ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24AUG1405

Effects of Neem Tree Leaf Extract (*Azadirachta I Ndica* A. *Juss*) as Defaunating Agent of Sheep (*Ovis Aries* L.) Fed Napier Grass (*Pennisetum Purpureum* Schumach)

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Abstract:- The effectiveness of neem tree leaf extract (NTLE) as defaunating agent was compared with sodium lauryl sulphate (SLS). Ten (10) sheep was used in the comparison of defaunating agents, the neem tree leaf extract was found to cause fluctuations in ruminal pH, similar to that of sodium lauryl sulphate, effectively reducing protozoal counts (from 296,000 cells/ml to 1,000 cells/ml) and increasing bacterial population (from 10,780 colony forming units/ml to 950,000 colony forming units /ml) in the rumen of sheep. It is therefore strongly advised to use neem tree extract as defaunating agents for sheep fed Napier grass to defaunated sheep in order to maximize food utilization and animal performance.

Keywords:- *Defaunation, Neem Tree, Rumen Protozoal, Rumen Bacterial.*

I. INTRODUCTION

The inventory data on goat and sheep in the country from the year 1999 to 2002 showed that sheep population in the country was less than 1% of the total sheep population in Asia which became a concern of the Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD). Hence, innovation that can assist the sheep industry became one of the Council's research priority areas, to solve nutritional problems leading to significant growth performance and production of the animal.

Like other ruminants, nutritional diet is the primary factor to be considered in sheep raising. As pointed out by Lee (2008), nutrition of the animal is related to its growth performance, and innovation such us rumen manipulation or bypass protein supplementation are promising strategies to provide efficient nutrition of the animal (Leng, 1991).

The presence of billions of bacteria, and thousands of protozoa, and fungi per ml rumen fluid as agents of digestion makes it possible f or ruminants to consume and utilize highly fibrous feeds (Muchira, 2020; Eugene et al., 2004). However, it is very important that the balance of microbial population in the rumen is maintained at a proper ratio to ensure efficient breakdown of consumed roughages. In instances when supply of amino acids (from protein) in the rumen is limited, the protozoan, which require amino acid-N f or growth and normal function (Schwab, and Broderick 2017; Jouany, 1996), will engulf bacteria f or amino acid supply. As a consequence, bacterial population declines, affecting the overall microbial Fermentation in the rumen by reducing the rate at which bacteria colonize ingested food particles (Owens and Basalan, 2016; Hungate, 1966; Coleman, 1975).

Manipulation of the microbial ecosystem by defaunation (process of making the rumen free of protozoa) increases bacterial population and the consequent capture of dietary nitrogen in the rumen, thereby improving the efficiency of feed utilization. The removal of protozoa in the rumen results not just in the increase of the amount of protein but also energy due to the elimination of nutritional competition between bacteria and protozoa in utilizing carbohydrate. Defaunation has been carried out using detergents, and the commonly used is sodium lauryl sulphate (Santra et al., 2007; Santra and Karim, 2000). The economic benefits of defaunation using these chemicals had been proven scientifically. Due to the high cost, pollution through accumulation of sodium ions and the dangers to people and animals due to its corrosive nature of using this chemical, there is then a need f or alternative defaunating agent.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24AUG1405

There is wide array of plants that can be used as defaunating agents (Monforte-Briceno et al., 2005; Serohi et al., 2009). Plant extracts containing high levels of saponins, flavonoids, and tannins had been found to reduce population of protozoa, decrease methane production and induce extensive stimulation of microbial metabolism resulting in enhanced flow of microbial protein from the rumen, increased efficiency of feed utilization, and improved nutrition of animal (Teferedegne, 2000; Patra et al., 2006; Tekeli et al., 2007). However, effectiveness of plant extracts as defaunating agent would depend upon the source, and the type and level of secondary metabolites present such as saponins, flavonoids and tannins (Patra et al., 2006). Neem (*Azadirachta indica A. Juss*) plant extract has high concentration of secondary metabolites and, therefore, can be a good alternative (Chauhan et al., 2004). Neem is a fast-growing tree, belonging to family *Meliacea*, and has a strong root system. It is tagged as "a tree f or solving the global problems" because of its potential in solving human health, agronomical and environmental problems. It is used as defaunating agent in buffalo as reported by Chaunhan et al., (2004).

The study's goals are to assess the effectiveness of sodium lauryl sulphate and neem tree extract as defaunating agents for sheep fed Napier grass.

II. MATERIALS AND METHODS

A. Preparation and Dosing of Neem Tree Leaf Extract

Fresh l eaves of neem tree were collected excluding the t wigs. The collected l eaves were weighed and washed with tap water to remove foreign particles. Juice of the neem tree l eaves was extracted with tap water as the extracting agent by the use of a blender. About 1 kilo of neem tree leaf and 1 liter of tap water or 1:1 ratio was used to extract 1 liter of concentrated neem tree juice (Fig. 2). The extracted juice was strained with cheesecloth and administered fresh to the animal, or stored for not more than 24 hours before administration. Only 200 ml of the juice extract was drenched to each animal per administration. The first dose was done for three (3) consecutive days at 7:00 A.M., the next dose was on the 6th day, and the third dose was on the 14t h day after the first dose.
20

B. Preparation and Dosing of Sodium Lauryl Sulphate Solution

Sodium laury lsulphate powder (Fig. 3) was dissolved in distilled water before being administered to the animal. The rate of administration was at 0.08 gram of powder dissolved in 10 ml of water per kg body weight (Santra and Karim, 2002) by drenching. The first dose was done for three (3) consecutive days at 7:00 A.M., followed by a second dose on the 6t h day, and a third dose on the 14t h day.

C. Preparation and Feeding of Experimental Animals

Prior to the conduct of the study, the animals were dewormed and given vitamin ADE through injection. The sheep in this experiment were fed *ad libitum* using napier grass (*Pennisetum purpureum* Schumach). The feeding of experimental animals was done for 14 days. Experimental animals were fed at 8:00 A.M. and at 4:00 P.M daily. Drinking water was made available at all times, and the animal shed was cleaned daily.

D. Dietary Treatments and Experimental Set-up

Ten (10) sheep were weighed and randomly distributed to the two treatments and confined individually in open-top metabolism cages (Betsil, 1985). The treatments consisted of t wo (2) types of defaunating agents as follows:

- Neem tree leaf extract
- Sodium lauryl sulpahte

E. Administration of Defaunating Agents

In this study, the method of Ankrah et al., (1990b) was employed for defaunation, with some modifications.

Day 1-3 Feed given was reduced to half of the *ad libitum* intake, and at the same time the defaunating agent was administered every day in 3 consecutive days by drenching.

Two (2) hours post-dosing in day 3, the animal was drenched with 200 ml of a substrate solution of flour and sugar to sustain the bacterial population in the rumen when the animals were fed half full.

Day 4 Start of feeding the experimental animals *ad ibitum* ration until the end of the experiment.

Day 6 The second dose of defaunating agent was administered to the animal, while being fed *ad libitum.*

Day 14 The defaunating agent was administered at 50% of the dose without feed restriction.

F. Rumen Fluid Collection

The 10 ml sample of rumen fluid was collected through stomach tubing using a human stomach tube (fringe 18 x 125 cm), as shown in Figure 4. The collection was done one hour after every dose of the defaunating agents (Jouany et al., 1981). The initial collection was done night before the administration of the first dose of the defaunating agents, and the second was done on third day which was done one hour after administering the last dose. The third collection was done at day seven, while the last collection was done at day fourteen (Rowe et al., 1985). This is the one recommended since defaunating agents can affect the rumen ecosystem, thus, there was sampling on the 3r d day during "adaptation period" and on the 9th day by which the rumen ecosystem was believed to have stabilized. All rumen fluid samples were added with 40 ml formal saline (9g sodium chloride/l) solution upon collection (Rowe et al., 1985).

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G. Data Gathered

➢ *Ruminal pH Determination*

The pH of the rumen fluid was determined using pen type pH meter. Determination of pH was done before and one hour after administration of defaunating agents to the animal during the first dose at day 1-3, during the second dose at day 6, t o t he last dosage at day 14.

➢ *Protozoal Count*

The collected rumen fluid sample was immediately placed into the test tube and was serially diluted to 1:10 dilutions (Rowe et. al., 1985) within one hour after collection. Protozoa was counted through the bright line standard 1/10 mm deep counting chamber method using a microscope and hand tally counter. The protozoal counts were then expressed as: Cell counts (cc/ml) = Number of protozoal cells x 2500 x dilution rate

➢ *Bacterial Count*

The rumen fluid was immediately placed into the test tube with rubberized cover and was brought to the laboratory. The rumen fluid samples were serially diluted from 1:10 to 1:1,000,000 dilutions (Rowe et. al., 1985) and poured into the prepared medium containing using MRS agar. The medium was incubated for 18 to 24 hours using anaerobic chamber (GasPak) system. The bacterial colonies were counted by the use of a Suntex Colony counter, and expressed as "colony forming unit's /ml fluids as follows:

Colony Forming Unit (cfu/ml) = Number of bacterial colonies x dilution rate

➢ *Analysis of Data*

All data were analyzed by unpaired t -Test using Statistical Package for the Social Science (SPSS) version 12 computer package.

III. RESULTS AND DISCUSSION

The study used a botanical leaf extract of neem tree as defaunating agent in comparison to sodium lauryl sulphate (SLS). The use of neem tree leaf extract (NTLE) is assumed to be ecologically friendly compared t o t he commercially available SLS.

A. Ruminal pH

As presented in Table 1 and Figure 1, the pH rapidly increased as defaunating agents were administered, then continuously declined in the second until the third dose. The result is expected f or SLS being an alkali, but NTLE exhibited similar effects as that of the commercial defaunating agent. It was also observed that rumen pH rapidly declined after the first dose of the defaunating agent in three consecutive days.

Detergents had been used effectively as defaunating agents (Santra et al., 1994). SLS had been primarily used as one of the ingredients in making commercial soap and tooth paste, therefore, it has the capacity to kill protozoa in the rumen. In the experiment of Benge (1986), NTLE and oil was utilized in making soap as well. NTLE appeared to be comparable to SLS in effecting sudden increases and consequent drop in rumen pH, therefore it has a great potential in killing protozoa in the rumen since protozoa are very susceptible to fluctuations in rumen pH. Within the normal limits of rumen pH from 6.2 to 6.8, population of protozoa in the rumen is high (Shriver et. al., 1986). However, the sudden rise in rumen pH after administration of defaunating agents eliminate protozoa, allowing bacteria to flourish rapidly with a consequent reduction in rumen pH (Santra et al., 1995).

Table 1: The Fluctuations in Rumen Fluid pH of Sheep Fed Napier Grass and Defaunated using NTLE and SLS.

Treatment	**1st dose day 1-3**		**2nd dose day 6**		**3rd dose day 14**	
	Before	**After**	**Before**	**After**	**Before**	**After**
Neem Tree Leaf Extract (A)	6.8	7.1	6.3	7.0	6.0	6.9
Sodium Lauryl Sulphate (B)	6.8	7.1	6.4	7.0	6.9	7.0
p-value			0.092	0.08	0.407	0.303

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24AUG1405

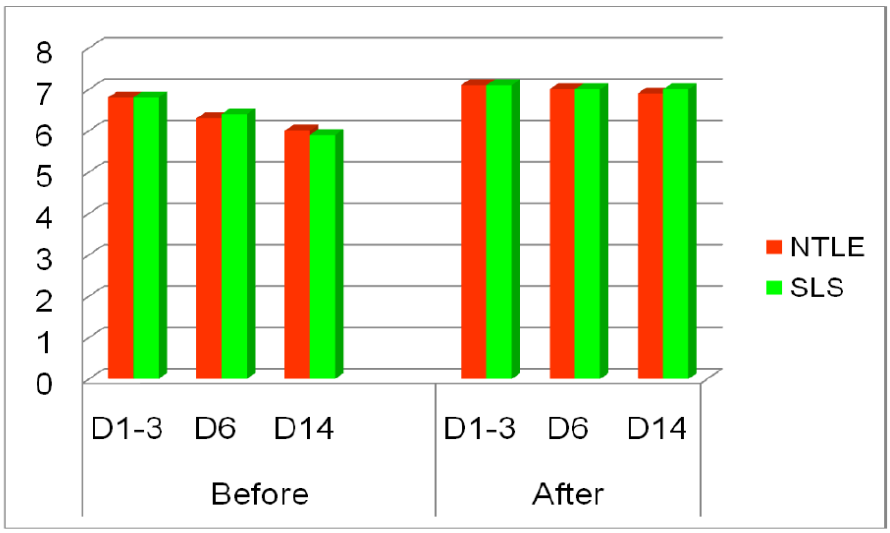

Fig 1: The Rumen pH Values Sheep Fed Napier Grass as Offered by the Type of Defaunating Agent

B. Rumen Protozoal Count

As presented in Table 2 and Figure 2, the influence of defaunating agents on the rumen pH apparently affected the protozoal population in the rumen. The protozoal count rapidly declined after the first dose of NTLE and SLS in three consecutive days, and decline continuously until the 3rd dose in day 14. The reduction in protozoal count by NTLE was comparable to that of SLS, showing no significant difference (P<.01) between the two defaunating agents.

However, the defaunation treatment, did not completely eliminate all protozoa in the rumen, and the possibility of a shift in the composition towards the more acid-resistant protozoa such as the *Isotrichia and Entodinium* species (Nagaraja and Towne, 1990) is the likely explanation. The mode of action on the NTLE defaunating agent on the protozoal population is not known, but the secondary metabolites present in NTLE (Biu et al., 2009) may have effectively affected the attachment of protozoa (Sarwar et. al., 1999), reducing its function to utilize fiber particles (Varga and Kolver, 1997; Santra and Karim, 2002), which eventually facilitated the killing action of the extract.

Table 2: The Ruminal Content Protozoal Count (cell Count /ml) in Sheep Fed Napier Grass Defaunated using NTLE and SLS

Treatment	**Initial**	**1st dose day 1-3**	**2nd dose day 6**	**3rd dose day 14**
Neem Tree Leaf Extract	2960,00	23,000	11,000	1,000
Sodium Lauryl Sulphate	330,500	21,000	11,250	625
p-value	0.171	0.495	1.000	0.422

Cc/ml – Cell count x 2500 x 10 (dilution)

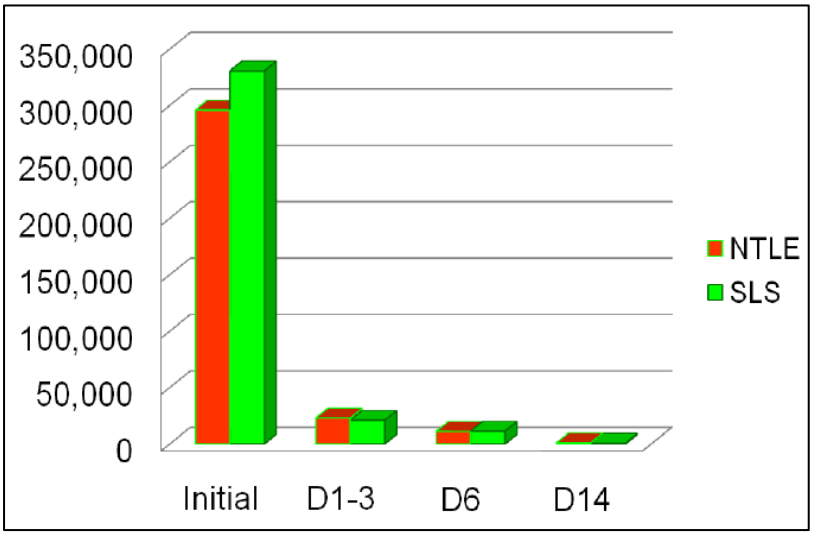

Fig 2: The Ruminal Protozoal Count Sheep Fed Napier Grass as Offered by the Type of Defaunating Agent.

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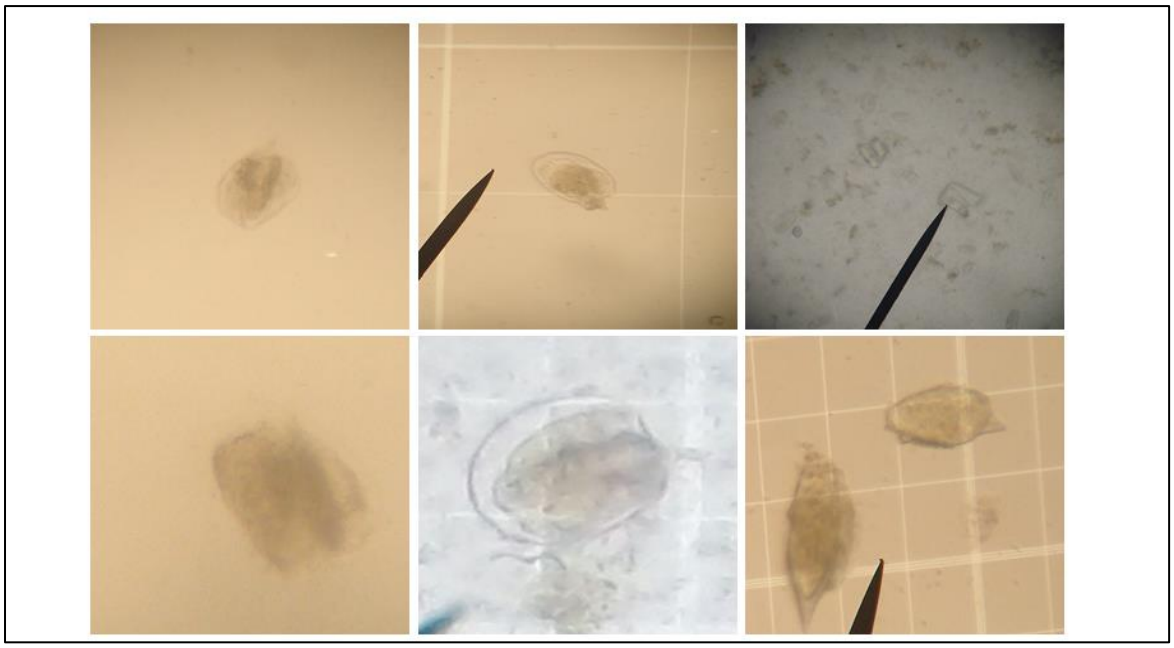

Fig 3: Photomicrograph of Species of Ciliated *Entodiniomorphid* protozoa Observed in the Rumen Fluid of Sheep in this Experiment.

C. Rumen Bacterial Count

The key benefit of defaunation is the consequent increase in bacterial population in the rumen which are considered as key microbes of the ruminant animal for the digestion of feedstuffs in the ruminant. They are much affected when protein deficient diet is fed to the ruminant animal and the protozoa in the rumen starts to feed on bacteria, reducing its population and, thus, its digestive action.

As shown in Table 3 and Figure 3, the bacterial population in the rumen fluid increased, with more rapid increase after the 2nd and 3rd dose of NTLE and SLS. Comparing this with protozoal count in Table 2, this is also the stage when protozoal count was at its lowest, indicating the inverse relationship of these organisms in the rumen. There was no significant difference between NTLE and SLS in effecting increases in bacterial population, indicating the effectivity of NTLE as a defaunating agent and its comparability to SLS. The moment protozoa lost their attachment to the fiber particles, bacteria must have penetrated aggressively into starch particles and utilized dietary nitrogen (Miresan et al., 2006), leading to the multiplication of the number of bacteria, especially amylolytic bacteria (Kurihara et al., 1978).

Table 3: The Rumen Bacterial Colony Forming Unit (cfu / ml) in Sheep Defaunated NTLE and SLS and Fed Napier Grass.

Treatment	**Initial**	**1st dose day 1-3**	**2nd dose day 6**	**3rd dose day 14**
Neem Tree Leaf Extract	10,780	12,480	702,000	950,000
Sodium Lauryl Sulphate	10,840	13,140	680,00	947,500
p-value	0.853	0.609	0.010	0.132

The figures appeared to be underestimation because these are expressed as colony forming units / ml rumen fluid (Figure 4) and not individual bacterial count. Beside, a large number of bacteria are non-culturable (Kamra, 2005) and may also depend on the type of diet. For example, the increased supply of rumen degradable protein, facilitate the growth of bacteria in the rumen allowing increases in its number, and lead to increased microbial protein synthesis.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24AUG1405

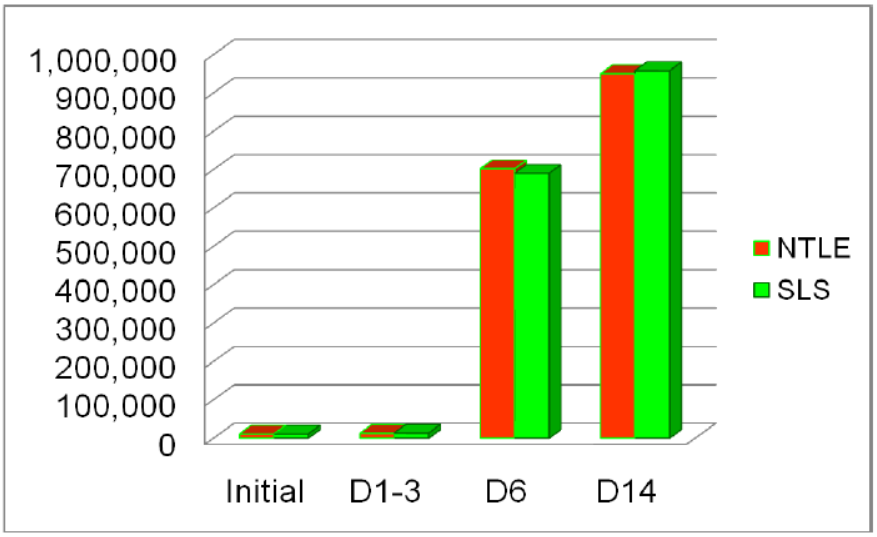

Fig 3: The Rumen Bacterial Count (cfu / ml) in Sheep Fed Napier Grass and Defaunted using NTLS or SLS.

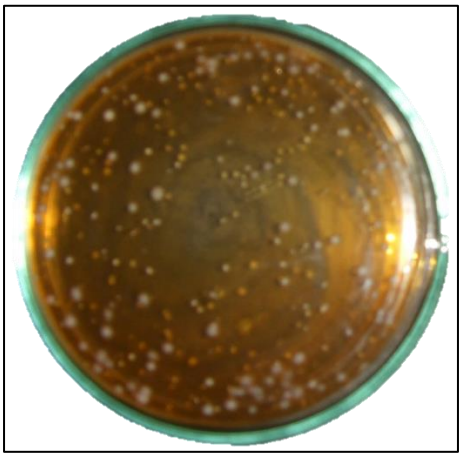

Fig 4: Bacterial Colony Forming Units (cfu) in Cultured Rumen Fluid of Sheep Fed Napier Grass and Defaunated using NTLE and SLS.

IV. ACKNOWLEDGMENT

I would like to express my sincere gratitude to the Department of Science and Technology - Science Education Institute (DOST-SEI) Advanced Science and Technology Human Resource Development Program (ASTHRDP) Philippine Council for Agriculture, Aquatic Resources Research and Development (PCARRD), and Carcar City College for providing me with the invaluable opportunity to be a graduate scholar. Your support has been instrumental in my academic growth and professional development.

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