian, mammalian, and human hosts (Sun et al., 2020). Through an intermediate species, coronaviruses acquire zoonotic potential that allows them to move from a reservoir species to other species, including humans (Cui et al., 2019). This zoonotic ability is evidenced by their genetic plasticity that promotes a high frequency of genetic changes (mutation and recombination) (Decaro & Lorusso, 2020), which also influence tissue tropism and pathogenicity (Vijaykrishna et al., 2007). In dogs, CCoV is one of the most important viral agents causing gastroenteritis. Canine coronavirus is an emerging infectious disease affecting animals of all ages (Kong et al., 2007). Occurring worldwide, it has been detected in various countries, such as the United Kingdom (Radford et al., 2021), Japan (Takano et al., 2015), China (Tian et al., 2021), Turkey (Timurkan et al., 2021), Italy (Zobba et al., 2021), USA (Licitra et al., 2014), and Spain (Decaro et al., 2006). In Brazil, the first outbreak was detected in 1989 by Mitika et al., with viral particles identification by transmission electron microscopy at the Biological Institute of São Paulo, SP, Brazil. Subsequently, other cases were reported (Dezengrini et al., 2007; Castro et al., 2010; Guirao et al., 2013).

Canine Enteric Coronavirus (CCoV) belongs to the *Alphacoronavirus* genus, *Coronaviridae* family, and *Nidovirales* order. They are pleomorphic viruses, spherical to elongated, with a characteristic envelope containing surface projections from the viral membrane in the form of a club, solar crown, or petal, measuring 75-160 nm in diameter (Belouzard et al., 2012; Li, 2016). The CCoV genome is single-stranded RNA and measures from 27.6 to 31 kilobases in length. It has four fundamental proteins in its envelope essential for infection. The S glycoprotein is responsible for the entry of infectious virion particles into the target cell through interaction with the host cell receptors, as well as providing the virion with a crown-like appearance. By binding to the nucleocapsid, the M protein acts in viral assembly organization, and the E protein operates in pathogenesis, assembly, and virus release. The N protein facilitates the interaction of the M protein during virion assembly, promoting increased viral transcription efficiency (Dhama et al., 2020). CCoV has 3 subtypes, types I and II included in the *Alphacoronavirus* genus, and type III (CRCoV - canine respiratory coronavirus), in the *Betacoronavirus* genus. Type II can be divided into subtypes IIa and recombinant IIb. CCoV types I and II cause mild asymptomatic enteritis or self-limiting, but pathogenic variants of pantropic II-a CCoV can cause severe disease (Decaro et al., 2012), characterized by loss of appetite, vomiting, profuse bloody and watery diarrhea with a putrid odor, abundant serosanguineous fluid in the abdominal cavity, accompanied by fever, anorexia, vomiting, prostration, and severe dehydration (Hagiwara et al., 1989; Catroxo et al., 1998, Buonavoglia et al., 2006; Zapulli et al., 2008). Mortality is low but can occur, especially in co-infected puppies with parvovirus, canine distemper virus, Ehrlichia canis, Isospora, or other intestinal pathogens (Pratelli et al., 2008; Catroxo et al., 2023). Transmission occurs via the oral-fecal route, through ingestion of contaminated food or water and by direct contact with an infected animal. The incubation period is 1 to 4 days, and the duration of the disease is 2 to 10 days in most dogs, with cases where symptoms intermittency is observed, with periods of improvement and apparent cure, followed by diarrheal episodes. The viruses reach the duodenum within 48 hours, reach the small intestine, penetrate enterocytes, and begin their replication in the cytoplasm of villous epithelial cells, causing their atrophy. Dogs can be carriers and shed the virus in feces for up to 6 months post-infection (Mitika et al., 1989; Licitra et al., 2014).

Canine coronavirus (CCoV) has a significant impact on veterinary activities in kennels and animal shelters due to the rapid spread of the virus, mainly contaminating young animals aged 2 to 5 months (Decaro & Buonovoglia, 2008), causing economic losses due to mortality and/or morbidity, especially in commercial production kennels (Guirao et al., 2013).

This study aimed to detect the presence of coronavirus particles in feces, rectal swabs, and organ fragments from dogs using transmission electron microscopy techniques.

II. MATERIAL AND METHOD

2.1 Clinical cases:

During routine clinical research carried out at the Biological Institute of São Paulo, SP, Brazil, from October 1994 to July 2016, 643 samples of feces, fecal swabs and fragments of organs from dogs were sent for investigation of viral agents. The animals, of different breeds, aged between 23 days and 17 years, of both sexes, came from São Paulo, SP, Brazil. The dogs presented clinical symptoms and signs of apathy, weight loss, liquid and yellowish or bloody diarrhea, gastritis, prostration, anorexia, vomiting, dipsia, nausea, gastroenteritis, hemorrhagic gastritis, colitis, hematochezia, hypoglycemia, lymphopenia, bronchial pneumonia, pulmonary edema, cough, cardiac dilation, leukopenia, fever, nasal secretion, increased ALT and AST and hypovolemic shock.

2.1.1 Outbreak description:

In 1998, an outbreak of gastroenteritis occurred in a kennel belonging to the Military Police of the State of São Paulo, SP, Brazil, of German Shepherd breed, with black fur. Of the 130 animals in the kennel, 40 were affected by a sudden incidence of profuse, bloody and watery diarrhea, with a putrid odor, accompanied by fever, anorexia, vomiting, prostration and severe dehydration. Most of the affected animals were mainly puppies aged 6 to 8 months and the rest of the animals were adults aged between 1 and 5 years, of both sexes. All dogs had been immunized with V8 and anti-rabies vaccine, according to the vaccination schedule (first dose between 6-8 months and annual booster). Adult animals maintained contact with fomites and other animals during routine work around the city. Parasitological and bacteriological examinations of the feces were negative.

All samples from routine clinical cases and the occurrence of the outbreak were processed using the negative staining technique (rapid preparation) for transmission electron microscopy.

2.2 Transmission Electron Microscopy:

2.2.1 Negative staining technique (rapid preparation):

In the negative staining technique, feces, fecal swabs and intestine fragments were suspended in 0.1M phosphate buffer at pH 7.0. Drops of the viral suspension were placed in contact with copper screens previously covered with collodion film and metallized by carbon. After 10 minutes, the screens were removed from the viral suspension, contrasted with drops of 2% ammonium molybdate and subsequently dried on filter paper (Brenner & Horne, 1959; Hayat & Miller, 1990; Madeley, 1997). Observations were made using a Philips EM 208 transmission electron microscope under a voltage of 80 kV.

Samples positive for coronavirus particles using the negative staining technique were processed using the immunoelectron microscopy technique.

2.2.2 Immunoelectron microscopy technique.

In this technique, copper grids, previously prepared with collodion film and stabilized with carbon were first incubated with protein A (1ml/ml) placed in contact with the virus-specific antibody. After, grids were washed in PBS drops, incubated with the viral suspension of the 287 positive samples, washed with drops of water and negatively stained with 2% ammonium molybdate, pH 5.0 (Berthiaume et al., 1981; Katz & Kohn, 1984; Doane & Anderson, 1987; Hayat & Miller, 1990).

III. RESULTS AND DISCUSSION

3.1 Clinical cases

A total of 643 samples of feces, fecal swabs, and intestinal fragments were processed using the negative staining technique and examined under a transmission electron microscope. Of these, 287 (44.63%) were positive for coronavirus. Of the 287 CCoV-positive dogs, 107 (37.63%) were female, and 108 (37.63%) were male. In 72 (25.08%) samples, the gender of the animals was not identified. Regarding the age of the animals, it ranged from 23 days to 17 years, with 71 (24.73%) samples from younger animals aged less than 11 months and 65 (22.64%) from animals aged over 11 months, with no significant difference between these parameters. Concerning co-infection, 52 samples (18.11%) were mixed with other agents. A total of 20 samples (38.46%) were co-infected with paramyxovirus, 21 (40.3%) with parvovirus, 3 (5.76%) with parvovirus and mycoplasma, 4 (7.69%) with mycoplasma, 1 (1.92%) with herpesvirus, 1 (1.92%) with parvovirus and paramyxovirus, and 2 samples (3.84%) with Ehrlichia. Approximately 11 (3.83%) dogs died, with 7 animals infected solely with coronavirus, 2 having dual infection with coronavirus and parvovirus, and 2 presenting triple infection with coronavirus, parvovirus, and mycoplasma.

3.1.1 Outbreak

Animals from the outbreak received treatment with fluid replacement and antibiotic therapy, and complete remission of symptoms was observed in all animals after 10-15 days.

3.2 Transmission Electron Microscopy

3.2.1 Negative staining (rapid preparation) technique

In all 287 positive samples examined under the transmission electron microscope, the presence of coronavirus particles was observed, which were rounded, or spherical (Figs. 1, 2, arrow), pleomorphic (Fig. 3, arrow), enveloped, containing typical radial projections in the form of a solar crown, with an average diameter of 140 nm (Figs. 1,2, arrow). The spikes of the envelope measured between 16 and 20 nm in length (Fig.2, big arrow). An internal spherical core, measuring 60 to 85 nm in diameter, was visualized in some particles (Fig.3, big arrow).

3.2.2 Immunoelectron microscopy technique.

In 287 (44.63%) out of 643 fecal samples, fecal swabs, and intestinal fragments analyzed, the antigen-antibody interaction was characterized by aggregation of viral particles (Fig. 4, arrow).

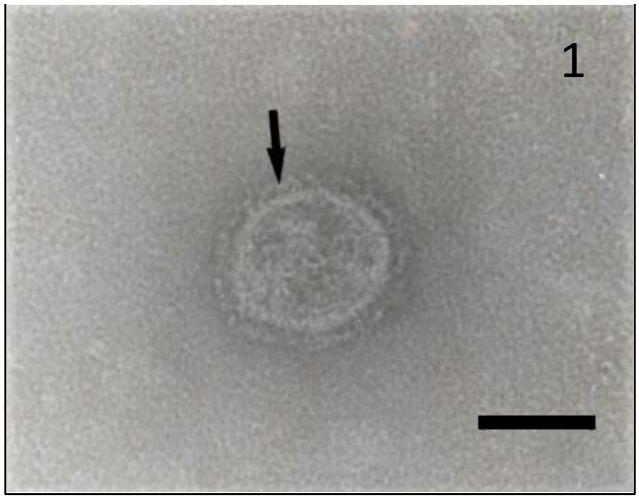

FIGURE 1: Negative staining of spherical coronavirus particle in dog feces suspension, showing envelope in the shape of a solar crown or petal (arrow). Bar: 80 nm.

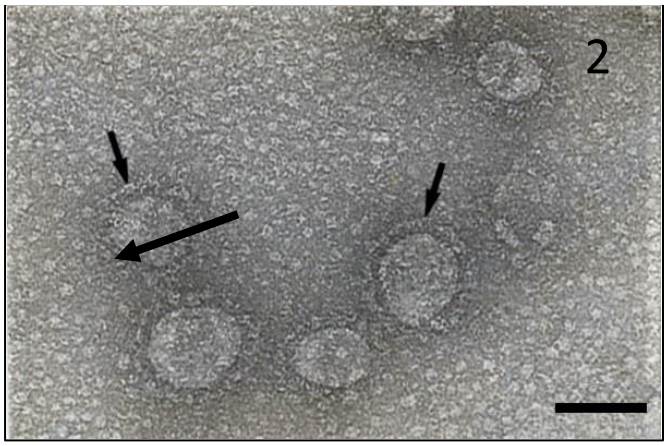

FIGURE 2: Negative staining of a group of spherical coronavirus particles, showing a solar corona-shaped envelope (minor arrow) and spikes of the envelope measured between 16 and 20 nm in length (big arrow). Bar: 100 nm.

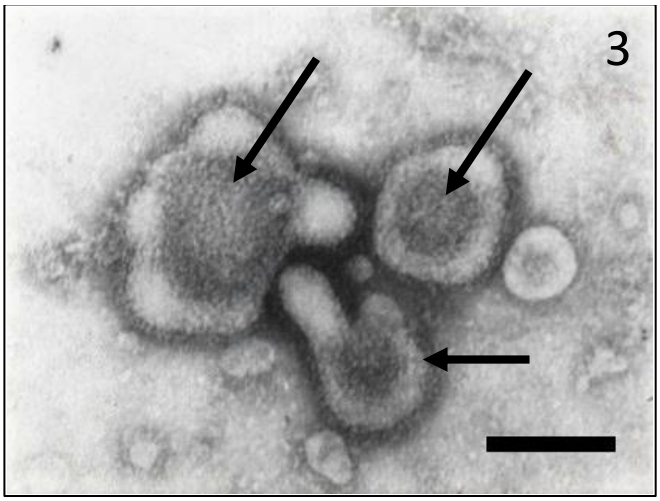

FIGURE 3: Negative staining of pleomorphic coronavirus particles (minor arrow), displaying an internal spherical core, measuring 60 to 85 nm in diameter (big arrow). Bar: 80 nm.

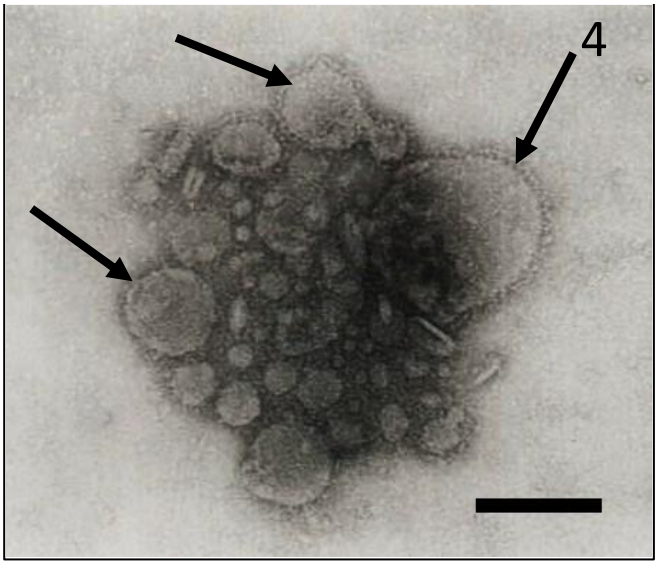

FIGURE 4: In the immunoelectron microscopy technique the coronavirus particles were aggregated by antigen-antibody interaction (arrows). Bar: 100 nm.

Canine coronavirus is an important disease that affects dogs in kennels, shelters or companions, causing everything from mild diarrhea to severe gastroenteritis (Zapulli et al., 2020). In this study, transmission electron microscopy techniques were applied to detect coronavirus particles in stool samples, fecal swabs and fragments of small intestine from clinical cases of dogs with diarrhea, sent to the electron microscopy laboratory of the Biological Institute to research the etiological agent. Around 287 samples were positive for coronavirus using the negative staining technique (rapid preparation), where the particles visualized were pleomorphic, rounded or elongated, enveloped, containing typical radial projections, in the shape of a solar corona, measuring on average 140 nm in diameter. According to previous reports, these ultrastructural aspects of particles from the *Coronaviridae* family were also reported by other researchers who used this technique in studies of canine coronavirus (Roseto et al., 1980; Vandenberghe et al., 1980; Williams, 1980; Hammond & Timoney, 1983; Mitika et al., 1989; Finlaison, 1995; Catroxo et al., 1998; del Amo, et al., 1999; Pratelli, 2008; Gan et al., 2021; Tian et al., 2021; Vieira et al., 2023). In some particles we were able to observe a spherical internal core, measuring 60 to 85 nm in diameter. Risco et al. (1996) reported the presence of this internal core, measuring around 65 nm in particles of swine transmissible gastroenteritis coronavirus and studies by immunogold mapping and protein analysis of purified cores showed that they consist of M and N proteins. The coronavirus particles in our study had envelope spikes measuring between 16 and 20 nm in length. According to Escors et al. (2001) and Locker et al. (1992), the long spikes of the 20 nm, which consist of the S glycoprotein, are present on all coronaviruses, whereas the short spikes, which consist of the HE (hemagglutinin-esterase) glycoproteins, are present in only some coronaviruses.

In the immunoelectron microscopy technique, a positive antigen-antibody reaction was detected in 287 samples of feces, fecal swabs and fragments of small intestine as a result of agglutination of a high number of viral particles. This technique was used previously by other researchers (Williams, 1980; Pensaert et al., 1981; Risco et al., 1996).

We found 44.63% positivity for coronavirus in dog samples from our study. Close percentages (40.8%) were reported in research conducted by Naylor et al. (2001) in kenneled dogs in Australia and 43.3% by van Nguyen et al. (2017) in Vietnam. In Brazil Castro et al. (2010) reported 47.8% of vaccinated dogs, 45.5% of the unvaccinated and 43.3% of the dogs with unknown historical vaccination. In other countries, CCoV positivity was quite variable, ranging from 4.3% in Bangladesh (Hossain et al., 2021), 4.8% in the Caribbean region (Navarro et al., 2017), 7% in Argentina (delAmo et al., 1999), 7.01% in Australia (Finlaison, 1995), 2.8% and 20.69% in United Kingdom (Staviski et al., 2010; Radford et al., 2021) respectively, 12.5% in France (Roseto et al., 1980), 33% in China (Dong et al., 2022), 34.61% in Italy (Decaro et al., 2004a), 62.5%-74.3% and 100% in Turkey (Yesilbag et al., 2004; Timurkan et al., 2021), respectively. In Brazil, positivity also varied between 22% (Guirao et al., 2013) and 50.4% (Dezengrini et al., 2007). Study carried out by Takano et al. (2016) in Japan showed that 88.9% of dogs were positive for CCoV-I and 7.4% for CCoV-II, while in Italy CCoV-IIa was found in 18% of dogs and CCoV -1 in 10.3% (Zobba et al., 2021). In another research carried out in a Zoological collection in United Kingdom, 100% of the *Speothos venaticus* studied were positive for coronavirus, showing that other canids are also susceptible (Rowland et al., 2021).

Considering age, the dogs in our study were between 23 days and 17 years old, 71 (24.73%) were animals aged up to 11 months and 65 (22.64%) were animals aged over 11 months, with no significant differences between these two parameters and confirming that dogs of all ages can be infected with CCoV, although puppies are more susceptible (Pratelli et al., 2008). Corroborating our results, Delamo et al. (1999) found a rate of 42.85% of positive animals between 6 weeks and 6 months and a similar percentage in dogs older than six months of age. Other authors reported the occurrence of higher percentages, such as 100% in adult animals (Hossain et al., 2021), 57% aged over 5 years (Dezemgrini et al., 2007), 52% over 3 years (van Nguyen et al., 2017) and 43.8% in dogs aged between 1 and 6 years (Castro et al., 2010). The average age of the animals studied by Radford et al. (2021) was 4 years. The highest number of occurrences, however, was mentioned in animals under 6 months old, although the percentage was lower (43%) (Dong et al., 2022), 46.4% under 1-year-old (Takano et al., 2016), between 1 to 6 months (Guirao et al., 2013), 4 days to 21 weeks (Licitra et al, 2014), 10 weeks old (Vandenberghe et al., 1980), and 2 weeks to 4 months (Yesilbaf et al., 2004).

We found that among the 287 positive samples, 108 (37.63%) were males and 107 (37.28%) were females, therefore there was no significant difference between these values, reinforcing the results of most authors who also did not report statistical differences (Dezemgrini et al., 2007; Castro et al., 2010; Zobb et al., 2021; Dong et al., 2022). Timurkan et al. (2021), however, found greater positivity (69.23%) in male dogs. Radford et al. (2021) also reported that the number of infected male dogs in their research was greater than that of female dogs.

The main clinical signs that we observed in the positive dogs in our study, such as apathy, prostration, weight loss, vomiting, gastroenteritis, dehydration and liquid or bloody diarrhea, were reported in other canine coronavirus studies (Vandenbergue et al., 1980; Tennant et al., 1991; Naylor et al., 2001; Stavisky et al., 2010; Licitra et al., 2014; Navarro et al., 2017; Radford et al., 2021; Rowland et al., 2021; Tian et al., 2021; Timurkan et al., 2021). Respiratory clinical signs such as bronchopneumonia and cough, as well as other less common signs, such as rhinitis, conjunctivitis, intestinal intussusception, and neurological signs, were mentioned by Vandenberghe et al. (1980) and Licitra et al. (2014).

Around 8 dogs (2.78%) in our research, aged between 1 day and 6 months, died. Licitra et al. (2014) reported 20% mortality in dogs 6 to 8 weeks old, while Tian et al. (2021) confirmed death in a 5-week- old dog and Timurkan et al. (2021) 1 month old. These data confirm that CCoV can cause mortality more frequently in puppies up to 21 weeks of age, especially when housed in kennels with a high population (Licitra et al., 2014).

Regarding mixed infections, a total of 20 samples (38.46%) were co-infected with paramyxovirus, however, Catroxo et al. (2023) found a rate of 5.51% and Zhao et al. (2016) 1.11%. Approximately 21 dogs in our survey (40.3%) were co-infected with parvovirus. Similar studies revealed a rate of 8.90% (Roseto et al., 1980); 5% (Delano et al., 1999) and 11.11% for CCoV II and CPV-2 and 2.77% for CCoV1a and CPV-2 (Zobba et al., 2021). Decaro et al. (2006) also reported the simultaneous detection of CCoV and CPV-2 in 40-day-old pups and Licitra et al. (2014) in a dog from their study. 7.69% of the dogs in our research were co-infected with Mycoplasma and two animals that died had a triple infection with coronavirus, parvovirus and Mycoplasma. Triple infection represented by the simultaneous presence of paramyxovirus, coronavirus and adenovirus was found in a dog, in the study by Decaro et al. (2004a). No occurrence reports the joint infection of coronavirus and Mycoplasma, however the concomitant presence of parvovirus and Mycoplasma has already been demonstrated by transmission electron microscopy, in young dogs, during an outbreak of hemorrhagic gastroenteritis with mortality (Cappellaro et al., 1995). Coronavirus facilitates transmission by other agents and these mixed, double or triple infections tend to worsen the clinical course of the disease and can lead to the death of infected animals (Decaro et al., 2006).

The animals from the Military Police kennel of the State of São Paulo, SP, Brazil, affected by the outbreak, were immunized with the V8 vaccine, which includes coronavirus. Little is known, however, about the immunological mechanisms involved in protection against CCoV enteritis (Decaro et al., 2004b). Several factors can contribute to vaccine failure, including deficient animal immune response, administration to immunocompromised, immunosuppressed or passively immune animals, use of ineffective vaccines or those with inadequate conservation or expired validity, among others (Pratelli et al., 2003).

Treatment for canine coronavirus is supportive, which includes maintaining hydro-electrolyte balance, control of secondary infections, prevention measures, through the institution of improvements in hygiene, efficient and adequate vaccination, intake of maternal colostrum, exemption from crowds in kennels, quarantine and isolation of sick animals (Dezengrini et al., 2007; Hass et al., 2008; Strottrmann et al., 2008).

Transmission electron microscopy applied to fecal suspensions, swabs and intestinal fragments can reveal the multiplicity of primary pathogens and predisposing factors involved in the installation of gastroenteric conditions (Hagiwara et al., 1989).

Through its various techniques, it is especially useful for quickly diagnosing agents in samples, allowing veterinary clinics to take immediate prophylactic and disease control measures (Goldsmith & Miller, 2009; Wolff & Bárcena, 2021).

Conjecturing that previous studies prove that several species of wild animals can be contaminated with coronaviruses (Catroxo et al., 2023), other research must be conducted to assess the impact of infections caused by these viruses in companion animals, kennels or wild animals and propose preventive and protective measures.

IV. CONCLUSIONS

Considering that canine coronavirus is an emerging infectious disease with an important impact on veterinary activities in kennels and animal shelters, causing economic losses due to mortality and/or morbidity, the use of transmission electron microscopy techniques allows for rapid and safe diagnosis, contributing to the immediate implementation of prophylactic measures, prevention and control of the disease, during routine procedures or in the event of outbreaks.

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Comparative Proximate and Selected Mineral Analysis of Two Edible Land Snail Species (*Archachatina Marginata and Archatina Fulica*)

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Abstract— *Comparative proximate and selected mineral analysis of two edible land snails (Archachatina Marginata, Achatina fulica were carried out using standard methods. Proximate analysis of the snail meat revealed that that moisture was slightly higher in A. marginata (58.90+0.01^a) and least in A. fulica (58.58+0.01^a) which was not significantly different from each other. The fat content of A. marginata (8.72+0.01^a) was slightly low and slight high in A. fulica (8.920.01^a) which is also not significant. The value for the ash was not significantly different in A. marginata (2.31+0.01^a) when compared to A. fulica (2.71+0.01^a) which was least. The protein content of A. marginata (26.62+0.02^a) was lower compared to A. fulica (27.40+0.02^a) which is also not significant. While, the carbohydrate content of A. marginata (3.45+0.01^a) was higher compared to A. fulica (3.250.01^a) meat which is also not significant. The result of the mineral analysis showed that A. marginata recorded highest value (mg/100 g) in Ca (1.77±0.07), Se (2.26± 0.14), Pb (2.73±2.81) and Zn (22.85± 0.15). While A. fulica the least values of 1.6±0.02, 1.86±0.08, 0.72±0.03 and 22.08±0.25 respectively. The values across species for all the minerals analyzed were not significantly different ($p<0.05$) from one another. The result indicates that the two edible land snail species (A. marginata and A. fulica) are nutritionally rich in protein and mineral nutrients which can be obtained at minimal cost. Hence, consumption for both the young and old should be encouraged as an alternative source of essential nutrients.*

Keywords— Proximate, Mineral, Land Snails, Protein.

I. INTRODUCTION

Snails are members of mullusca phylum, like slugs, oysters, squids and cuttle-fish; they live widely spread across fresh water, seas and land. Land based species prefer moist areas. They can be seen commonly through spring and autumn where rainfall is abundant (Robert, 2009).

African giant land snails (*Achatina fulica*) and edible garden snails (*Helix aspersa*) are among the various species of snails in the Southern and other parts of Guinea savannah in Nigeria where the atmospheric weather conditions and vegetation are favorable for their survival (odaibo, 1997). These creatures enjoy moist environment and are found easily at night. Snails generally exhibit sluggish movement over short distances which are dependent upon the temperature, food and nature of the soil (Ebenso, 2002; Ebenso and Okafor, 2002; Ebenso, 2003B). During their movement these invertebrates produce unpleasant odor via deposition of their saliva and feaces on the plants which distastes man and even herbivores from feeding on these contaminated plants (Ahmed and Nabil, 2012).

Snail meat has been reported to be highly nutritious because it is rich in the essential amino acids, vitamins and minerals but low in fats and cholesterol. It is also reported that the land snail meat is very rich in protein which could be as high as 14.52% (Yusuf and Oseni, 2004). Other values like 12.87% and 12.2 g/100g of protein have been reported for the garden and apple snails respectively. However, the nutritional composition of snail might vary depending on its feeding habits, species and sexual conditions.

There are different breed of edible land nails found in Nigeria. The two giant land snails common to Nigeria are *Archachatina marginata* and *Archachatina archachatina*. Archachatina marginata has definite shell coloration and it is wider at the posterior end compared to others. The foot is usually dark brown in colour. It is the most common breed found in western Nigeria. *A. achatina* has a brown shell with conspicuous zigzag streaks and a narrow apex. The foot is grey in colour. *A. fulica* is of small size and the fleshy part could be whitish o dark brown. It as low economic value compared to the two giant land snails. It is also known as garden snails. The mineral composition of snail meat varies also depending on their diet, species and environment where they are found. However, therefore, this study focused on the comparative proximate composition and selected mineral analyses of *Archatina fulica and Achatina marginata*.

II. MATERIALS AND METHODS

2.1 Collection of Samples:

Two different species of life snails *Archatina marginata* and *archatina fulica* were purchased from a snail farm in Afikpo, Afikpo North Local government Area of Ebonyi state. A total of ten (10) snails, five (5) each of *Archatina marginata* and *archatina fulica* species was used for this study. The Snails was identified and authenticated by a Zoologist, in Science Laboratory Technology Department, Akanu Ibiam Federal Polytechnic Unwana.

2.2 Preparation of sample:

The giant African Land Snails was washed with clean water to remove dirt and dust particles on the shells. The shells were knocked open at the apex. The inner content (i.e. fleshy body) of the snails was separated from the shells by a mechanical means involving the use of a spirally coiled rod inserted to remove the fleshy body. The fleshy body was oven dried at 90^{o}C. It was crushed and sieved using 0.4mm mesh (Gary, 2004). This was used for the digestion and proximate determinations

2.3 Digestion of snail sample:

One gram of the sample was weight into a 200cm Kjeldehl mask and 20ml of digestion mixture (1:1 mixture of 8M HCl: 8 $MHNO_3$) was added. The mixture was allowed to stand for 24 hours before digestion was done under reflux for 45 minutes. The sample was heated a little to remove the brown fumes of NO_2. The solution was then allowed to cool and made up to 50cm3 with distilled water and then stored in a treated polythene bottle for AAS analysis (Onianwa, 2001).

2.4 Proximate Analysis:

The moisture content was determined according to standard methods of AOAC (2010). Ash determination was carried out according to the method described in AOAC (2010). Crude protein was determined using the Kjeldahl methods of AOAC (2010). The fat content was determined using soxhlet extraction method according to AOAC (2010). The carbohydrate was done by difference according to oyenuga 1968, as follows % Carbohydrates = 100- (% moisture+ %fat+ %ash+ %protein+ crude fibre).

2.5 Mineral Composition Determination:

Major element such as Zn, Se, Cu, Cr, Cd was carried out using Atomic Absorption Spectrophotometer (AAS) as previously done by Usunobun and Okolie (2015).

A 0.5 g mashed sample was weighed into a 100ml pyrex connicall flask. 5ml of thee wet acid digestion reagent (H_2SO_4-selenium-salicylic acid) wsa added and allowed to stand at ambient temperature for about 16 hours. The sample was placed on a digestion clock, and heated at 20^{o}C for about two hours. The sample was removed from the block and about 5ml of conc perchloric acid added then placed back on the digestion stand, temperature rained to about 80^{o}C-150^{o}C. The digestion continued until a profuse white perchloric fumes emerged showing a clear digest indicating the completion of the digestion. The sample was removed from the hot plate, allowed to cool and made up in a 100ml volumetric flask with distilled water. The digest was used for the determination of Zn, Se, Cu, Cr and Cd using Atomic Absorption Spectrophotometer (AAS).

2.6 Statistical Analysis:

All the values obtained from proximate and selected mineral analysis of the two edible land snail species was statistically analyzed using one factor randomized design ANOVA as described by Mahoney (1986).

III. RESULTS

TABLE 1
THE RESULT OF THE PROXIMATE COMPOSITION OF THE SNAIL

Proximate compounds (%)	***A. marginata***	***A. fulica***
Moisture	58.90+0.01	58.58+0.01
Fat	8.72+0.01	8.920.01
Ash	2.31+0.01	2.71+0.01
Protein	26.62+0.02	27.40+0.02
Carbohydrate	3.45+0.01	3.250.01

Values are expressed as mean ± standard deviation of the triplicates. [a,b] Means along the same row with different superscripts are significantly different (p<0.05).

As shown in table 1, above, the proximate composition of the snail meat revealed that moisture was slightly higher in *A. marginata* (58.90+0.01) and least in *A. fulica* (58.58+0.01) without significance. The fat content of *A. marginata* (8.72+0.01) was slightly lower without significance than *A. fulica* (8.920.01). The value for the ash was slightly lower in *A. marginata* (2.31+0.01) when compared to *A. fulica* (2.71+0.01). The protein content of *A. marginata* (26.62+0.02) was lower compared to *A. fulica* (27.40+0.02) which is also not significant. While, the carbohydrate content of *A. marginata* (3.45+0.01) was higher compared to *A. fulica* (3.25+0.01) meat which is also not significant.

TABLE 2
THE RESULT OF THE SELECTED MINERAL ANALYSIS IN THE SNAILS

Mineral (Mg/100g)	***Archachatina maarginata***	***Archatina fulica***
Cu	6.27±0.18	6.58±0.20
Ca	1.77±0.07	1.6±0.02
Se	2.26± 0.14	1.86±0.08
Pb	0.73±0.08	0.72±0.03
Cd	0.01±0.00	0.01±0.00
Zn	22.85± 0.15	22.08±0.25

Values are expressed as mean ± standard deviation of the triplicates. [a,b] Means along the same row with different superscripts are significantly different (p<0.05).

The results of the mineral analysis showed that *A. marginata* recorded highest value (mg/100 g) in Ca (1.77±0.07), Se (2.26± 0.14), Pb (2.73±2.81) and Zn (22.85± 0.15). While A. fulica the least values of 1.6±0.02, 1.86±0.08, 0.72±0.03 and 22.08±0.25 respectively. The values across species for all the minerals analyzed were not significantly different ($p<0.05$) from one another

IV. DISCUSSION

It indicates that *A. marginata* contains crude protein of 26.62% which is slightly lower than that of *A. fulica* 27.40%. However, the crude protein value of both *A. marginata* and *A. fulica* is higher than other livestock meat like mutton, duck and chicken which have protein contents of 16.9, 18.6 and 20.5% respectively (Ogungbenle, and Omowole, 2012). The fat (8.72%) content obtained from *A. marginata* is also lower than 8.92% of *A. fulica*. The fat content obtained from *A. marginata* and *A. fulica* is also lower than 9.6% for egg, 21.4% for mutton and 23.0% for duck products respectively (Babalola and Akinsoyinu, 2009). However, the present result is higher than that of periwinkle (1.32%) and *Oryctes rhinoceros* larva (0.55 – 0.68%) (Okaraonye and Ikewuchi, 2009). The low fat content makes *A. marginata* and *A. fulica* meat an ideal diet for hypertensive patients and those that have fat related diseases like arteriosclerosis (Ugonna et al., 2020). The moisture content of *A. marginata* (58.90%)

is slightly higher than that of *A. fulica* (58.58%). These values were significantly lower than the values (73.67% and 79.28%), reported by Ogungbenle and Omowole (2012) for *A. marginata* and *A. fulica* respectively and also lower than the value reported by Wosu (2003) and Eneji *et al*., (2008) for snail meat. The ash content which is an indication of the rich minerals contents that are beneficial to man of *A. marginata* 2.31% is higher than 2.71% of *A. fulica*, however, this is significantly lower than that of periwinkle (9.56%) and *Oryctes rhinoceros* larva (12.70- 15.25%) (Okaraonye and Ikewuchi, 2009). The Carbohydrate content of *A. marginata* (3.45%) is slightly higher than 3.25% of *A. fulica*. This value is very low when compare to the values of 24.81 – 30.95% reported by Soniran *et al*. (2013) for Snail meat. Hence Snail meat (*A. marginata* and *A. fulica*) could be said to be a poor source of carbohydrate.

The result of the selected Minerals analysis of the two edible snails is shown in table 4.2 the result indicate that Snails meat are rich in calcium. A value of 1.77±0.07mg/ 100 g gotten for AM is a pointer to this. Calcium is involved in calcification of bones and teeth. Its shortage therefore can affect the structure of bones which become weakened. Calcium ions are needed for blood clotting and successful functioning of nerves and muscles (Fox and Cameron, 1980). The high content of calcium in the two species of the edible land snail investigated suggests that consumption of snail can increase the calcium in the body and contribute tremendously to the blood clotting process. Zinc is needed in the body to help the pancreas produce insulin, to allow insulin to work more effectively and to protect insulin receptors on cells Okaka and Okaka, 2001). Therefore the presence of zinc in the snail meat could mean that it can play valuable roles in the management of diabetes, which results from insulin malfunction. Magnesium ions are known hormone activators in type 2 diabetes their presence in the studied plants can be beneficial in managing this disease. This results is also in agreement with other previous work of Uboh et al. (2010) and Fagbuaro et al. (2006), who reported that the concentration of calcium and zinc were consistently high in different species of snail. Also, the mineral composition of the two species of the edible land snail can compare favourably with the mineral contents of some lean domestic livestock meats (Aganga *et al.,* 2003). Copper is an important essential elements that helps in interconversion of the major neurotransmitters, dopamine, noradrenaline, and adrenaline, and in pigment production. Zinc-Cu interaction has shown hypothesis of ischemic heart disease, which proposes that decreased Cu intake with excessive Zn may play an etiologic role in cardiac deaths in both animals and man (Akan *et al.,* 2010; Davies *et al.,* 2013). Copper deficiency results in kinky and steely hair syndrome in humans and abnormal wool in sheep (Ugonna *et al.,* 2020). Cadmium is considered as the most non-essential and highly toxic heavy metal. It is the heavy metal which is situated in between of zinc and mercury in the periodic table, having similar behavior to zinc. It is usually found as the impurity in zinc or lead deposits and therefore, it is primarily produced as the by-product of zinc or lead smelting. These important minerals found in this study shows that snail is a good source of minerals which are vital for healthy growth of the body, helping muscles, nerves and proper metabolism of body, therefore should be incorporated in the diets of man and its animals particularly the growing ones.

Cadmium possess various usuful applications such as in electroplating, batteries, plastics, paint pigments, television screens, cosmetics, galvanizing steel and metal coatings. It is also known to enter naturally through volcanic eruptions, river transport, weathering and human activities like, smelting, mining, incineration wastes and manufacturing of fertilizers. Primary exposure sources of cadmium include food and tobacco smoking. The crops are able to accumulate the cadmium levels due to the high rates of soil-to-plant transfer via cation exchange and intracellular transport. Therefore, consumption of staple foods such as rice, wheat and other leafy vegetables significantly contribute to the human cadmium exposure. Also, consumption of sea foods such as fishes, oysters, molluscs, crustaceans also lead to the human exposure (Jhumi and Pammi, 2017). Chronic exposure to the metal can lead to kidney disorders, anemia, emphysema, anosmia (loss of sense and smell), cardiovascular diseases, renal problems, and hypertension (Akan *et al.,* 2010; Davies *et al.,* 2013).Therefore, the low Cd level in the two species of the edible land snail can be consider to b save for consumption. Lead, like cadmium has been reported not to have any known function in human biochemistry or physiology, and do not occur naturally in living organisms (Collin et al., 2018). Hence dietary intakes of these metals, even at very low concentrations can be very harmful because they bioaccumulate. From our finding, *A. marginata* accumulate more lead (0.73±0.08mg/100g) than *A. fulica* (0.72±0.03mg/100g) this shows that, consumption of *A. fulica* meat is relatively safer than *A. marginata* in terms of lead toxicity. In conclusion, from the present study, the two species of snail (*A. marginata* and *A. fulica*) are nutritionally rich in protein and mineral nutrients which can be obtained at minimal cost, considering the high cost and associated high fat content risk of beef, poultry and other higher meat proteins. Hence, consumption of the two species of giant land snails should be encouraged for both the young and old, as an alternative source of essential nutrients at a lower cost.

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Influence of Integrated Nutrient Management with differential substitution of Farm Yard Manure on Grain Yield and Straw Yield of dual purpose *rabi* fodders

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Abstract— *A field experiment was carried out at the Research Farm of Division of Agronomy of Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, Chatha during the rabi season of 2017-18. The soil of the experiment was sandy clay loam in texture, slightly alkaline in reaction, low in organic carbon, available nitrogen and medium in available phosphorus and potassium. The experiment was carried out in randomized block design with 12 treatments and 3 replications. Three different dual purpose rabi fodders viz. Oat, Barley and Wheat were subjected to four integrated nutrient management treatments. The treatments consisted of oat with recommended dose of fertilizers + 25%N through FYM(T_1), oat with 75% recommended dose of fertilizers + 50% N through FYM (T_2), oat with 50% recommended dose of fertilizers+ 75% N through FYM (T_3), oat with 25% recommended dose of fertilizers+100% N through FYM (T_4), barley with recommended dose of fertilizers+ 25% N through FYM (T_5), barley with 75% recommended dose of fertilizers+50% N through FYM (T_6), barley with 50% recommended dose of fertilizers+75% N through FYM (T_7), barley with 25% recommended dose of fertilizers+100% N through FYM (T_8), wheat with recommended dose of fertilizers+25%N through FYM (T_9), wheat with 75% recommended dose of fertilizers+50% N through FYM (T_{10}), wheat with 50% recommended dose of fertilizers+75% N through FYM (T_{11}), wheat with 25% recommended dose of fertilizers+100% N through FYM (T_{12}). Among all the treatments, recommended dose of fertilizers along with 25% N through FYM proved superior in terms of grain and Straw yield. Similarly, the lowest values were obtained where 25% of recommended dose of fertilizers along with 100% of N through FYM was applied.*

Keywords— INM, Rabi fodders, dual purpose, grain yield, straw yield, harvest index.

I. INTRODUCTION

Agriculture is the backbone of the Indian economy and about 58% of the Indian population depends on agriculture (http://www.ibef.org). Due to diverse agro-climatic conditions, successful production of different types of crops could be possible. Different cereals, pulses, oilseeds, beverages, spices etc. are grown on Indian soils. However, we are still far behind in the production of forage, both quantatively and qualitatively for feeding the large livestock population (Sharma, 2008). With the decline in the size of agricultural land holdings and growing food security concern, there is no scope to increase area under fodder production. In order to meet the problems of land shortage and fodder scarcity, cultivation of dual purpose fodders could be a better option. Therefore, growing dual purpose cereal forages can serve both the concerns of growing food demand and food shortage. The demand and supply of green fodder can be covered upto a certain extent by growing dual purpose cereal varieties (Hundal *et al.,* 2014). Hence, it is very essential to maximize the quantity and quality of dual purpose fodder

production per unit area and time by proper management of grassland, pasture and also by utilising proper agro-techniques for fodder production (Jat *et al*., 2015).

II. MATERIALS AND METHODS

The experimental study was carried out at Research Farm of Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Main campus Chatha, during *rabi* season of the year 2017-18. Geographically, the experimental site is situated in the sub-tropical Shiwalik foothills of Jammu and Kashmir at 32^{o} 39′ N latitude and 74^{0} 53′ E longitude at an elevation of 332 meter above mean sea level. The soil of the experiment was sandy clay loam in texture, slightly alkaline in reaction, low in organic carbon and available nitrogen but medium in available phosphorus and potassium with electrical conductivity in the safer range. The research site is endowed with hot and dry early summers followed by hot and humid summers and cold winters. The contribution of South-West monsoon rains which are usually received from June to September is about 75 percent, where as the remaining 25 percent of rains are received in the form of few showers of cyclonic winter rains from December to March with mean annual rainfall of about 1174mm. The experiment was laid out in Randomized Block Design with twelve treatments replicated thrice. The treatments comprised of three different dual purpose *rabi* fodders *viz*. oats, barley and wheat. Full dose of Phosphorus and Potassium along with half dose of N were applied as basal dose through urea, Diammonium phosphate (DAP) and Murate of potash (MOP) respectively and the remaining half was applied in two equal splits, one at first cut and second at 30 days after first cut.

III. RESULTS AND DISCUSSIONS

Persual of the data revealed that application of recommended dose of fertilizers along with 25% N through FYM recorded significantly highest grain yield which was statically at par with 75% recommended dose of fertilizers + 50% N through FYM in all dual purpose fodders under study whereas the application of 25% recommended dose of fertilizers+100% N through FYM recorded lowest grain yield in different dual purpose *rabi* fodders. Among different fodders under experimentation, wheat was found to be the most superior in attaining maximum grain yield of 35.88 q/ha under treatment T_9 followed by barley recording grain yield of 30.66q/ha under treatment T_5 and oats 19.62 q/ha under treatment T_1. Application of 25% recommended dose of fertilizers along with 100% N through FYM resulted minimum grain yield. Maximum straw yield was obtained with the application of recommended dose of fertilizers along with 25% N through FYM whereas the lowest straw yields recorded with the application of 25% recommended dose of fertilizers+100% N through FYM in all the dual purpose *rabi* fodders under study. Comparison of different dual purpose *rabi* fodders under study revealed that wheat was the most superior in attaining maximum straw yield of 47.55 q/ha under treatment T_9 followed by barley 38.12q/ha under treatment T_5 and oats 30.45 q/ha under treatment T_1 whereas the application of 25% recommended dose of fertilizers along with 100% N through FYM recorded minimum straw yield in oats (17.05 q/ha) under treatment T_4 followed by barley (24.25q/ha) under treatment T_8 and wheat (32.07 q/ha) under treatment T_{12} respectively. This might be due to the reason that the combined use of FYM along with inorganics supplied readily available nutrients which improved the formation of assimilates and their translocation from source to sink which ultimately resulted in maximum grain and straw yield. Among all the treatments of Integrated Nutrient Management, harvest index showed parity with each other whereas among different dual purpose *rabi* fodders, significantly highest harvest index was recorded in barley (45.05%) followed by wheat (42.99%) and oats (39.19 %). Similar findings have been reported by Sharma and Kumar (2009), Shinde *et al*., (2017), Saharan *et al*. (2018) and Jadhao *et al*.(2019).

TABLE 1

EFFECT OF INTEGRATED NUTRIENT MANAGEMENT ON GRAIN AND STRAW YIELD (q/ha) OF DUAL PURPOSE *RABI* FODDERS

Treatments	Grain Yield (q/ha)	Straw Yield (q/ha)
T_1 - Oats with recommended dose of fertilizers + 25 % N through FYM	19.62	30.45
T_2 - Oats with 75 % recommended dose of fertilizers + 50 % N through FYM	16.76	27.78
T_3 - Oats with 50 % recommended dose of fertilizers + 75 % N through FYM	13.51	22.35
T_4 - Oats with 25 % recommended dose of fertilizers + 100 % N through FYM	9.18	17.05
T_5 - Barley with recommended dose of fertilizers + 25 % N through FYM	30.66	38.12
T_6 - Barley with 75 % recommended dose of fertilizers + 50 % N through FYM	28.28	35.98
T_7 - Barley with 50 % recommended dose of fertilizers + 75 % N through FYM	23.72	29.07
T_8 - Barley with 25 % recommended dose of fertilizers + 100 % N through FYM	16.77	24.25
T_9 - Wheat with recommended dose of fertilizers + 25 % N through FYM	35.88	47.55
T_{10} - Wheat with 75 % recommended dose of fertilizers + 50 % N through FYM	33.03	42.87
T_{11} - Wheat with 50 % recommended dose of fertilizers + 75 % N through FYM	29.22	37.85
T_{12} - Wheat with 25 % recommended dose of fertilizers + 100 % N through FYM	21.47	32.07
Sem (±)	1.04	1.63
CD (5%)	3.07	4.78

IV. CONCLUSIONS

Based on one season study, it can be concluded that the application of recommended dose of fertilizers along with 25% N through FYM proved superior in terms of grain and straw yield. Among different dual purpose *rabi* fodders, wheat was found to be superior followed by barley and oat respectively.

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Characterisation of Orchards and Diseases of Sugar Cane (*Saccharum Officinaruml.*) in Côte D'ivoire: Case of Sugar Complexes of Borotoukoro and Zuenoula

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Abstract— *As part of a study to develop a bacterial biocontrol agent against phytopathogens of sugarcane (Saccharum officinarum L.) in Côte d'Ivoire, an orchard diagnosis was carried out by conducting a survey in two sugar complexes, Zuenoula and Borotoukoro. The aim of this work was to collect socio-demographic data on sugarcane growers and to identify the pathologies encountered in these orchards. A sample of 220 growers was surveyed, with 110 growers per sugar complex. The results of this study showed that the majority of growers are Ivorian (95.5-100 %), with a high percentage of men (80-90.9 %) and more than half are illiterate (46.4-53.6 %). The frequency of phytosanitary treatments is limited to an average of two applications of chemical pesticides per year. None of the growers use biocontrol agents to treat their plantations. Symptoms of fungal diseases (smut, red snot and Pokka boeng) and viral diseases (mosaic dash) are present in the sugarcane plantations surveyed.*

Keywords— Survey, Sugar cane, sociodemographic data, pathologies, Côte d'Ivoire.

I. INTRODUCTION

Sugar cane (*Saccharum officinarum* L.) is a plant whose cultivation is very widespread in the world because of its importance in terms of food, economic and energy. It is a perennial grass from the Poaceae family. It is generally grown in tropical and subtropical regions, mainly for the exploitation of sugar (sucrose) contained in its stems. Indeed, the latter can have a saccharin richness of up to 19% each **(Péné *et al*., 2012).** World sugar consumption is growing continuously, hence the need for increased sugar production. For example, the 2021-2022 sugar season ended with sugar production of 184.4 Mt, up 2.5 Mt from the previous year **(FranceAgriMer, 2022)**. Apart from beets and sweeteners, sugar cane alone accounts for 80% of global sugar production **(FAOSTAT, 2018)**. In Côte d'Ivoire, its cultivation is carried out by two private companies (SUCAF-CI and SUCRIVOIRE) on a distribution area of more than 25,000 ha located in the north and central-west of the country **(Kouamé *et al*., 2009).** According to **Traoré et al., (2019)**, the country is ranked 53rd world and 16th African. Cane production amounts to about 330,000 t of sugar, or 50% of consumption in the WAEMU (West African Economic and Monetary Union). In the medium term, the WAEMU market offers good development prospects for Ivorian sugar companies **(Kouamé *et al*., 2009)**.

Despite its socio-economic importance, the cultivation of sugar cane in Côte d'Ivoire, its culture faces biotic constraints due to viruses, stem-boring insects such as Eldana saccharinaWalker, bacteria such as *Xanthomonas albilineans* and fungi such as *Sporisorium scitamineum*, responsible for anthrax (**Kouamé *et al*., 2010).** On the other hand, it faces a decline in biological soil fertility due to monoculture sugar cane farming practices carried out for more than 30 years in the various sugar complexes **(Mauboussin, 1988; Marion, 2000)**. All these factors play a very important role in lowering sugar cane yields in the country's sugar complexes **(Péné *et al*., 2012).** In the sugar complexes of Borotoukoro and Zuenoula, producers are confronted with various diseases of fungal, viral and bacterial origin. These diseases may have a negative impact on the yield of the canned crop in Côte d'Ivoire. As part of a study for the development of a bacterial biocontrol agent against these diseases, The aim of

this study was to collect sociodemographic data on sugar cane producers and to identify the pathologies encountered in these orchards.

II. MATERIAL AND METHODS

2.1 Material:

The material used for this study consisted of a survey sheet with a questionnaire, pens and a GPS for taking geographical coordinates.

2.2 Methods:

2.2.1 Study area:

This study was carried out in two sugar cane production areas: the Zuenoula Integrated Agricultural Unit (IAU), located between 7°30 and 7°40 north latitude, and between 6°50 and 6°15 west longitude and the Integrated Agricultural Unit (UAI) of Borotoukoro, located between latitude 08°31 North and longitude 7°17 West **(Figure 1)**.

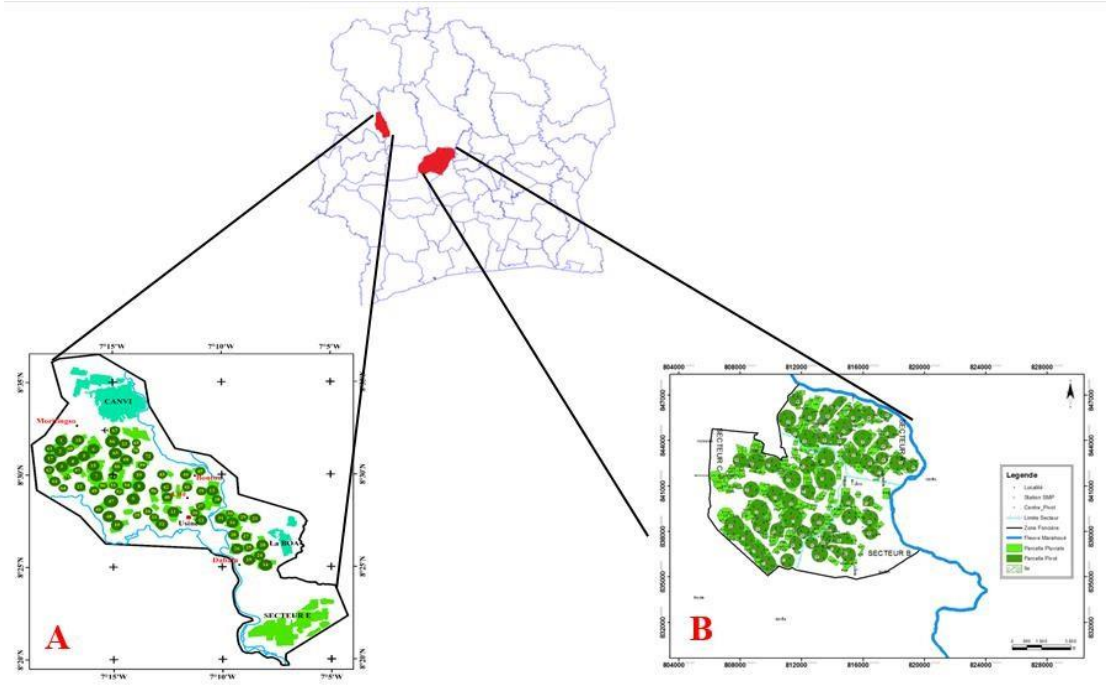

FIGURE 1: Study area

A- UAI Borotoukoro Plot Map and B-UAI Zuenoula Plot Map

2.2.2 Conducting the survey

The survey was carried among sugar cane producers in the Borotoukoro and Zuenoula sugar complexes. interviewed, or one hundred and ten (110) producers per complex. The information was collected using a questionnaire sheet addressed to producers. The interviews focused on the identity of the producers, the description of the farmers, the method of harvesting sugar cane, the typology of the diseases, the losses due to the diseases and the methods of control used against these diseases.

III. RESULTS

3.1 Socio-demographic characteristics of producers:

The socio-demographic profile of sugar cane producers interviewed in the localities of Borotoukoro is recorded in Table I.It appears that of the 110 people interviewed in Borotoukoro, 95.5% were Ivorians and 90.9% were men. In terms of educational attainment, 53.6% of those surveyed were illiterate. The dominant age group was between 41 and 60 with a rate of 58.2%. Almost all the people surveyed regularly produced sugar cane and the cultural experience was mostly over 15 years with a rate of 45.5%. On the other hand, in Zuenoula all the people interviewed had the Ivorian nationality. Of the 110 respondents, 80% were male. In terms of educational attainment, 46.4% of those surveyed were illiterate. The dominant age

group was between 41 and 60 with a rate of 49.1%. 108 of the respondents produced sugar cane on a regular basis, and the majority of their farming experience was over 15 years (**Table II).**

TABLE 1
SOCIODEMOGRAPHIC PROFILE OF SUGAR CANE PRODUCERS IN THE BOROTOUKORO AREA

Variable	Categories	Staff (n=110)	Percentage (%)
Nationality (n=110)	Ivorian Foreign	105 5	95,5 4,5
Sex (n=110)	Man Woman	100 10	90,9 9,1
Age group (n=110)	18-40 years 41-60 years Over 61 years	26 64 20	23,6 58,2 18,2
Level of education (n=110)	None Primary Secondary Superior	59 23 28 0	53,6 20,9 25,5 0,0
Cultural experience (n=110)	4-6 years 7-10 years 10-15 years Over 15 years	9 17 34 50	8,2 15,5 30,9 45,5

TABLE 2
SOCIODEMOGRAPHIC PROFILE OF SUGAR CANE PRODUCERS IN THE ZUENOULA AREA

Variable	Categories	Staff (n=110)	Percentage (%)
Nationality (n=110)	Ivorian Foreign	110 0	100 0,0
Sex (n=110)	Man Woman	88 22	80,0 20,0
Age group (n=110)	18-40 years 41-60 years Over 61 years	18 54 38	16,4 49,1 34,5
Level of education (n=110)	None Primary Secondary Superior	51 31 27 1	46,4 28,2 24,5 0,9
Cultural experience (n=110)	1-3 years 4-6 years 7-10 years 10-15 years Over 15 years	1 2 16 27 64	0,9 1,8 14,5 24,5 58,2

3.2 Types of diseases observed in sugar cane plantations in surveyed areas:

The most reported pathologies in the two sugar cane complexes were mosaic with a rate of 90.0% in the locality of Zuenoula against a rate of 86.4% in the locality of Borotoukoro. Thus, 87.3% of the 110 people surveyed in Zuenoula say that they observed on the plots of Coal against a rate of 83.6% in the locality of Borotoukoro. 76.4% of people observed Pokka boeng in the locality of Zuenoula against a rate of 56.4% of people surveyed in the locality of Borotoukoro. However 68.2% of producers surveyed in Zuenoula confirmed the presence of other pathologies against a rate of 52.7% in the locality of Borotoukoro **(Table III and IV)**.

TABLE 3
DISEASES OBSERVED IN THE BOROTOUKORO AREA

Variable	Categories	Staff (n=110)	Percentage (%)
Coal (n=110)	Yes	92	83,6
	Non	18	16,4
Pokka boeng (n=110)	Yes	62	56,4
	Non	48	43,6
Mosaic (n=110)	Yes	95	86,4
	Non	15	16,6
Other diseases (n=110)	Yes	58	52,7
	Non	52	47,3

TABLE 4
DISEASES OBSERVED IN THE ZUENOULA AREA

Variable	Categories	Staff (n=110)	Percentage (%)
Coal (n=110)	Yes	96	87,3
	Non	14	12,7
Pokka boeng (n=110)	Yes	84	76,4
	Non	26	23,6
Mosaic (n=110)	Yes	99	90,0
	Non	11	10,0
Other diseases (n=110)	Yes	75	68,2
	Non	35	31,8

3.3 Means of combating diseases:

In both complexes, all surveyed producers claimed that the only means of controlling these diseases was essentially chemical. No planter uses bacterial biocontrol agents.

3.4 Causes of post-harvest losses:

In the locality of Zuenoula, 71.8% of the producers surveyed believe that the losses are due to microorganisms against 62.7% producers in the locality of Borotou-koro. For losses due to pests in the locality of Zuenoula on 110 productions questioned, 75 producers or a rate of 68.2% think that the losses are due to pests against 57 producers or a rate of 51.8% in the locality of Borotou-koro, 53.6% of producers questioned in the locality of Borotou-koro found that the losses are due to bush fires against a rate of 22.7% in the locality of Zuenoula. In both localities, most of the producers questioned agree that in addition to the causes already listed, there are other reasons that can explain the post-harvest losses, a rate of 89.1% in the locality of Zuenoula against a rate of 70,0% in Borotou-koro **(Table V and IV)**.

TABLE 5
CAUSES OF POST-HARVEST LOSSES IN BOROTOUKORO

Variable	Categories	Staff (n=110)	Percentage (%)
Microorganisms (n=110)	Yes	69	62,7
	Non	41	37,3
Pests (n=110)	Yes	57	51,8
	Non	53	48,2
Bush fires (n=110)	Yes	59	53,6
	Non	51	46,4
Other causes (n=110)	Yes	77	70,0
	Non	33	30,0

TABLE 6
CAUSES OF POST-HARVEST LOSSES IN ZUENOULA

Variable	Categories	Staff (n=110)	Percentage (%)
Microorganisms (n=110)	Yes	79	71,8
	Non	31	28,2
Pests (n=110)	Yes	75	68,2
	Non	35	31,8
Bush fires (n=110)	Yes	25	22,7
	Non	85	77,3
Other causes (n=110)	Yes	98	89,1
	Non	12	10,9

IV. DISCUSSION

The objective of this work was to collect sociodemographic data of sugar cane producers and identify the pathologies encountered in these orchards. The results of the investigation revealed that the majority of sugar cane producers in the Borotou-Koro and Zuenoula areas are men and have Ivorian nationality. These results could be explained by the fact that field work is exclusively reserved for men because of their painful nature. According to FAO (2011), in agricultural regions of Africa, men often dominate the agricultural workforce. However, it is important to note that more than half of those surveyed are illiterate. In Côte d'Ivoire, the lack of schooling of a large segment of the population explains the high level of illiteracy. This can have a negative impact on access to agricultural information and good agricultural practices. Similar results were obtained in **Koffi's (2018)** work on risk factors for pineapple fruit spoilage in production areas. Producers have a dominant age range between 41 and 60, suggesting that sugar cane is an agricultural activity that attracts mainly middle-aged farmers. This could have implications for the transmission of agricultural knowledge and practices to younger generations. The sector is therefore part of sustainability. The growers' growing experience is also a key factor. In both localities, a significant proportion of producers with a cultivation experience of more than 15 years, suggesting a thorough knowledge of sugar cane cultivation. The high rates of diseases, such as Mosaic, Coal and Pokka Boeng, in both localities underline the endemic nature of these diseases. Managing these diseases in sugar cane cultivation remains a major challenge. The Mosaic, in particular, is a major concern with a prevalence rate of 90% in Zuénoula. Similar results have been observed in Africa, particularly in Nigeria where coal, red snot and mosaic have been detected on sugar cane plots **(Wada *et al.*, 2016)**. These diseases are caused by various pathogens, including fungi and viruses **(Rott *et al.*, 2000; Baudin, 1963)**. Finally, other diseases have been cited by producers such as red snot or red rot, rust, gum, scald leaves and annular spots. Producers identify several causes of post-harvest losses, including microorganisms and pests. These post-harvest losses are major problems for sugar cane producers in both localities. According to **Kouamé *et al.* (2010)**, biotic constraints in sugar cane cultivation can be summarized as insect pest attacks and microorganisms. Anthropogenic activities such as bush fires have also been reported as a cause of losses, particularly in Borotou-Koro. Producers in both communities are aware that there are other reasons for post-harvest losses, including weeds and maintenance of crop plots. Indeed, a maintenance time of 4 months is necessary so that the cane does not suffer a measurable yield loss following competition induced by weeds **(Traoré *et al.*, 2019)**. In addition, the soil between cropping cycles and the use of heavy harvesting machinery have led to physical, chemical and biological degradation of sugarcane soils and, as a result, the development of various soil organisms harmful to sugarcane growth according to **Péné *et al.*, (2012)**.

V. CONCLUSION

This study contributed to the provision of socio-demographic data and the pathologies encountered in sugarcane production in Côte d'Ivoire. The study revealed that sugar cane cultivation is a male activity, practised by illiterate people on village plantations. Crop losses are caused by micro-organisms responsible for diseases such as smut, red glanders and Pokkah Boeng. The overuse of chemical pesticides could result in the development of resistance to plant pathogens, hence the need to develop biological control through the development of biopesticides.

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Response of Dairy Cow on Different Types of Feeding Diet: A Review

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Abstract— *This research critically investigates dairy cows responses to various feeding diets, including forage-based diets, total mixed rations (TMR), and new dietary supplements. Dairy cows are fed a variety of diets, each with its own role and effects. The review explains the varied consequences of various feeding regimens on dairy cow performance, health, and environmental sustainability through a thorough examination of contemporary literature. Milk yield, composition, and quality, as impacted by various dietary components, are important topics of study. The review also investigates the nutritional adequacy and digestibility of alternate feed sources in dairy cow diets, such as crop residues and byproducts, to provide light on their potential as sustainable feed solutions. From this review article we have learned that if green fodder and concentrate is given to dairy cow as part of mixed diet then overall health or milk production of the animal will be improved and when feed mixture of dry fodder or concentrate or mineral mixture or biphosphate is given along with green fodder, the animal is healthy or gives good milk production and well prepared for the next calving.*

Keywords— Animals, Dairy, Cow, Feeding, Milk.

I. INTRODUCTION

For thousands of years, dairy production has played a significant role in the agricultural landscape. About 70% of people live in villages in India, a For thousands of years, dairy production has played a significant role in the agricultural landscape. About 70% of people live in villages in India, a country with a predominately agrarian economy where animals are essential to everyday life. High- quality foods like milk, cheese, butter, ghee, and others are produced by livestock. India is the world's biggest consumer of milk and milk products in addition to being one of the top producers of milk worldwide. To meet domestic demand, we must import a sizable quantity of milk products due to the shortage in supply. Animal husbandry and agriculture coexist in a symbiotic connection whereby the former supplies livestock with food and straw, while the latter provides milk, manure, and labor for a variety of agricultural tasks. India's socioeconomic landscape is changing thanks in large part to the dairy industry. In addition to offering better nutritional benefits, it has produced a great deal of job chances.

With a rise in domestic output, dairy production is essential to the livestock industry and the country's economy Azage *et al.,* (2013). Regarding employment, income creation, and consumption, this sector provides a substantial and widely held section of the rural population with their means of subsistence. Additionally, milk and milk products contribute to nutritional energy requirements, high-quality protein, minerals, and vitamins, especially in vegetarian diets for humans. Górska *et al.,* (2019). Dairy cattle form a unique niche among animals used for human purposes. They were chosen from among cattle to serve a single, highly specialized function: producing fluid milk. There are no other livestock breeds that have undergone as much systematic and prolonged selection for milk production as dairy cattle, despite the fact that many dairy cattle are equally valuable as meat or draught animals. Cattle are raised for dual (meat and milk) or even triple (meat, milk, and draught) purposes in many regions of the world. Cattle breeds usually exhibit the results of having so many goals. Breeds of cattle raised primarily for milk production, particularly in Europe and North America, are known as dairy cattle breeds.

Five essential nutrients—protein, carbs, fats, minerals, and vitamins—are needed by cattle. Each nutrient plays a unique purpose in the body. For example, protein is necessary for body growth and to repair wear and tear on the body. It also plays a significant role in the production of milk. The primary sources of protein include sunflower meal, groundnut cake, and oil cake.

The body needs carbs as a source of energy to carry out its basic functions. Fats, on the other hand, supply about 2.5 times the energy of proteins and carbohydrates combined. Animals obtain their fat from oil seed cake and feed additives such as bypass fat. Maize, pearl millet, etc. Since maize has a larger energy content than other grains, ground maize is employed as an energy source in the majority of animal feeds. For dairy animals to produce and reproduce well, about fifteen minerals are needed, including calcium, phosphorus, magnesium, sulfur, copper, and cobalt. Dairy animals may produce certain vitamins internally, such as B complex, C, K, and D when exposed to sunshine, but we also need to provide them external sources of vitamins A and E, which can be obtained from minerals mixed with green feed.

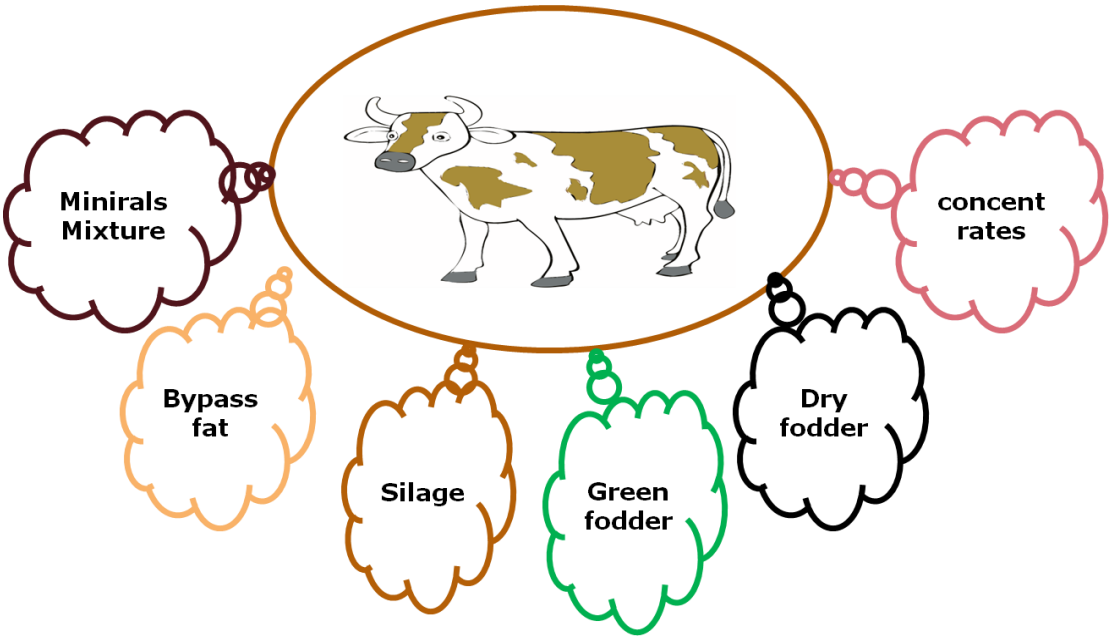

FIGURE 1: Scientific feeding of dairy animal

TABLE 1

Breed	Origin	Milk Production
Holstein Friesian	Holland.	7200-9000 Kg
Jersey	France.	5000-8000 Kg.
Red Sindhi	Pakistan	1700 -3400 Kg
Sahiwal	Pakistan	1350 - 2100 Kg
Gir	India	900 - 1600 kg

1.1 Milk production of dairy cow kg per lactation according to icar.org:

The way dairy cows are raised plays a vital role in milk production, and a high- grain diet has a significant impact on milk production.

High-grain diets, characterized by a higher proportion of grains such as corn or barley, have been shown to affect dairy cow performance and lead to changes in fat and protein content in milk.

Research shows that diets based on barley and corn generally produce higher fat yields compared to concentrates, highlighting the importance of dietary composition on milk production.

Additionally, research shows that a high-fat diet improves milk production efficiency, as evidenced by lower dry matter intake and increased milk and butterfat production. On the other hand, dairy cows' milk quality has been demonstrated to be impacted by diets based on forage. The fatty acid profiles of milk can be impacted by the quality of the forage consumed; dairy cows fed maize stalks produced milk with a higher concentration of particular fatty acids. In addition, it has been discovered that diets with higher-quality alfalfa and a lower concentrate % produce milk that is equivalent to diets with lower-quality alfalfa and a higher concentrate percentage, highlighting the significance of forage quality in preserving

milk productivity. These studies show that adding forage-based diets to dairy cattle's diets can significantly affect the content and quality of their milk. Since balanced diet gives dairy cows the nutrients they need to produce large amounts of milk, it is crucial for increasing milk productivity.

Moreover, the diet's composition—in particular, its level of fat, protein, and carbohydrates—is essential for supplying dairy cows with the nutrients they need to produce a lot of milk.

II. GREEN FODDER

Green fodder is fed fresh either after cutting it from the field or after cutting it at home. Hence there is no need for its storage. Green fodder contains abundant amounts of protein, vitamins, carbohydrates, fiber and mineral salts. For dairy cattle, green grass is a cost-effective source of nutrition. It is quite easy to eat and digest. Green fodder's microorganisms aid in enhancing crop residue digestion in mixed feeding systems. Additionally, it promotes animal health and increases the effectiveness of animal reproduction. The cost of producing milk may be decreased if cows were fed more green grass. Green fodder output must be increased by increased usage of improved fodder seeds in order to close the gap between supply and demand. Farmers must implement the following farming strategies in order to guarantee the supply of green fodder throughout the year. Green fodders are rich in nutrients and also the primary source of Vitamin 'A'. Animal immunity is increased when green fodder is fed in a balanced manner. Better vision and the health of the respiratory system depend on vitamin A. It is crucial to the upkeep and functionality of the mucous membrane. It is necessary for the maintenance of pregnancy, placenta shedding, and other reproductive processes. It is necessary for the gastrointestinal system; lack of it results in diarrhea, poor nutrient absorption, etc. It is necessary for the urinary tract; insufficient amounts result in kidney, ureter, and bladder stones. Twenty thousand I.U. of vitamin "A" are excreted from each litre of milk during lactation. Green fodder lowers milk production costs by reducing concentrate feeding. The microorganisms found in green fodders are mostly responsible for its digestibility and palatability. It supplies vital nutrients needed for animal reproduction, health, and the production of milk and meat. In use, it is a laxative. A cheap source of vitamin "A" is green fodder. In addition, it contains dry matter, crude protein, all digestible elements, and minerals. Additionally rich in phytochemicals and oxidants, green fodder is crucial for growth, reproduction, health, and productivity.

III. DRY FODDER

Dry fodder is the fodder which is made by drying crop residues, Hay, stovers like jowar kadbi, and straws with a moisture content of 10-15%, such as paddy straw, wheat bhussa, and Karad grass, are the main sources of dry feed. Adult cattle need three to six kg of dry feed per day in total. The amount and quality of dry feed have a big impact on how much milk dairy animals produce. Superior dry fodder improves rumen health and microbial activity, which in turn improves milk yield and composition. It does this by having an ideal nutritional content and digestibility. On the other hand, subpar dry feed can negatively impact the effectiveness of milk production and the health of the herd. Research by Johnson & Associates (2018). The wellbeing and general health of dairy cattle are greatly impacted by the quality of their dry feed. Sufficient fiber from dry hay is necessary for healthy rumen function and guards against digestive issues like bloat and acidity. However, low-quality dry fodder might put cattle at risk for dietary deficits and metabolic disorders. Mycotoxins can have a negative impact on milk quality and offer a health concern to dairy calves. They are often found in moldy or contaminated dry feed. Study conducted by Kholif *et al.* (2017)

IV. SILAGE

Silage is a type of fodder made from green foliage crops which have been preserved by fermentation to the point of acidification. It can be fed to cattle, sheep, and other animals Animals that ingest sugarcane top silage treated with urea and molasses more effectively than those that feed sugarcane top (SCT) silage without additives would gain greater weight each day, according to Silvester *et al.* (1976). Compared maize silage with and without hay at low and medium dry matter levels. The low dry content corn silage matter (24%) decreased intake and output; however, this effect was countered by include hay in the diet. According to Kumar *et al.* (2019), fermented silage is as nutritious as green fodder since it keeps the nutrients in their natural state, making it just as useful for animals.

V. CONCENTRATE

A concentrate mixture containing protein supplements such as oil cakes, energy sources such as cereal grains (maize, jowar), tapioca chips, and laxative feeds such as brans (rice bran, wheat bran, gram husk) is commonly utilized. The concentrate mixture can be fed before milking, half in the morning and half in the evening. Half of the roughage diet can be fed in the

morning after the animals have been watered and cleaned. The remaining half is fed in the evening, following milking and watering. High yielding animals can be fed three times per day (roughage and concentrate). Increasing the frequency of concentrate feeding will aid in maintaining normal rumen motility and optimal milk fat levels. Overfeeding concentrates may cause off feed and dyspepsia. Abrupt changes in the feed should be avoided. Grains should be ground to a medium fineness before being fed to cattle. It is critical to assess the production benefit of a "feed to yield" strategy in comparison to minimal concentrates, not only on milk production but also on other crucial variables such as BCS and metabolic condition. Hillset *et, al.*, (2015).

VI. MINERAL MIXTURE

Mineral mixture is a specially formulated blend of essential minerals that dairy animals require for proper growth, reproduction, and overall health. The major minerals include calcium, phosphorus, magnesium, potassium, sodium, chlorine and sulphur. Among those needed in trace amounts are iron, zinc, manganese, copper, iodine, cobalt and selenium. Mineral mixture gives with the diet according to cattle different types of stages. Mineral deficiencies, imbalances, and toxicity can lead to reproductive issues in animals, as minerals are crucial for their health and reproduction. The quantity of minerals in frequently available feed stuffs is vary, and the majority of them may not be sufficient to meet the requirements of animals at different phases of production. Bhanderi *et al.,* (2016). Dietary mineral supplementation can boost dairy cow productivity and generate more cash for producers. Large-scale implementation is necessary to improve dairy animal performance.

VII. BYPASS FAT

Dietary fat that resists lipolysis and bio hydrogenation by rumen microbes but is digested in the lower digestive tract is referred to as bypass fat, rumen protected fat, or inert fat. Oil seed and oil seed cake are main source of bypass fat diet. Erickson *et al.* (1992) found that supplementing Ca-LCFA improved hemicellulose digestibility, resulting in increased NDF and decreased ADF digestibility in cows during early lactation. According to Naik et al. (2009).supplementing dairy animals' diets with bypass fat increases milk output by 5.5-24.0%. Elliott *et al*. (1996). This study aimed to assess the impact of feeding lumen-bypass fat on milk supply, composition, and economics in early-mid lactating dairy cows in the field. Supplementation with bypass fat exhibited no negative effect on rumen fermentation, feed intake, nutritional digestibility, or other blood markers in dairy cows.

VIII. RESULTS AND DISCUSSION

Dairy cows are fed different diets, each of which has its own role and different effects like green fodder contains Vitamin A and other vitamins and easy to digest which increases milk production, silage and Dry fodder works well in the shortage of fodder or works on the quality of milk. It is cheap or good fodder for non-milking animals. Limited bypass fat diet keeps the animal's joints or digestive system healthy and does not affect milk production. Concentrate or mineral mixture is an improvement diet for lactating cows that can increase both the quality and quantity of milk or it can improve the overall health of the animal. From this review article, we have found out which fodder does what work and a good diet is necessary for good dairy and milk production.

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