***Original Research Article***

***Leucaena leucocephala*-based pellets effect on Djalonke sheep gastro-intestinal parasites**

**Abstract**

**Aims:** Synthetic chemical molecules have long been used to control gastrointestinal parasites. However, this chemical control has led to parasite resistance developments within populations. Moreover, because of their toxicity for the environment and the residues’ risk in animal products, calls for a reduction in the use of these chemicals in livestock production are becoming more and more recurrent. So, the experiment tested *Leucaena leucocephala* leaves anti-helminthic properties. **Place and Duration of Study:** The experiment was conducted at the National Polytechnic Felix Houphouët Boigny (INP-HB) Yamoussoukro, at the Graduate School of Agriculture experimental farm. It lasted from April to September, 2022, 6 months. **Methodology:** First, some pellets were made with dried *Leucaena leucocephala* leaves. Secondly, plant phytochemicals were assessed. Third, the pellets were served to 12 lambs. Alongside, a second animals’ group received Levamisole synthetic anti-helminthics. During the experiment, the feces were collected in animals’ anus, at the start, 3 and 6 months after. The parasites were counted per gramme of feces. Forth, the lambs were weighed, while checking feces parasites. **Results:** The pellets were rich in total polyphenols, condensed tannin, and total tannin for 2.796 mg GAE/g, 95.317 mg catE/g, and 81.017 mg TAE/g, respectively. *Leucaena leucocephala*-based pellets reduced *Haemonchus contortus* charge from 533 at 3 months to 316 at 6 months. Similarly, Coccidia number was reduced from 2833 at 3 months to 1300 at 6 months (p=0.002). But these pathogens increased in lambs’ feces receiving Levamisole synthetic anti-anthelminthic, during the same period. For example, *Haemonchus contortus* eggs per gram of feces increased from 1016 after 3 months to 1450 after 6 months. To end, the lambs fed on Leucaena leucocephala-based pellets weighed 25.27 kg while the second group treated with the synthetic anti-helminthic weighed 20.07 kg. so, there was 5.2 kg weight loss (p=0.02). Also, hematocrit assessment showed significant improvements in animals receiving *Leucaena leucocephala*-based pellets for 31.16 vs. 20.16 for the control (p<0.0001). **Conclusion:** The results demonstrated *Leucaena leucocephala*-based pellets’ anthelmintic function, and it can be used in sheep farming without any adverse effect.

***Keywords***: *Haemonchus contortus*, Djallonké lambs, *Leucaena leucocephala*

1. **Introduction**

In Côte d'Ivoire, agriculture is the main sources of growth and development (MIRAH, 2014). Moreover, it employs almost the entire working population and contributes 34% to total GDP (MIRAH, 2014). Despite the efforts made by both state and private structures to develop livestock farming, this sector remains a secondary activity, contributing around 4.5% to agricultural GDP and 2% to total GDP (Koffi-Koumi et al., 2001; MIRAH, 2014; Kouakou et al., 2015). In 2019, animal protein needs were covered at 34.1% for beef, 98.8% for poultry and 70.9% for small ruminants (MIRAH, 2022). According to MIRAH (2022), the sheep number was estimated for 218,208 heads, concentrated in the central for 40% and northern for 37% regions (Apala et al., 2020). In order to cover its needs in meat, Cote d'Ivoire imports sheep from Sahelian countries (Yao and Kallo, 2015). This deficit can be linked to two major factors whose are the feeds and parasitism (Akouedegni et al., 2019). Among the two factors, gastrointestinal parasitism is the first major difficulty in the small ruminant breeding. It causes major economic losses through reduced milk, meat or wool production, or through morbidity and mortality in small ruminants (Qamar et al., 2011; Fitzpatrick, 2013; Ali et al., 2019). In fact, this parasitism often causes anaemia in sheep and goats, a drop in productivity, and it can lead to heavily infested young animals’ death (Githigia et al., 2001). Also, its gastro-intestinal parasitism can lead to 50% production potential loss in tropical zones (Mahieu et al., 2009). Since a long time, synthetic anthelmintics are the main methods of controlling these parasites (Emanfo et al., 2022).

However, resistance to synthetic anthelmintics has led to the widespread emergence of parasites chemical-resistant strains (Getachew et al., 2007). Importantly, these antibiotics are toxic to the environment and the risk of residues in animal products (Cooper et al., 2011; Tsiboukis et al., 2013). Many international structures are calling for these chemical uses’ reduction in livestock production. So, alternative methods to synthetic anthelmintics are being developed to control digestive parasitism in animals (Getachew et al., 2007). One of these alternatives is the use of bioactive molecules contained in plants (Akouedegni et al., 2019). For instance, condensed tannins contained in certain plants have been shown to be effective against ruminant gastrointestinal parasites (Häring et al., 2008; Brunet et al., 2011; Mueller-Harvey et al., 2019). However, these plant part quantities ingested by the animals would need to be significant to achieve a significant effect on parasites (Marie-Magdeleine et al., 2020). In addition, gastrointestinal strongylosis causes severe disturbances to digestive physiology, leading to an increase in the host's feed requirements. Improving the feed ration to cover the additional requirements associated with the nematodes’ presence would help to improve the host's response to parasitism. Supplementing sheep with pellets made with tanning plants should therefore help to improve the feed ration. So, the essay assumed that these plants’ consumption level by the animals could have an effect against digestive parasites. Then, some *Leucaena leucocephala* plants’ parts were incorporated at different proportion in sheep feed pellets.

**2. Materials and Methods**

This study was carried out at National Polytechnic Institute Felix Houphouët Boigny (INP-HB), on the Graduate School of Agriculture experimental farm in Yamoussoukro during the rainy season, from the end of March to September, 2022. This environment is characterized by two main dry seasons and two rainy seasons. The rainy seasons are between April-June and September-October. Average rainfall over the period 1991-2020 was estimated at 1,175 mm. Also, average temperatures for the coldest month in August and the hottest month in March over the same period are 25.7 °C, and 28.9 °C, respectively.

Pelleted feeds were produced with *Leucaena leucocephala* leaves. Leaves were harvested at the flowering stage and dried under cover at room temperature. The dried fodder was ground and mixed with other raw materials such as corn, cottonseed cake, soybean cake, oyster shells, cane molasses, and salt. The floury mixture was pelletized with 6 mm for the diameter, and 3 mm for the length, using a pelletizer. Then, the pellets were dried under cover.

**2.1. Leucaena leucocephala leaves-based pellets chemical analyses**

Two solvents were made with distilled water mixed with ethanol and methanol at 30/70 (v/v), respectively. Following Yao et al. (2023) approach, the maceration was carried out. So, 1 gram of *Leucaena leucocephala* leaves’ powder was macerated at room temperature for 45 min with 60 mL hydroalcoholic solution. Erlenmeyer flasks containing hydroalcoholic solutions were placed on a magnetic stirrer. To obtain a homogeneous solution, magnetic rods were dipped into each solution at 1,100 rotations per minute speed. Each mixture was filtered through absorbent cotton and stored in a refrigerator at 4°C until use.

**2.2. Phenolic compounds analyses**

These phenolic compounds are condensed tannin, total tannin, and total polyphenols.

**2.2.1. Condensed tannins**

Condensed tannins were determined through vanillin-in-acid method (Price et al., 1978). It is based on the vanillin ability to react with condensed tannin units in the presence of acid to produce a colored complex measured at 500 nm. In fact, vanillin's reactivity with tannins involves only the first polymer unit. In details, 3 mL of 4% methanolic vanillin were added to 50 µL of hydroalcoholic extract. The resulting mixture was then stirred and made up to volume with 1.5 mL concentrated hydrochloric acid. Finally, the solution was left to react for 15 minutes in the dark before reading. The readings were repeated 3 times. Absorbance was measured with a UV spectrophotometer set at 500 nm, against a blank solution containing 4% vanillin in methanol. Catechin was used as a standard, and results were expressed as micrograms of catechin equivalent per milliliter (µg CatE/mL). These results were converted to milligrams of catechin equivalent per gram of matter (mg CatE/g) (Equation 1).

$Ce(mg catEg^{-1})=\frac{C\_{L}}{0.546}$, Equation 1

Where, Ce is the condensed tannin content in the sample, and CL, the concentration read with the spectrophotometer.

**2.2.2. Total polyphenols**

Total polyphenols were determined using Kouamé et al. (2021) approach. Exactly, 30 µL of extract were mixed in 2.5 mL of Folin-Ciocalteu reagent diluted 1:10. The mixture was left in the dark at room temperature for 2 minutes. In addition, 2 mL of 75% sodium carbonate (NaCO3) solution was added to the mixture. The resulting solution was incubated at 50°C for 15 minutes and then cooled to room temperature. Thereafter, the absorbance was read with UV-visible spectrophotometer set at 760 nm. The readings were repeated 3 times, with gallic acid at different concentrations used as the reference standard. Finaly, total polyphenol contents were expressed in milligrams of gallic acid equivalent per liter of extract (mgEAG/L extract). These results were converted (Equation 2) to milligrams of gallic acid equivalent per gram of matter (mg GAE/g).

$Ce\left(mg GAEg^{-1}\right)=\frac{10^{-4}}{9.06}C\_{L}$ (Equation 2)

Where, Ce is the total polyphenol content in the sample, and CL, the concentration read with the spectrophotometer.

**2.2.3. Total tannins**

The samples’ total tannin content was determined according to Hossain et al. (2020) methodology. So, 7.5 mL distilled water was added to 100 µL extract to obtain a solution. After, 0.5 mL of pure Folin-Ciocalteu and 1 mL of 35% sodium carbonate were added to the previous solution. Next, 0.9 mL distilled water was made up to the volume after the reagents’ addition. The mixture was well shaken, and kept for 30 minutes at room temperature. Finally, absorbance was read at 700 nm on a UV-visible spectrophotometer. Tannic acid concentrations of 0; 1.95; 3.9, 7.81; 15.62, 31.25; 62.5; 125; 250; 500 and 1,000 µg/mL were used to plot the calibration curve and as a standard. Total tannin levels were expressed in microgram tannic acid equivalent per liter of extract (µg TAE/L). These contents were converted into milligrams of tannic acid equivalent per gram of matter (mg TAE/g ; equation 3).

$Ce\left(mg TAE g^{-1}\right)=\frac{1}{0.6}Cal$ (Equation 3)

Where, Ce is the total tannin content in the sample and Cal, the concentration read by the spectrophotometer.

**2.2.3. Lamb management**

The experiment involved 24 Djallonké lambs aged around 3 months and average live weight was 11.6 kg. The animals were identified by numbered ear tags and divided into two homogeneous groups of twelve. They were fed on natural pasture. One group received *Leucaena leucocephala*-based feed supplements. In this experiment group, each animal got 30 g pellet-feeds per kg of live weight per animal per day for 6 months. Importantly, this group did not receive any synthetic anthelmintics. The control group received dried cassava peels and cooking salt for six months. The control animals were internally dewormed once a month with a synthetic levamisole anthelmintic until 6 months. Also, the lambs were weighed individually every month for six months to monitor their growth.

**2.2.4. Parasite control**

The lambs’ parasitic charge was monitored every month, for six months by quantitative coproscopy analysis. Specially, droppings were taken directly from each animal rectum, from the start to the end, every month. The samples were placed in individual numbered plastic bags, stored in a cooler containing dry ice and sent to the laboratory for coprological analysis using the McMaster quantitative technique with a 1.2 density NaCl solution to identify the animals' internal parasites and estimate their parasite content in eggs per gram of feces (EPG). In fact, this technique is versatile, as it can detect all parasites’ forms, Cestodes, Nematodes, Trematodes eggs or larvae (Raynaud et al., 1970).

**2.2.5. Hematocrit determination**

At the experiment start and every month, during 6 months, individual blood samples were collected directly from each animal jugular veins, using heparinized EDTA tubes fitted with a needle, then placed in a cooler containing dry ice. The collected samples were transported to the laboratory for hematocrit determination.

**2.2.6. Statistical analysis**

The results’ statistical analyses were carried out using a single-factor model. The pellet-feeds’ effect on fecal parasite excretion and animal growth was processed using R STUDIO software version R.4.2.2. Hematocrit expressed as a proportion was analyzed using the Chi-square test. Parasite EPG means, animal weight and animal growth were analyzed and compared using the STUDENT t-test at 5% significance level.

**3. Results and discussion**

**3.1. *Leucaena leucocephala*-based pellet total polyphenol**

*Leucaena leucocephala* leaves’ granules total polyphenol content results are presented on Table 1. Ethanol solvent was the most effective, followed by methanol. The total polyphenol contents obtained with ethanol and methanol in the pellets were 3.311±0.31 mg GAE/g, and 2.796 ±0.22 mg GAE/g, respectively. The results showed that ethanol is the most effective solvent for extracting polyphenols from pellets, compared to methanol. These results were similar to those obtained by Mahood et al. (2023), who showed that ethanol is the most efficient solvent for extracting polyphenols from *Crocus sativus* for 7.29 mg GAE/g.

**3.2. *Leucaena leucocephala*-based pellet condensed tannin**

*Leucaena leucocephala-*based pellet condensed tannin content is on Table 1. The condensed tannin extracted from the pellets were 85.714±92.140 mg catE/g, and 95.317±92.744 mg catE/g for the ethanolic and methanolic extracts, respectively. These condensed tannin contents are high irrespective of the polar solvent used in this study. Ghedadba et al (2014) have shown that polar solvents have the capacity to diffuse within the plant powder, to reach the plant matrix and therefore recover a lot of secondary compounds as possible. These condensed tannins presence in the granules suggests their ability to play an important role in the gastrointestinal parasites control in small ruminants (Marie-Magdeleine et al., 2020).

**3.3. *Leucaena leucocephala*-based pellets’ total tannin**

Total tannin contents (Table 1) extracted from *Leucaena leucocephala* pellets were 55.756±7.492 mg TAE/g and 81.017±7.492 mg TAE/g for the ethanolic and methanolic extracts, respectively. This difference shows that hydro methanol is a better solvent than hydro ethanol for total tannin extraction. This finding confirmed Masriani et al. (2023) observations. For example, they showed that methanol is the more efficient solvent for tannin extraction than ethanol. They explained that, because methanol contains fewer carbon atoms, which means that the compounds bound by these solvents have different polarities. According to Masriani et al. (2023), the more polar the solvent is in the extraction process, the higher the tannin quantities extracted is. Nevertheless, whatever the solvent used to extract total tannins from pellets, herein obtained results were high. These values would be explained by the fact that tannin extraction depends largely on the solvent (Ali-Rachedi et al., 2018).

**Table 1: *Leucaena leucocephala*-based pellets’ total polyphenol, condensed tannin and total tannin contents**

|  |  |  |
| --- | --- | --- |
| **Secondary compounds** | **Solvents** | **Contents** |
| Total polyphenols (mg GAE/g) | Ethanol | 3,31±0.31 |
| Methanol | 2,796 ±0.22 |
| Condensed tannins (mg catE/g ) | Ethanol | 85,714±92.140 |
| Methanol | 95,317±92.744 |
| Total tannins (mg TAE/g ) | Ethanol | 55,756±7.492 |
| Methanol | 81,017±7.492 |

**3.4. *Leucaena leucocephala*-based pellets effect on gastrointestinal parasites**

The study identified various parasite species in the lambs’ feces (Table 2). The main parasite species were *Haemonchus contortus*, *Moniezia sp* and *Eimeria* genus, coccidia oocysts, in sheep feces. Coprological analyses showed that the parasitic species found in the feces of the two animals’ groups were identical. This similarity could be explained by the fact that these animals shared the same grazing areas. These results confirm Achi et al. (2003) assertions, who justify the gastrointestinal parasites similarity in small ruminants in a given locality because they shared same pastures. Some works carried out on sheep in central Côte d'Ivoire by Apala et al (2020) revealed *Haemonchus*, *Trichostrongylus*, *Strongyloides*, *Cooperia, Oesophagostomum* and *Eimeria genera* presence. *Trichostrongylus*, *Strongyloides*, *Cooperia, Oesophagostomum* genus absence could be linked to their low prevalence in central Côte d'Ivoire. They mentioned 8.6% *Trichostrongylus*, 8.2% *Strongyloides*, 8% *Cooperia* and 0.2% *Oesophagostomum* genera. At the experiment start, lambs from both groups had practically the same parasite charges for *H. contortus*, *Moniezia sp*, and *coccidia* (p>0.05). According to Githiori et al. (2005) scale, 133 EGF initial *H. contortus* parasite charge was low. In fact, they were receiving synthetic helminthics.

With the same animals, *H. contortus* parasite charge increased over time. For instance, from 133 EGF at the start, the parasites charge reached 1450 EGF at the end. This eggs per gram of feces increase in the animals’ feces though they were receiving chemical anthelmintics could be explained by this anthelmintic ineffectiveness on *H. contortus* parasite. These observations were made by Geurden et al (2014), who observed levamisole chemical anthelmintics ineffectiveness on parasite populations in sheep in France, Greece and Italy. At the beginning, low EGFs may be due to the hypobiosis phenomenon. In fact, the first samples were taken at the end of March, which is the end of the dry season and the start of the rainy season. This phenomenon was observed by Bonfoh et al. (1995). Also, the animals in the experience had very low *Haemonchus contortus* EGFs from the start for 200 EGF, and six months after animals had 316 EGF.

**Table 2: *Haemonchus contortus*, *Moniezia spp* egg excretion trends, and Coccidia in the feces**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gastrointestinal worms’ charges | *Leucaena leucocephala*-based pellets | Synthetic levamisole anthelmintic | SE\* | p-values |
| *Haemonchus contortus* essay start | 200 | 133 | 38.15 | 0.76 |
| *Haemonchus contortus* 3 months after  | 533a | 1016b | 146.63 | 0.038 |
| *Haemonchus contortus* 6 months after | 316c | 1450d | 201.05 | 0.002 |
| *Moniezia* sp, essay start | 286 | 300 | 32.11 | 0.4 |
| *Moniezia* sp, 3 months after | 100 | 125 | 20.17 | 0.33 |
| *Moniezia* sp 6 months after | 150 | 86 | 9.40 | 0.23 |
| Coccidia number, essay start | 6266 | 5483 | 220.07 | 0.06 |
| Coccidia number, 3 months after | 2833e | 1500f | 162.85 | 0.04 |
| Coccidia number, 6 months after | 1300 | 995 | 8.15 | 0.89 |

SE\*: Standard error of means, egg excretion means with distinct letters are statistically different from each other (p<0.05)

*Leucaena leucocephala*-based pellets were highly effective against these parasites. These pellets’ effectiveness may be due to the *L. leucocephala* presence, because of its condensed tannins. The pellets effectiveness could be explained by *Leucaena leucocephala* high incorporation rate for 60% in the feed. Indeed, *L. leucocephala* vermifuge effects appear at very high incorporation levels (Marie-Magdeleine et al., 2020). In addition, *Moniezia sp* and *coccidia* parasitic charges were practically low in both groups. These results showed that *Leucaena leucocephala*-based consumption was effective in systematically reducing gastrointestinal parasitic infestations in sheep.

**3.5. *Leucaena leucocephala*-based pellet effect on animal hematocrit**

Hematocrit values were similar for both animals’ groups at the essay start, at around 18 (p>0.05, Table 3). According to Polizopoulou (2010), the animals were anemic. For example, anemia occurs when the hematocrit value is below 24%. Herein essay showed that animals fed on *Leucaena leucocephala*-based pellet had significantly increased hematocrit values to 31.16%, while animals treated with the chemical anthelmintic had hematocrit values below 24%. The control animals’ low hematocrit values could be explained by *H. contortus* hematophagous nature. Animals, receiving *Leucaena leucocephala*-based pellet, high hematocrit values were due to tannins action.

Table 3: lambs’ hematocrit values as a treatment and time functions

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Hematocrit trends | *Leucaena leucocephala*-based pellets | Synthetic levamisole anthelmintic | SE\* | p-values |
| Hematocrit, essay start | 18 | 17.67 | 15.71 | 0.927 |
| Hematocrit, after 3 months | 25.66 | 18.16 | 16.9 | 0.071 |
| Hematocrit, after 6 months | 31.16 | 20.16 | 0.88 | <0.0001 |

**3.6. *Leucaena leucocephala*-based pellets effect on animal weight**

Figure 1 shows animals’ weight. It shows that sheep fed on *Leucaena leucocephala*-based pellet experienced continuous and significant increases in weight gain over the experiment (p<0.05). Weight gains for the pellet group rose from 10.38 kg at the experiment start to 25.27 kg at the end. While, weight gains for the control group, receiving levamisole synthetic helminthic, rose from 10.50 kg to 20.07 kg. So, lambs in the pellet grew 1.55 times faster than those in the control group. *Leucaena leucocephala*-based pellet animals’ group evolution reflected tannins effect in slowing down gastrointestinal tract infestations (Hoste et al., 2011) and strengthening their immune defenses.

Figure 1: Lambs’ weight (kg) according to treatment, and time

To: essay start; T3: 3 months after, T6: 6 months after; Témoin: Control group

**4. Conclusion**

The work aimed to determine *Leucaena leucocephala* leaves and pellets polyphenolic compounds content, for the gastrointestinal parasites control in Djallonké sheep. The results showed that *L. leucocephala* leaves pellets improved animal growth by 14.88 kg weight gain over 6 months. Also, sheep fed on *L. leucocephala* leaves pellets had very low EGF and better hematocrit values. Thus, the study showed that *Leucaena leucocephala* leaves can be used to improve productivity in sheep farms, alongside a good gastrointestinal worms’ control. In short, *Leucaena leucocephala* leaves have anti-helminthics function.

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