**_Original Research Article_**

# ANTHELMINTIC POTENCY OF NEEM (*Azadirachta indica*) LEAF MEAL ON WEST AFRICAN DWARF (WAD) SHEEP


## ABSTRACT

A 90-day study was conducted to determine the response of semi intensively managed West African dwarf sheep to concentrate supplement containing varying levels of neem leaf meal (NLM). Twenty (20) West African Dwarf sheep aged 5 to 6 months with an average weight of 10kg were used in a Completely Randomized Design with animals grouped into four treatments of five replicates each balanced for weight. The animals were allowed to graze on natural pastures predominantly made up of *Panicum maximum* in the morning with a daily supplementation of 100g concentrate diet containing varying levels of neem leaf meal at 0, 5, 10 and 15%. Blood samples were taken from the animals before the commencement of the experiment and at the end of the experiment. At the start of the experiment, faecal samples were collected from each animal to determine the faecal egg count and this was repeated once in three weeks for the 90 day experimental period. There was significant ($P < 0.05$) difference in the haematology indices studied with no definate pattern. The inclusion of NLM in the diets of West African Dwarf sheep significantly ($P < 0.05$) reduced the faecal egg counts across the treatments with a percentage reduction range of 33.38 to 88.00% for sheep on 0% and 5% NLM, respectively. This study, however, concluded that neem leaf inclusion at 5% in West African dwarf sheep's diet had effects on the overall performance of the animals with a potential improvement in drastic reduction in faecal egg counts.



**key word:** Haematology, faecal egg counts, *A. indica*, West African Dwarf Sheep.

# INTRODUCTION

Throughout the world, internal parasites pose one of the major health limitations for grazing animals. Although there are numerous internal parasites, only a few of them account for the majority of problems for grazing livestock.

Helminth infections in small ruminants are serious problems of the developing world, particularly where nutrition and sanitation are poor (Faye *et al.,* 2003). Helminthosis is a primary factor in the reduction of productivity of these animals through mortality and reduced weight gains (Gatongi *et al*., 1997). While some studies have reported that goats are more susceptible than sheep to a similar challenge, others have reported that sheep usually suffer heavier worm burdens because of the difference in their grazing habits (Tinar *et al.,* 2005).

Economic losses are caused by gastrointestinal parasites in a variety of ways: they cause losses through lowered fertility, reduced work capacity, involuntary culling, a reduction in feed intake and lower weight gains, lower milk production, treatment costs, and mortality in heavily parasitized animals.

Prevention rather than cure is the philosophy used in developing control programs against gastrointestinal nematodes. It should be assumed that worms cannot be eradicated from the environment and livestock will continually be reinfected. However, infections can be limited to the extent that they will not cause economic loss to the producer. A combination of treatment and management is usually necessary to achieve control (David, 2010).

Sheep and goats farmers rely heavily on anti-parasitic drugs, or anthelmintics to control internal parasites in their small ruminant flocks. A wide variety of anthelmintics, covering the entire range of chemical groups, are used for the treatment of nematode parasites of sheep and goats. However, due to the serious problem of anthelmintic resistance (Chandrawathani *et al*., 2004), there is growing demand for alternative methods of parasite control to reduce the dependence on these drugs.

In 1999, a survey of 39 sheep farms and 9 goat farms found that the majority had worm populations resistant to all classes of drugs (Chandrawathani *et al*., 1999). From this investigation, it was clear that anthelmintic resistance was rapidly increasing.

Neem leaf (*Azadirachta indica)* is efficient as an antibiotic, anthelmintic and growth promoter when added to the feed of ruminants. Preliminary studies done by Chandrawathani *et al.,* (2000) showed that feeding Neem foliage is safe, eco-friendly, cheap and palatable to sheep. *Ad libitum* feeding of fresh Neem leaves produced 82% reduction in worm eggs of sheep and a further trial on a limited number of sheep showed that Neem produced a significant reduction in worm burdens (Chandrawathani *et al.,* 2002).

This study however investigate the effect of varying inclusion of Neem leaf meal in promoting growth and reducing helminth infections in West African Dwarf sheep grazing natural pasture.

## MATERIALS AND METHODS

### Experimental Site

The experiment was carried out between February and April in the Sheep unit of Federal College of Forestry, Forestry Research Institute of Nigeria (FRIN), Jericho hill, Ibadan, Oyo State. It is located on the latitude $07^{0}23'32''$N and longitude $03^{0}51'44''$E with altitude 212m above sea level. The rainfall pattern is bimodal with peaks around June to July, and September to October. The mean annual rainfall is about 420 mm in 109 days with mean maximum and minimum temperature of about 34 $^{0}$C and 24 $^{0}$C respectively. Mean relative humidity ranges from about 82% between June and September to approximately 60% between December and February (FRIN, 2014).

### Experimental Animals

Twenty (20) growing West African Dwarf (WAD) sheep aged 5-6 months with average weight of 10kg were purchased from markets within Ibadan. The animals were quarantined for a period of 30 days. The experimental pens were disinfected with diazintol solution before the arrival of the animals. For the period of the experiment, the sheep were managed using semi intensive management system. They were allowed to graze on natural pastures which predominantly *Panicum maximum* in the morning from 8am and returned to their individual feeding pens after grazing for about five hours.

### Procurement and Processing of Experimental Materials

Fresh Neem leaves samples were obtained from Neem trees in and around the Forestry Reasearch Institute of Nigeria, Ibadan. The leaves were chopped for effective drying. The chopped leaves were sun dried for 3-4 days until they are crispy. The dry leaves were milled using a hammer mill to produce leaf meal before they were incorporated into the concentrate supplement at 0g, 5g, 10g, 15g Neem leaf/ 100g concentrate/animal/day respectively and fed to the animals before going out to graze for a period of 90 days.

### Animal Grouping and Treatment

The animals were grouped into four treatments of five replicates each balanced for weight namely;

Treatment 1: 0g of Neem leaf /100g concentrate/animal/day

Treatment 2:  5g of Neem leaf/100g concentrate/animal/day

Treatment 3: 10g of Neem leaf/100g concentrate/animal/day

Treatment 4: 15g of Neem leaf/100g concentrate/animal/day

The animals were supplemented daily with 100g concentrate experimental diet composed of maize, wheat offals, palm kernel cake, soyabean meal, bone meal with salt and premix, Neem leaf was added at varying levels (Table 1). Fresh, clean water was given to the animals *ad libitum*.

**Table 1: Composition of the Experimental Diets fed to sheep**

| Ingredients (%) | Diets | | | |
|---|---|---|---|---|
| | 0%NLM | 5%NLM | 10%NLM | 15%NLM |
| Neem leaf meal (NLM) | 0 | 5 | 10 | 15 |
| Maize | 24 | 24 | 24 | 24 |
| Palm kernel cake | 20 | 15 | 10 | 5 |
| Soyabean Meal | 14 | 14 | 14 | 14 |
| Wheat offals | 38 | 38 | 38 | 38 |
| Bone meal | 2.5 | 2.5 | 2.5 | 2.5 |
| Salt | 1 | 1 | 1 | 1 |
| Premix | 0.5 | 0.5 | 0.5 | 0.5 |
| Total | 100 | 100 | 100 | 100 |

## DATA COLLECTION

### Faecal collection

Before the commencement of the experiment, faecal samples were collected from each animal to determine the faecal egg count and this was repeated once in three weeks for the 90 days experimental period. Hand gloves were used on the hands and the hand was dipped inside the rectum of the animals to collect fresh faeces. Three grammes of each collected faecal samples were ground and mixed with 42ml of water. A saturated solution was poured into the mixture of faeces and water to float the eggs following the modified McMaster method described by Miller *et al*., (1998). A sample obtained from this was collected and put into both compartments of McMaster counting chamber/slide and then viewed under the microscope. The number of eggs within each viewed area was multiplied by 100 to get the actual number of eggs per gram.

### Blood samples collection

Blood samples were taken from the animals before the commencement of the experiment and at the end of the experiment. Blood samples were collected via the jugular vein puncture using a 10ml hypodermic syringe. Five milliliters of the blood was infused into collection bottles containing Ethylene Di-amine Tetra-acetic acid (EDTA) for serum and the remaining 5ml into collection bottles without anti-coagulants for plasma and taken to the laboratory for analysis. Blood parameters namely packed cell volume and haemoglobin concentration (HB) were determined following the procedure outlined by Schalm *et al*., (1995). Red blood cell and total white blood cell were determined using haemacytometer (Dacie and Lewis, 1984). Serum biochemical parameters like serum urea nitrogen and serum total protein were determined by haemacytometer (Dacie and Lewis, 1984).

## Statistical Analysis

All data collected were subjected to one way analysis of variance in a completely randomized design according to SAS (1999) and means were separated using the Duncan Multiple Range Test (Duncan, 1955).

## RESULT AND DISCUSSION

### Pre-haematology and serum indices of WAD sheep fed varying inclusion level of Neem leaf meal

Table 2 shows the pre-haemtology values of the animals; urea nitrogen, packed cell volume, haemoglobin concentration, red blood cell, white blood cell and serum total protein.

The values for the urea nitrogen range between 32.15mg/dl to 47.50mg/dl while packed cell volume range between 27.25% to 32.75%. The haemoglobin concentration ranges between 8.75 g/dl to 10.92 g/dl 4.15×$10^6$/µl to5.03×$10^6$/µl, 3.53×$10^3$/µl to 5.42×$10^3$/µl and 5.19 g/dl to 5.51 g/dl are the value range for red blood cell, white blood cell and serum total protein respectively.

The packed cell volume values obtained at the pre-haematology were within the physiological range of 27.0 – 45.0 % given by Jain (1993), slightly higher than the range of 25–30% reported by Opara *et al.,* (2010). In contrast to this, Taiwo and Ogunsanmi (2003) reported higher values of 35.5% and 36.9% for clinically healthy West African dwarf sheep. The haemoglobin concentration ranges between 8.75 to 10.92g/dl which falls within the range of 9–15 g/dl reported by (Kaneko, 1997; Patra *et al*., 2003), but higher than the values of 5 to 6 g/dl obtained by Belewu and Ogunsola (2010) for goats. The red blood cell counts falls within the range of 4.3 – 5.03 ×$10^6$/µl the counts reported in this study fell below the range of 10.25–12.85 × $10^6$/µl (Ajala *et al.,* 2000), 9.2–13.5 g/dl (Tambuwal *et al.,* 2002), 9.9–18.7 /dl by (Taiwo and Ogunsanmi, 2003). The white blood cell count falls between 3.53× $10^3$/µl – 5.42 ×$10^3$/µl. The WBC counts were similar among the treatment groups and fell within the normal range (5 to 11g/dl) reported by Scott *et al.,* (2006) for sheep. The total serum protein of the animals falls between the range of 5.19-5.51mg/dl.

**TABLE 2: Pre-haematology and serum indices values of West African Dwarf sheep fed concentrate containing varying inclusion levels of Neem leaf meal**

| Parameters | 0%NLM | 5%NLM | 10%NLM | 15%NLM | ±SEM |
|---|---|---|---|---|---|
| Packed Cell Volume (%) | 29.75 | 28.25 | 27.25 | 32.75 | 1.61 |
| Haemoglobin Concentration (g/dl) | 9.77 | 9.00 | 8.75 | 10.92 | 0.34 |
| Red Blood Cell ($\times10^6$/µl) | 4.30 | 4.39 | 4.15 | 5.03 | 0.15 |
| White Blood Cell($10^3$/µl) | 4.18 | 3.53 | 4.25 | 5.42 | 0.08 |
| Urea Nitrogen (mg/dl) | 34.81 | 39.28 | 47.5 | 32.15 | 1.83 |
| Serum Total Protein(g/dl) | 5.50 | 5.19 | 5.36 | 5.51 | 0.09 |

NLM- Neem leaf meal

**Post haematology and serum indices of WAD sheep fed concentrate supplement containing varying inclusion level of Neem leaf meal**

Table 3 shows the post haematology values of WAD sheep fed varying inclusion levels of NLM; urea nitrogen, packed cell volume, haemoglobin concentration, red blood cell, white blood cell and serum total protein. Animals on the control (0% NLM) (25.62mg/dl) had the lowest urea nitrogen at the post haematology while 15% NLM had the highest urea nitrogen (33.39mg/dl).

For the packed cell volume (PCV), the values are 26.25%, 31.25%, 24.25% and 27.25% for 0% NLM to 15% NLM respectvely. 5% NLM (31.25%) had the significantly highest packed cell volume at the post haematology. PCV was significantly higher at 5% inclusion level of NLM than other treatment groups.

For haemoglobin concentration, 10% NLM (10.42g/dl) had a significantly higher ($P<0.05$) value when compared to other treatments. The values range between 7.59g/dl to 10.42g/dl from 0% NLM to 15% NLM. For the red blood cell count, 10% NLM ($8.80\times10^6$µ/l) had the highest red blood cell count while 0% NLM ($7.59 \times 10^6$µ/l) had the lowest red blood cell count, the values are $7.59\times 10^6$µl, $8.71\times 10^6$µl, $8.80\times 10^6$µl and $8.43\times 10^6$µl for 0% NLM to 15% NLM respectively. 15% NLM had the highest white blood cell count ($6.82\times10^3$/µl).

The post haematology values for all the parameters monitored differ among the dietary treatment. Urea nitrogen at the post haematology decreased across the treatment compared to the pre haematology except for treatment 4 which increased by 1.24% Although, Blood urea level was slightly higher for treatments with NLM inclusion compared to the control (0% NLM), they were within the normal range. This could be due to the higher crude protein contents of NLM supplemented treatments, in which there was improvement in the crude protein content by the treatment materials confirming the observation by Coles (1986) that high dietary protein is associated with increase in urea level.

Sheep fed 5% NLM had a higher PCV at the post haematology compared to pre-haematology, it increased by 3.00% compared to other treatments that decreased at the post haematology. Although, there was reduction in the PCV of treatment1, 3 and 4 at post haematology, PCV of this work still falls within the range of 21-35% and 20.10-48.00% reported for West African Dwarf goats and Afec-Awassi sheep by Daramola *et al.,* (2005) and Jawasreh *et al.,* (2010) respectively. This indicated that the PCV has not been affected in all the treatments. It further showed that in all the treatments, animals did not suffer from anaemia or dehydration. This confirms the report of The Merck Veterinary Manual (1998) that a low PCV value was an indication of anaemia while sharp increase in PCV is most often caused by dehydration.

Sheep fed 5%NLM (10.42%) had the highest Haemoglobin concentration. Animals fed 5% NLM had a higher value at the post haematology compared to the pre haematology, it increased by 1.42 while other treatments decreased as compared to the post haematology. The values reported in this study were within the range of 7-15 and 8.15-10.75 $gdL^{-1}$ reported for West African Dwarf goats and West African Dwarf sheep by Daramola *et al.,* (2005) and Akinyemi *et al.,* (2010), respectively. Ogbuewu *et al.,* (2010) reported the highest haemoglobin concentration at 5% inclusion level of neem leaf meal in the diet of rabbits. The implication of the values obtained in this study is that the dietary proteins were of high quality (Abu *et al.,*1998).

The haemoglobin concentration (Hb) in the blood of the studied animals showed a similar pattern of variation as with PCV. Mean Hb concentration was higher in animals fed 5% NLM than in other treatments. With the relatively higher Hb concentration observed in 5% NLM, the dietary treatment seemed to be capable of supporting high oxygen carrying capacity blood in the sheep.

The post haematology values of the red blood cells increased across all the treatments compared to the pre haematology. The RBC counts reported in this study fell below the range of 10.25–12.85 × $10^6$/µl obtained by Ajala *et al.,* (2000), 9.2 – 13.5 ×$10^6$µ/l reported by Tambuwal *et al*. (2002) and 9.9 – 18.7 ×$10^6$/µl by Taiwo and Ogunsanmi (2003).

The non significant value of red blood cells (RBC), packed cell volume (PCV) and hemoglobin (Hb) of the sheep on NLM diets relative to the control group is an indication that the animals were not anemic. The PCV and Hb values of sheep in the test diets were not different from the control group. This tends to confirm the report of Talebi *et al*. (2005) that nutrition affect the blood profiles of animal and this implies that up to 15% inclusion of NLM had a positive effect on the relative quantity of blood cell as well as total volume of blood.

Meanwhile, the white blood cell values at the post haematology are above the range of 2.23-3.48×$10^3$/µl reported by Ukanwoko *et al.,* (2013). White blood cell in animal possesses phagocytic function (Campbell and Coles, 1986) and differential white blood cell counts were used as an indicator of stress response and sensitive biomarkers crucial to immune function (Graczyk *et al.,* 2003). The white blood cell values at 5% NLM was the least in this study disagrees with the findings of Ososanya *et al.,* (2014) that recorded a highest white blood cell value for WAD ewes fed water-washed neem fruit supplemented diet at 5%.

Sheep fed 5% NLM had an increase in the serum total protein in post haematology compared to the pre-haematology while the 0% NLM, 10% NLM and 15% NLM had a decrease in the post haematology compared to the pre haematology. Animals fed 5% NLM (5.32g/dl) had the highest serum total protein while 0% NLM (4.61g/dl) had the lowest serum total protein. The values were within the range of 5.0-12.3(g/dl) but lower than 6.3-8.5 (g/dl) reported for Afec-Awassi sheep and West African Dwarf goats by Jawasreh *et al.,* (2010) and Daramola *et al.,* (2005), respectively. The implication of this result is that the highest increase in total protein in the serum of the experimental animals in 5% NLM would suggest that protein synthesis was efficient. The serum protein concentration indicates the balance between anabolism and catabolism of protein in the body. The serum protein concentration at any given time in turn is a function of hormonal balance, nutritional status, water balance and other factors affecting health (Abdel Hameed *et al.,* 2011).

**TABLE 3: Post-Haematology and serum indices values of WAD sheep fed concentrate supplement containing varying inclusion levels of Neem leaf meal (NLM)**

| Parameters | 0%NLM | 5%NLM | 10%NLM | 15%NLM | SEM |
|---|---|---|---|---|---|
| Packed Cell Volume (%) | 26.25[b] | 31.25[a] | 24.25[b] | 27.25[ab] | ±0.97 |
| Haemoglobin Concentration (g/dl) | 8.78[b] | 10.42[a] | 8.10[b] | 9.10[ab] | ±0.32 |
| Red Blood Cell ($\times 10^6$/µl) | 7.59[c] | 8.71[ab] | 8.80[a] | 8.43[b] | ±0.24 |
| White Blood Cell ($\times 10_3$/µl) | 5.28[b] | 4.53[c] | 6.00[ab] | 6.82[a] | ±2.37 |
| Urea Nitrogen (mg/dl) | 25.62[b] | 32.31[a] | 31.94[b] | 33.39[ab] | ±2.42 |
| Serum Total Protein (g/dl) | 4.61[b] | 5.32[a] | 4.86[b] | 5.26[a] | ±0.19 |

[a,b,c] Mean values followed by different letters in the same row are significantly different (P≤ 0.05)

## FAECAL EGG COUNT

Table 4 shows the faecal egg count (egg/gram) of the animals among the dietary treatments. The graphical pressentations are obtained in Figure 1.

At the onset of the experiment, the faecal egg count of the animals were 0% NLM (800.00), 5% NLM (833.33), 10% NLM (533.33) and 15% NLM (533.33) which reduced (P<0.05) at the end of week 12.

By the end of week 12, animals in 5% NLM (100), 10% NLM (133.33) and 15% NLM (113.33) showed a reduction in FEC; NLM administered in this study caused a significant reduction in the worm burden of the sheep while the animals in 0% NLM (533.33) which is the control diet were not effectively dewormed. The study showed that all the animals were naturally and heavily infested with worms at the beginning of the experiment. Administration of the neem leaf meal shows a significant reduction (P<0.05) in the faecal egg counts of the animals.

At the end of this study, there was a significant reduction in FEC of animals supplemented with NLM based concentrate. The reduction in Faecal egg count of animals in this study corroborates earlier findings of Chandrawathani *et al.,* (2000) which reported 82% reduction in worm eggs in animals fed fresh neem leaves *ad libitum* and a further trial on a limited number of animals showed that neem produced a limited worm burdens (Chandrawathani *et al.,* 2002).

In another study, Chandrawathani *et al*., (2006) evaluated the anthelmintic effect of Neem on nematode parasites of sheep, the result of study shows that for FEC there was significant difference between the control group and the treated group, worm burden estimations showed that the number of parasites was significantly higher in the control group compared to the treated group. This result indicated that feeding neem has an effect on the worm numbers of sheep. The result in this study contradicts the study conducted by Khadijah *et al.,* (2005) on the use of fresh Neem which showed no significant difference in faecal egg count compared with control sheep, although the control sheep had higher mean faecal egg counts.

This result may be affected by feeding systems such as free pasture grazing on contaminated pastures as the animals are constantly challenged with infective larvae from pasture, so faecal egg counts may increase.

Results of highly significant reduction in the EPG count in lambs fed Neem leaves were also reported by Arunachal *et al.,* (2002). However, Costa *et al.,* (2006) reported no anthelmintic activity while feeding the Neem leaves for three months to sheep and as Niezen *et al.,* (1998) observed reduction in EPG count of *Trichostrongulus species* by Sulla feeding in ewe lambs, Hordegen *et al.,* (2003) also observed a reduction in egg counts of *Haemonchus concortus* with the seeds of Neem. Kahiya *et al.,* (2003) also observed similar decrease in EPG counts feeding *Acacia karoo* diets. However, Pietrosemoli *et al.,* (1999) did not find any differences in EPG count by feeding Neem leaves up to 40% level as blocks in calves.Similar to the effectiveness of neem leaves in lowering the worm count (Niezen *et al.,* 1998). Hordegen *et al.,* (2003) also reported reductions in worm burden while feeding sulla and seeds of neem respectively. The variability in faecal egg counts within the NLM fed group may be due to differences in terms of physiological conditions of each animal and its ability to utilize the medicinal properties in neem.

**TABLE 4: Faecal egg count (egg/gram) of West African Dwarf sheep fed concentrate supplement containing varying inclusion levels of neem leaf meal**

| Weeks | 0% NLM | 5% NLM | 10% NLM | 15% NLM | ±SEM |
|---|---|---|---|---|---|
| **0** | 800 | 833 | 533 | 533 | |
| **3** | 633[a] | 533[b] | 466[c] | 600[a] | 83.33 |
| **6** | 600[a] | 333[c] | 366[c] | 400[b] | 5.53 |
| **9** | 566[a] | 122[d] | 233[c] | 333[b] | 3.33 |
| **12** | 533[a] | 100[b] | 133[b] | 113[b] | 9.17 |
| **% FEC Reduction** | 33.38[b] | 88.00[a] | 75.05[a] | 78.80[a] | 3.21 |

[a, b,c] mean values followed by different letters in the same row are significantly different (P≤ 0.05)
NLM- Neem leaf meaf; FEC- Faecal egg count

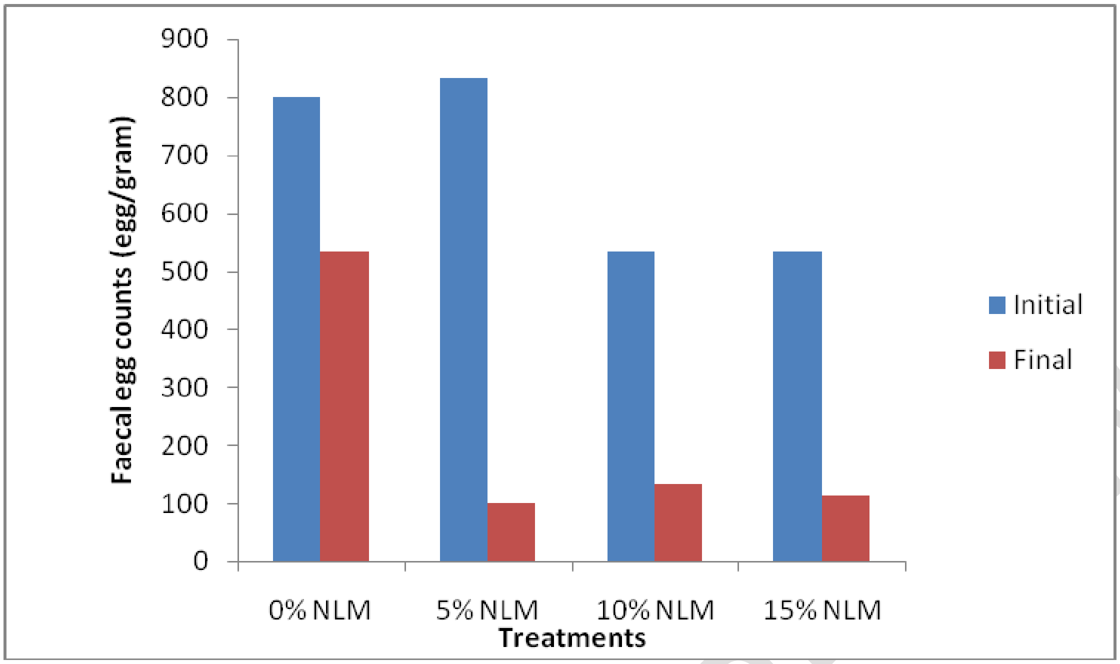


**Figure 1: The average initial and final faecal egg count of sheep fed neem leaf meal (NLM)**

## CONCLUSION

Animals supplemented with neem leaf meal (NLM) based concentrate diets had significant reduction in their faecal egg count compared to the control treatment (without NLM). However, animals on 5% NLM had the highest % faecal egg count reduction value. Sheep on 5% NLM had the best haematological values for packed cell volume, haemoglobin concentration and red blood cell, at the post haematology than other diets. Instead of farmers using anti-parasitic drugs or anthelmintics to control internal parasites in their small ruminant flocks which have residual effect on the populace consuming the meat of these animals, possible anthelmintic potential of medicinal plants such as Neem tree (*Azadirachta indica*) could be exploited.

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