Effect of Synthetic and Organic Insecticide on Chemical Composition and In-vitro Gas Production of Cowpea Haulms

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ABSTRACT

Farmers often use insecticides to manage pests on cowpea farms, raising concerns about their long-term residual effect on the chemical composition and digestibility of crop residues used as ruminant feed and on human health. This study examined the effect of synthetic and organic insecticides on the chemical composition and *in vitro* gas production of cowpea haulms. Field trials across two cropping seasons and laboratory analyses were used to assess the effect of insecticide application on cowpea haulms. Insecticide application significantly influenced the chemical composition of cowpea haulms ($p > 0.05$). Both synthetic and organic insecticide-treated haulms had significantly higher crude protein contents than the control group ($p > 0.05$). All treatment groups exhibited high levels of crude protein (CP) (157.2–213.4 g/kgDM), crude fat (78.8–103.7 g/kgDM), and ash (118.5–128.1 g/kgDM). The neem extract treatment had the highest CP content (213.4 g/kgDM), while the control group had the lowest (14.46%). Moderate *in vitro* digestible organic matter (IVDOM) (43.39 - 45.71%) and metabolizable energy (ME) values (6.09 - 6.68 MJ g/kg) were also observed. The microbial population was significantly decreased in the neem oil extract and Dursban treatment groups compared to that in the control group ($p<0.05$). Cowpea haulms can serve as supplementary feed to improve the utilisation of poor-quality feed resources during the dry season, thereby enhancing ruminant growth performance. However, the persistence of pesticides in plant matter post-harvest hinders microbial digestion and fermentation in the rumen when animals consume plant residue.

Keywords: Cowpea haulm, chemical composition, microbial population, insecticide, *in vitro* gas production

1.0. INTRODUCTION

Cowpea, a legume crop, is primarily grown for its nutritious leaves and grains, which are consumed by humans. The haulm can be used as an organic fertilizer to enhance soil productivity or as a fodder for livestock to enhance low-quality forage (Singh et al., 2003). Research has shown that including cowpea haulms in the diet of sheep, along with cereal straw, enhances growth performance (Savadogo et al., 2000). Cowpea cultivation is often considered a risky investment because of the damage caused by insect pests at different stages of the crop life cycle (Jackai & Adalla, 1997). Black cowpea aphid, Aphis craccivora Koch (Homoptera aphididae); cowpea flower thrips, Megalurothrips sjostedti Tryb; Maruca vitrata; Riptortus dentipes Fab; Clavigralla tomentosillis Stal.; and nezara virdula L are among the most serious field insect pest species that infest cowpea in the Guinea savanna agroecological zone (Singh et al. 1990). In the United States, an estimated 720 million animals die annually because of pesticide use. Eagles are the animals most unintentionally impacted by hazardous applications. Rachel Carson first highlighted the dangers of DDT use (Mahmood et al., 2016). The nesting populations of 11 bird species on British farms decreased by one crore owing to a drop in plant and invertebrate diversity. In 1999, Europe listed one hundred and sixteen (116) bird species. Researchers have linked the strategic use of insecticides at certain times and places to a decrease in bird populations. Between 1990 and 2014, the number of common agricultural birds declined in Sweden, France, and Europe (Garcês et al., 2020). Several pesticides, such as lambda-cyhalothrin, dimethoate, and cypermethrin are recommended as complete measures to protect cowpea crops (Afun et al., 1991). This technique is frequently used in Ghana and other sub-Saharan African countries. According to Singh et al. (1990), maruca can cause yield losses of 20–80%. The common control method for these cowpea insect pests is the application of synthetic insecticides when the insect population reaches a certain threshold, beyond which there is economic loss (Abudlai *et al.*, 2001). These pesticides are hazardous to life and their residues can be introduced at all levels of the food chain (Adedire & Ajayi, 1986; Adedire & Ajayi, 1996). The misuse of pesticides may leave residues in the haulms of cowpeas at levels that are toxic to livestock and could indirectly affect human health when such animals or their products are consumed (Karbo et al., 2001; Karbo *et al.*, 2005). In a study visit to the Next Generation farm in the Upper East Region of Ghana, the farm's veterinary personnel reported the death of sheep after feeding them haulms from a pesticide-sprayed field. Most farmers in Ghana are resource-poor and require pest management strategies that are cost-effective, sustainable, and maintain grain yield and haulm quality with less toxicity from chemical residues used as feed for ruminants (Konlan *et al.*, 2016).

There must be a significant trade-off between public health and the environment if pesticides boost the production of food and fibre while reducing vector-borne illnesses. Many of these compounds are detrimental to humans, animals, and the environment. Every nation's health risks disproportionately affect the poor and those exposed to pesticides, and no community is pesticide-free (Hashimi *et al*. 2020). Each year, pesticide poisoning claims the lives of almost one million individuals, particularly formulators, sprayers, mixers, and loaders. Pesticides, raw ingredients, hazardous solvents, and inert transporters can harm industrial workers more easily (Zacharia, 2011). Pesticides can enter the body via dust, aerosols, vapours, contaminated food, and drinks. Spraying pesticides may contaminate water, damage the ecosystem, and spread. The toxicity, duration, and intensity of pesticide exposure affects human health. Pesticide exposure is higher in farm labourers and their families. Pesticides are present in the adipose tissues of all people (Yazicioglu *et a*l., 2013). Kids are more vulnerable to the negative effects of pesticides than adults are because of their immune system development. Because of their intimate contact with the ground and propensity to consume strange items, children may be particularly susceptible. Lead exposure and mouth-to-mouth contact in children change with age (Yazicioglu *et al*., 2013).

However, there is a lack of extensive studies on the impact of pesticide-contaminated cowpeas or forage on the microbial population, nutritional quality, and in vitro kinetics of haulms. Rumen microbial fermentation supplies approximately 50%-70% of the energy requirements of ruminant animals, making it crucial to emphasise the importance of rumen microbes in ruminant nutrition (Gutenmann et al., 1968). The objective of this study was to investigate the influence of three distinct pesticides at varying dosages on the levels of gas generation in vitro and on some anticipated rumen fermentation characteristics. Ruminant animals consume approximately 50%–75% of their caloric intake via rumen microbial fermentation. Rumen microorganisms play a vital role in the nutrition of ruminant animals, and any element that disturbs the microbial ecology of the rumen might affect digestion and decrease an animal's performance. In this study, three distinct pesticides were used at various doses to observe their impact on gas production in the laboratory and other anticipated fermentation measurements in the rumen.

2.0. MATERIALS AND METHODS

2.1. Study Areas and Experimental Design

The cowpea was cultivated in Dukma Garden, Bolgatanga Municipality of the Upper East Region, Ghana, during the 2021 and 2022 cropping seasons. A Randomised Complete Block Design (RCBD) with three treatments (Control, Dursban, and Neem oil) was replicated four times (4 each). The plots measured 2 m^2 with an interval of 1m between plots to avoid the overlap of spray drifts with adjacent plots. Bolgatanga Technical University's Department of Ecological Agriculture Research Laboratory conducted the laboratory analyses.

2.2. Application of Pesticides

Dursban and Neem Seed Oil (azadirachtin) were the two main chemicals used in cowpea plants. Spraying was done every two weeks with a dosage of 1.5 mL to 1.8 mL of water. This dosage was calculated from the recommendation dosage of 250 ml to one hectare

The same dosage of neem oil extract was used to determine its infectivity against pests infesting cowpeas.

2.3. Chemical and In-vitro Analyses

The cowpea haulms were harvested and oven-dried for 48 h at 60 °C. The dried haulms were then milled through 1 mm screens using a centrifugal sieve (Retseh® ZM 200) for chemical, microbial, and *in vitro* gas production analyses. The basic procedure of AOAC (2008) was used to determine dry matter (DM), ash, and crude protein (CP) contents. The micro-Kjeldahl technique was used to obtain the nitrogen, which was multiplied by 6.25 to get the crude protein. The technique and procedure of Theodorou *et al*. (1994) were adopted for *in vitro* gas production at 24 h and 72 h. A randomised complete block design (RCBD) was used for the gas production and repeated four times using run as block. The samples (200 mg) were incubated in 50 ml test tubes containing buffered rumen fluid under anaerobic conditions. Fresh rumen fluid was obtained from slaughtered cattle in Bolgatanga Abattoir and filtered under continuous flushing with carbon dioxide (Ansah *et al*., 2018). The filtered rumen fluid was then mixed with McDougall's solution. The pressure of the fermentation gas in the incubation tubes was measured using a digital manometer for 72 h. The *in-vitro* digestible organic matter (IVDOM) was calculated by using the equation of Menke and Steingass (1988). IVDOM (%) = IVDOM (%) = 14.88 + 0.8893 GP + 0.0448 CP + 0.651% Ash (Menke, 1988).

The metabolisable energy (ME) was estimated using the equation described by Menke *et al*. (1979); ME (MJ/kg DM) = 2.20 + 0.136*GP + 0.057*CP + 0.0029CF.

where GP = gas produced at 24 h, CP = crude protein, and CF = crude fat. The 72-hour *in vitro* gas produced data were fitted to the exponential degradation curve of Orskov and McDonald (1979) using GraphPad Prism 7.2. The degradation parameters (b and c) were extracted from the model: Y = b (1-exp^{-ct}), where Y is the gas volume at time t (ml/h), b is the asymptote gas production (ml/200 mg), and c is the fractional rate of gas production (ml/h).

2.4. Microbial Count

The modified methods described by Maturin and Peeler (2001) and Adzitey *et al.* (2019) were used to analyse the dried haulm for the total microbial count, lactic acid bacteria, and mould. To obtain a neat solution, 10 g of each sample was added to 90 ml of 1% buffered peptone water. A series of dilutions (10-1–10) in 9 ml of clean distilled water. Then, 100 µL of each series of dilutions was spread out and placed on duplicate plate count agar, de Man, Rogosa, and Sharpe plates, and potato dextrose agar for the lactic acid bacteria, mould, and microbial counts, respectively.

2.5. Statistical Analysis

The data were subjected to analysis of variance using GenStat 18.2. A fixed-effects one-way ANOVA model was used: Yij= µ+ Vi+ eij. Where Yij is the observation, μ is the experimental mean, Vi is the treatment effect, and eij is random error. Significant differences among the treatment means were separated using Tukey's HSD test at the 5% significance level.

3.0. RESULT

The chemical composition of the cowpea haulm among the treatment groups is presented in Table 1. There was no significant difference ($p > 0.05$) in dry matter (DM) content among the treatment groups. The crude protein concentration was significantly influenced by pesticide application. The neem oil extract had a higher mean crude protein content than the other treatments. The Ether extract was significantly influenced by insecticide application ($P<0.001$). The ether extract content was in the range of 78.8 – 103.7 g/kgDM with the neem extract recording the highest values. Furthermore, an organic solvent may also dissolve the residual wax of pests in the control and Dursban-treated haulms, influencing the ether extract content.

In vitro gas production, organic matter digestibility (IVDOM), and fermentation characteristics of the treatments are presented in Table 2. The mean IVDOM values of the haulms studied were higher in the neem oil extract-treated group (45.71%); however, there were no significant differences ($P < 0.178$) among the treatment groups. The in vitro digestible organic matter ranged from 43.39 to 45.71%. Metabolisable energy did not differ significantly ($P>0.05$) among the treatments. The values were similar ranging from 6.09 to 6.68 MJ g/kg (DM) in the treatment groups. The asymptote gas production (b) and rate of gas production did not differ ($P> 0.05$) among the treatment groups.

Table 1: Chemical composition of the experimental cowpea haulms

	Treatment				
Parameter (g/kgDM)	Control	Neem oil extract	Dursban	SED	p. value
Dry matter	952.0±0.65	950.0±0.84	950.7±0.86	0.28	0.774
Organic matter	881.5±0.37a	871.9±0.34ab	869.7±0.51b	0.40	0.012
Crude Protein	157.2±0.28c	213.4±0.04a	183.4±0.02b	0.89	<.001
Ether Extract	103.7±0.08a	78.8±0.09b	77.86±0.11b	0.58	<.001
Crude Fibre	228.0±0.21	207.1±0.08	224.1±0.21	2.48	0.655
Ash	118.5±0.37b	130.3±0.34b	128.1±0.51ab	0.40	0.012
GE (kcal/g/DM)	345.1±3.69	352.7±3.9	364.0±5.02	21.54	0.678

[a,b] Means within the same row with different superscripts are significantly different ($P< 0.05$)

Table 2: *In-vitro* digestible organic matter (IVDOM), kinetics and gas production of cowpea haulm

	Treatment				
Parameter	Control	Neem oil extract	Dursban	SED	p. value
IVDOM (%)	43.39±1.89	45.71±1.83	45.62±2.718	1.39	0.178
ME (MJ g/kgDM)	6.09±0.58	6.68±0.53	6.53±0.69	0.52	0.509
b (ml/200mgDM)	24.54±3.55	26.14±3.21	26.97±2.65	2.35	0.458
c (ml/h)	0.026±0.01	0.029±0.01	0.027±0.1	0.002	0.065

Table 3 shows the microbial count. The haulms studied had a higher mean total microbial count value (7.74 cfu/g) in the control group than in the insecticide treatment groups (Table 3). The lactic acid bacterial counts varied from 4.41 to 6.43 cfu/g. The mould count was significantly higher in the control group than in the pesticide-treated groups (P<0.001). Insecticide application to cowpeas had a significant effect on the total microbial, lactic acid bacteria (LAB), and mould counts. There was a significant reduction in the microbial population compared to the control treatment.

Table 3: Microbial count (log cfu/g) of the cowpea haulm

	Treatment				
Parameter (cfu/10^{-5})	Control	Neem oil extract	Dursban	SED	p. value
Total viable count	7.74±0.82^{a}	6.51±0.32^{b}	6.91±0.75^{ab}	0.34	0.033
Lactic acid Bacteria	6.43±1.23^{a}	5.97±0.77^{a}	4.41±0.36^{b}	0.42	<.001
Mould	4.05±1.35^{a}	2.42±0.83^{b}	0.71±0.40^{c}	0.49	<.001

^{a,b} Means within the same row with different superscripts are significantly different ($P< 0.05$)

4.0. DISCUSSION

A high dry matter content is beneficial for rumen function in ruminants as it provides a substrate for microbial fermentation processes (Oni *et al.*, 2008). This is particularly beneficial for microbial activity in sheep, resulting in increased digestion and nutrient utilisation (McDonald *et al.*, 2011). The crude protein (CP) content of all insecticide-treated groups was significantly higher than that of the control group. The CP values ranged from 157.2 to 213.4 g/kg DM. The neem oil extract group had the highest CP content, whereas the control group had the lowest. The high apparent CP content in the neem oil extract-treated haulms may be attributed to the presence of nitrogen in the chemical structure of the pesticides. The reported CP content for the treatment groups was above the minimum requirement of 60–80 g/kg DM for successful microbial growth in ruminant animals (Van Soest, 1982). The crude protein content for all treatment groups were within the NRC (2007) recommended total dry matter intake of 120 g/kg DM CP for ruminant growth and maintenance. The quality of forage is more important than its quantity in ruminant production. The CP recorded in this study was above the range of 136.3 – 178.0 g/kg/DM crude protein reported by Mosimanyana and Kiftewalid (2006) for cowpea haulm. The chemical concentration in feed is influenced by several variables, such as species, harvesting date, fertilisation, soil, and environmental factors (Mahmut *et al*., 2010; Ansah *et al*., 2010; Ramirez *et al*., 2004; Kulik, 2009). The chemical composition of the cowpea haulm reported in this study is within the range reported for cowpea haulms. The significant effect of insecticide application on chemical composition might also be due to the chemical reactions induced by insecticide application. Higher CP and ME values indicate that haulms can be used as supplementary feed to enhance the utilisation of poor-quality feed resources during the dry season to improve the growth performance of ruminants.

The application of synthetic and organic pesticides had no adverse effect on *in vitro* digestible organic matter (IVDOM), gas production, and metabolisable energy, even though there was a marginal increase in neem and Durban pesticides compared to the control group. This could be attributed to the lower toxicity and potential release of inorganic sulphur, nitrogen, and phosphorus in the compound by rumen microflora, which are used as substrates for microbial proliferation (Cook, 1957). This observation aligns with the report of Williams et al. (1963), who observed an increase in gas production. Schwartz et al. (1957), reported no significant reduction in dry matter digestibility of cowpea haulm when levels of parathion and malathion were used to control pests in cowpea production. However, this observation contrasts with the report by Antwi *et al.* (2014), who documented a decrease in gas production and digestibility when lambcyhalothrin was applied to cowpeas. The results obtained in this study could be explained by the less toxic nature of the pesticides and, hence, the ability of the rumen microbes to break down the substrates. The high crude protein content in the neem extract treatment group could act as cellulolytic microbes with the required degradable protein to digest the dry matter. The IVDOM recorded in this study was slightly higher than the 41.13 to 43.44% reported by Alagma (2016) for different varieties of cowpea haulms. Higher gas production from microbial fermentation with corresponding low digestible organic matter suggests potential inefficiency in the digestibility of the diet (Tenakwa *et al*., 2022). This can lead to emission of enteric methane and carbon dioxide into the atmosphere when fed to ruminant livestock. The ME recorded in this study for the supplemented diet was within the ME recommended by NCR (2007).

The application of pesticides resulted in a reduction in the microbial population of haulms. This indicates that pesticide application suppressed microbial growth in the present study. This is explained by the nature of the compound and the toxicity of pesticides that influence the growth of sensitive microorganisms (Nagy *et al*., 1964), which is significant in the microbial population. Microorganisms play significant roles in feed digestion, the fermentation of plant structures, and non-structural nutrient fields in ruminants (Durand and Ossa, 2014). The detection of lactic acid bacteria in the haulm improves fermentation, digestion, and gut health of ruminants (Pessione, 2012).

5.0. CONCLUSION AND RECOMMENDATION

The chemical composition values of the cowpea haulms demonstrated their potential as a supplementary feed to enhance the use of low-quality feed resources during the dry season to boost ruminant growth. The pesticides applied to cowpea crops did not inhibit gas production and fermentation of haulms. The application of pesticides inhibits microbial growth in cowpea haulms. The application of pesticides to cowpea crops significantly decreases the microbial population in the cowpea haulms. The use of pesticides in food and forage crops should be drastically reduced to reduce their incorporation into and residuality in the food chain, thereby saving both humans and animals from the toxic effects of these chemicals. Alternative, integrated, and biodegradable pest management measures and strategies should be adopted as these are environmentally safe. Plants on which pesticides have been applied should be harvested at least two weeks after application to enable the excretion of these chemicals by the plants.

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Contribution of authors

All authors have made important scientific contributions to the study and have assisted with the drafting or revision of the manuscript according to the definition of an author.

Halidu Agolisi Mamudu: Methodology, Investigation, Data analysis and writing - original draft**. Ayimbire Abonuusum:** supervision, conceptualisation, writing – review, and editing.

Badiwon Charles: Methodology and Investigation**.** **Hendrick Essel**: Data curation and Investigation. **Abeba Theresa**: Data curation and investigation.

Competing Interest

The authors declare that they have no conflicts of interest.

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