

# In Vivo Anthelmintic Activity of *Ceratotheca sesamoïdes* Endl Whole Plant Powder

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**Abstract:** Small ruminants are an important lever for livestock production in Burkina Faso. With a numerically important livestock population, small ruminants contribute to the fight against poverty and participate greatly in the country's economy. Despite its importance, the development of small ruminant breeding is strongly hindered by parasitic diseases due to gastrointestinal nematodes. The appearance of parasite resistance to anthelmintic molecules makes the treatment of gastrointestinal parasitosis more and more problematic. In order to provide rural farmers with an effective and accessible biological alternative, *C. sesamoïdes* whole plant powder in vivo anthelmintic efficacy was tested on mossi sheep artificially infested. Mossi sheep was artificially infested with 3200 L3 larvae of *H. contortus* 30 days before the beginning of the experiment. Two (2) treated batches of 6 sheep each received respectively 14g/kg of body weight and 7g/kg of body weight while one negative control batch of 6 sheep without treatment and one positive batch of 6 sheep treated with levamisole were constituted during the 21 days of experimentation. The Fecal Eggs Count (FEC) reduction rate was high during treatment and reached 87.08% and 74.91% at D21 respectively for the dose of 14g/kg body weight and 7g/kg body weight. Statistical analysis revealed no significant difference between the two doses tested and between the two doses and the positive control, whereas the difference was significant ( $P < 0.05$ ) compared to the negative control. The animal's weight evolution was not significant ( $P > 0.05$ ) compared to the negative control. The mean hematocrit level varied between D0 (27.66) and D21 (30.5) for the 14g/kg body weight dose while that of the animals treated with the 7g/kg body weight dose decreased slightly between D0 (27.33) and D21 (26.66). Biochemical blood tests show no suspicion of hepatic and renal impairment. Thus, *C. sesamoïdes* could be used as an alternative for the treatment of small ruminant's gastrointestinal nematodes in the form of whole plant powder in Burkina Faso.

**Keywords:** Bioactive Forages, Anthelmintic, *H. contortus*, *C. sesamoïdes*

## 1. Introduction

Small ruminant breeding is the second most practiced activity in the livestock sub-sector after poultry farming in

Burkina Faso. The importance of small ruminants can be measured by its livestock population, which was estimated to 15,635,000 head of goats and 10,442,000 head of sheep in 2018 [25]. Also, small ruminant farming is an important

source of animal protein with an estimated to 13,716,000 tons of meat in 2018 for all small ruminants in Burkina Faso [25]. In addition, small ruminants are easily mobilized savings for the herder and contribute about 32% of the 30 billion provided by livestock to the economy of Burkina Faso [5, 15, 34].

Despite its importance, small ruminant production is low due to inadequate supervision and poor animal health [18]. Parasitic diseases due to Gastrointestinal Nematodes (GIN) are the most damaging to the poor performance of small ruminants in Burkina Faso. Among the NGIs, Gastro-Intestinal Strongyles (GIS) represent the most dominant parasitic pathologies of small ruminants [2]. Several GIS parasites of small ruminants are known but *Haemonchus contortus* is the most widespread. This haematophagous parasite lodged in the abomasum of small ruminants causes severe anaemia that can lead to reduced production and death in animals [27, 14, 22, 7, 35, 6].

The control of GISs in small ruminants is essentially based on the use of anthelmintic substances [16]. However, inefficiency of these anthelmintic molecules due to parasite resistance and their ecotoxicities are increasingly reported worldwide [33, 13]. In addition, the use of these anthelmintic molecules is expensive and geographically inaccessible for rural farmers [19].

This situation leads to the search for alternative and/or complementary control methods to chemical treatments. Several methods have been explored, including the use of bioactive forages. Studies carried out in many African countries have proven many woody forage species anthelmintic efficacy [18, 3]. The anthelmintic properties studies of annual grasses such as *C. sesamoides* are rare in Burkina Faso. *C. sesamoides* also called false sesame is an herbaceous plant widespread throughout tropical Africa and grows on sandy soils and rarely in rocky areas [36]. Several studies have shown the use of this sticky plant in human and veterinary pharmacopoeia as well as the presence of triterpenic saponoside, tannins, calcium and phosphorus in this plant [11, 36, 8, 28, 4].

The purpose of this to evaluate the anthelmintic activity of *C. sesamoides* whole plant powder through the measurement of Fecal Eggs Count (FEC) of sheep artificially infested with *H. contortus* L3 larvae.

## 2. Material and Methods

### 2.1. Vegetal Material

The whole plant of *C. sesamoides* was collected in the villages of Katchari and Mamasiol in the urban commune of Dori early in the morning between the end of September and mid-October. Whole plant samples were dried in a room at DRREA-Sahel for 2 weeks at room temperature and then transported to CREA Kambouinsin in the commune of Ouagadougou. One sample was identified at the National Herbarium of Burkina Faso (HNBF) under number 8758. The samples were then ground using a Retsch type SM 100 grinder with a mesh size of 5 before being used as whole plant powder for the *in vivo* test.

### 2.2. Animal Material

#### 2.2.1. Sheep

Twenty-four (24) Mossi sheep between 12 and 24 months of age and with an initial weight between 12.3 and 24.5 kg were used for the *in vivo* tests. All animals were paid at the livestock market of Kaya, capital of the Centre-Nord region. The animals were not dewormed during the three months preceding the experiment.

#### 2.2.2. L3 Larvae

The infesting L3 larvae of *H. contortus* were obtained by culturing fresh eggs in the sterilized's fecal of sheep mixed with wood's sawdust for 14 days at 31°C. After 14 days the culture was mounted in gauze and then placed on the Baermann device to get the L3 larvae.

### 2.3. Methods

#### 2.3.1. Experimental Device

The experimental set-up was carried out according to the method of [10]. Thirty (30) days before the beginning of the experiment, all animals were artificially fed 3200 *H. contortus* larvae orally through a naso-esophageal tube in two phases of 1600 larvae each, spaced one week apart. One week before the start of the *in vivo* tests, the animals were weighed and the degree of infestation was determined and then the animals were divided homogeneously into 4 batches of 6 sheep each according to the weight and the Fecal Eggs Count (FEC). The batches included, 2 batches treated with two different doses of *C. sesamoides* whole plant powder, 1 untreated negative control batch and 1 positive control batch treated with levamisole.

Table 1. Experimental setup.

Batches	Number	Weight (kg)	FEC	Dose (g/kg of body weight)	Concentrated food (g/animal)
C. SesamoidesA	6	18.95	2 400	14	200
C. sesamoides B	6	18.94	1 800	7	200
negative Control	6	16.37	2 500	No	200
positive Control	6	16.50	3 900	Levamisole (1/2 bolus for 25 kg)	200

The experiment lasted 21 days during which all animals received concentrate (SN Citec cattle feed) in the form of pellets in the morning at 6:00 am. For the treated batches, the concentrate was mixed with the equivalent amount of the set

treatment dose. In the afternoon, all animals were fed corn bran for the duration of the experiment. The animals were provided with water and the mineral lick ad libitum during the experimental period. The treatment in the form of *C.*

*sesamoïdes* whole plant powder was administered to the treated batches on the first four days (D1, D2, D3, D4) and then repeated on the 7<sup>th</sup> day after the end of the first treatment (D11, D12, D13, D14). At the end of the first treatment the animals grazed on natural pasture for 6 days before the second treatment was repeated. After the last day of the second treatment, the animals were still grazing on natural pasture until the 21<sup>st</sup> day of the experiment.

### 2.3.2. Measured Parameters

The main parameters collected for this study are: clinical data concerning the general condition of the animals, parasitological and hematological data.

1. During the period of the experiment, the clinical data concerning the general condition of the animals after the administration of the whole plant powder of *C. sesamoïdes* were evaluated by direct observations and recorded on a form.
2. The weight growth of the animals of different batches was measured from individual weighing at D0, D4, D7, D14 and D21. Weighing was done to fasting using a MARECHALLE-PESAGE cattle scale for small ruminants equipped with an electronic indicator.
3. Parasitological data were obtained by individual sampling of feces directly from the rectum of the animals at D0, D4, D7, D14, D21. Individual coproscopies were performed and *H. contortus* egg excretion was quantified by the Mac-Master technique by triturating 3g of feces in 42 mL of NaCl density 1.2 to allow egg flotation. The sensitivity for one egg found was 50. The Fecal Eggs Count (FEC) was thus determined.

FEC: number of eggs obtained in the two compartments X 50.

The Fecal Eggs Count Reduction Rate (FECR%) was determined according to the formula used by Kaboré [18]:

$$\text{FECR}(\%) = \left( \frac{\text{FEC before treatment} - \text{FEC after treatment}}{\text{FEC before treatment}} \right) \times 100$$

1. The hematocrit rate variation of the animals in each batches were determined by drawing blood through the jugular vein of the animals using heparinized tubes at D0, D7, D14, and D21 of the experiment. Then each

sample was used to fill a capillary tube with 4/5<sup>th</sup> hematocrit. The filled capillary tubes were centrifuged at 9000 rpm for 5 minutes using a HAEMATOKRIT 210 hematocrit centrifuge. In conjunction with the blood collection with heparinized tubes, a collection with dry tubes was performed on D0 and D21 of the experiment for each animal. The dry tubes were centrifuged using a SIGMA 3-15K centrifuge at 3000 rpm for 10 minutes and the serum was collected in 1.8 mL cryotubes for biochemical analysis.

2. The degree of anemia of each animal was determined by evaluating the FAMACHA score by comparing the coloration of the ocular conjunctiva of each animal with a card showing different colorations of the ocular conjunctiva in relation to the degree of anemia of the animal.

### 2.4. Statistical Analysis

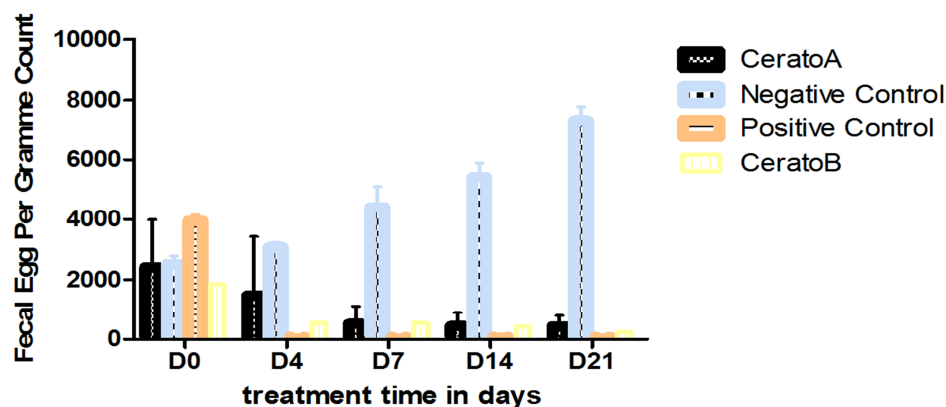
Data analysis was carried out according to the method of [10]. The collected data were entered into Excel 2016 which was used to calculate the means and standard deviations of the different measured parameters as well as the animals' Fecal Eggs Count reduction (FECR) rate. Then the data of the different days of follow-up were subjected to a one-factor analysis of variance (ANOVA I) followed by a multiple comparison of means at the threshold of 5% by the method of Tukey's using the interface Rstudio Version 1.4.1717 with the packages Rcmdr Version 2.7-1 of the software R Version 4.1.0.

Prism 5.0.0.288 software was used to produce the graphics.

## 3. Results

### 3.1. Fecal Eggs of *H. contortus* Reduction Rate

No apparent clinical signs of toxicity (salivation, skin reaction, diarrhea) in the animals during the treatment time with *C. sesamoïdes* whole plant powder were observed. The evolution of FEC showed a significant reduction over time compared to the negative control batch ( $P < 0.05$ ) (Figure 1).



CeratoA: dose 14g/kg body weight; CeratoB: dose 7g/Kg body weight.

Figure 1. Animal' Fecal Egg Count after *C. sesamoïdes* doses treatment.

The table 2 shows us a significant reduction in the FEC rate level of animals treated with the different doses of *C. sesamoïdes* powder from D4 and throughout the treatment

time. This reduction was significant compared to the negative control batch ( $P < 0.05$ ).

**Table 2.** FEC Reduction rates of the animals in the different batches.

Batches	FEC Reduction Rate			
	D4	D7	D14	D21
CeratoA	70.27d	75.84d	77.12d	87.08d
CeratoB	53.34d	71.94d	75.90d	74.91d
Positive Control	99.36d	99.57d	98.78d	99.35d
Negative Control	-24.01c	-77.28b	-118.39b	-195.07a
P	2e-16			

P: Probability; a, b, c, d: difference between column,

*CeratoA*: dose 14g/kg body weight; *CeratoB*: dose 7g/Kg body weight.

### 3.2. Weight Evolution

The treatment with whole plant powder of *C. sesamoïdes* resulted in a low average daily gain (ADG) with a decrease

of this ADG at the last day (D21) of the experiment. The weight evolution of the animals of the different batches was not significant compared to the controls ( $P > 0.05$ ) (Table 3).

**Table 3.** Animals weight evolution in the different batches.

Batches	GMQ (D0-D4)	GMQ (D0-D7)	GMQ (D0-D14)	GMQ (D0-D21)
CeratoA	135g±0.21a	185.7g±0.14a	117.8g±0.31a	46.1±0.05a
CeratoB	170g±0.19a	135.7g±0.06a	139.2±0.11a	46.1g±0.03a
Positive Control	280g±0.09a	218.5g±0.06a	32.1g±0.02a	37.1g±0.03a
Negative Control	227.5g±0.1a	161.4g±0.09a	-10±0.04a	-25.7±0.01a
P		0.222		

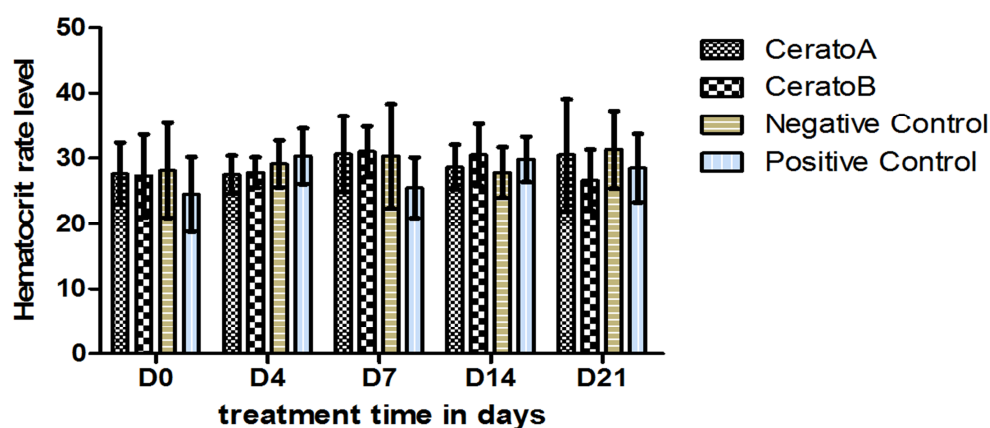
P: Probability; a: no difference between the columns,

*CeratoA*: dose 14g/kg body weight; *CeratoB*: dose 7g/Kg body weight.

### 3.3. Hematocrit Rate Level Evaluation

Figure 2 shows a slight increase in the mean hematocrit level of animals treated with both doses of *C. sesamoïdes* with a slight increase between D0 (27.66) and D21 (30.5) in the batch treated with 14g/kg body weight while the mean

hematocrit level in the batch treated with 7 g/kg body weight decreased slightly between D0 (27.33) and D21 (26.66). However, the statistical analysis was not significant between the treated batches and compared to the controls ( $P > 0.05$ ).



*CeratoA*: dose 14g/kg body weight; *CeratoB*: dose 7g/Kg body weight.

**Figure 2.** The hematocrit rate level evolution during treatment.

### 3.4. Evolution of FAMACHA

Figure 3 shows us a slight decrease in FAMACHA score

in animals treated with different doses of *C. sesamoïdes* at D21 compared to the score recorded at D0. The statistical analysis is significant compared to the negative control ( $P < 0.05$ ) at D14 and D21.

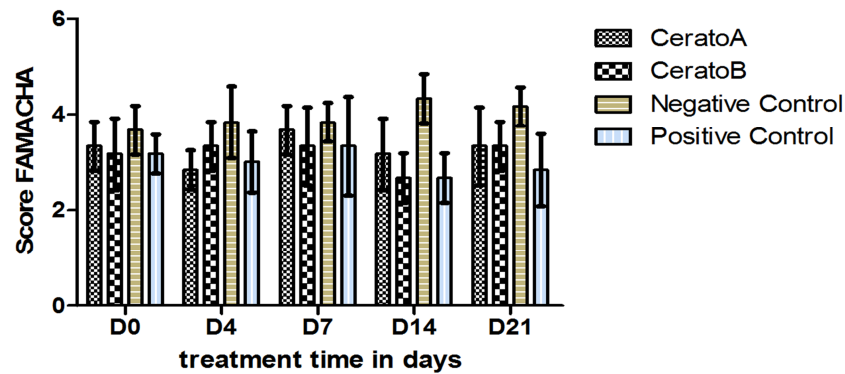


Figure 3. Evolution of FAMACHA score during treatment.

### 3.5. Effect of Treatment on Hepatic Enzyme Metabolism and Renal

Table 4 shows that the treatment with the two doses of *C. sesamoides* whole plant powder did not lead to a hepatic and renal damage in the animals. Indeed, the values of Aspartate Aminotransferase (ASAT) and Alanine Aminotransferase (ALAT) at D0 and D21 of the treated

animals are all situated in the range of the usual values of references as regards the hepatic function. Also, creatinine values, which is one of the most reliable biochemical markers of glomular function, were within the ranges of the different usual and reference values at D0 and D21. Statistical analysis of these parameters at D0 and D21 between the treated batches and the negative controls was not significant ( $P>0.05$ ).

Table 4. Effect of treatment on liver and kidney function.

Treatment		Creatinine ( $\mu\text{mol/l}$ )	ASAT (UI/l)	ALAT (UI/l)
Cerato A	J0	89.34 $\pm$ 9.70a	131 $\pm$ 50.71a	20.2 $\pm$ 7.82a
	J21	88.31 $\pm$ 11.76a	119.16 $\pm$ 31.18a	21.83 $\pm$ 7.93a
CeratoB	J0	79.36 $\pm$ 10.31a	134.5 $\pm$ 37.22a	18.16 $\pm$ 6.46a
	J21	83.31 $\pm$ 14.31a	158.83 $\pm$ 45a	21.5 $\pm$ 7.23a
Negative Control	J0	78.85 $\pm$ 8.45a	119.66 $\pm$ 36.10a	20.66 $\pm$ 5.98a
	J21	83.8 $\pm$ 6.36a	125.16 $\pm$ 38.20a	20.83 $\pm$ 3.12a
Positive Control	J0	79.06 $\pm$ 12.29a	130.4 $\pm$ 32.99a	23 $\pm$ 7.41a
	J21	81.25 $\pm$ 7.56a	129.5 $\pm$ 63.17a	19.33 $\pm$ 2.5a
P		0.61	0.82	0.94
Reference values		160-168 A; 53-115 C	71-209 B	30 $\pm$ 4 A

A: [21]; B: [31]; C: [12]; P: Probability; a: no difference between the columns; CeratoA: dose 14g/kg body weight; CeratoB: dose 7g/Kg body weight.

### 3.6. Effect of Treatment on Energy and Mineral Metabolism

Table 5 shows that the treatment with the two doses of *C. sesamoides* whole plant powder did not lead to an imbalance of the energetic and mineral metabolism. Indeed, the various

parameters measured are all located within the limit of the usual values of references D0 and D21. Statistical analysis of the different parameters at D0 and D21 and between treated batches and negative controls was significant ( $P<0.05$ ) except for the triglyceride parameter which was only measured at D21 ( $P>0.05$ ).

Table 5. Effect of treatment on energy and mineral metabolism.

Treatment		Glycemic (mmol/L)	Cholesterol (mmol/L)	Calcium (mmol/L)	Triglycerides (g/L)	Totals Proteins (g/L)
Cerato A	J0	2.4 $\pm$ 0.71a	3.4 $\pm$ 0.88b	3.57 $\pm$ 0.55c	ND	145.5 $\pm$ 17.9c
	J21	2.8 $\pm$ 0.57a	2.81 $\pm$ 0.56ab	2.23 $\pm$ 0.28a	0.5 $\pm$ 0.14a	96.6 $\pm$ 17.3b
CeratoB	J0	3.3 $\pm$ 0.9a	2.4 $\pm$ 0.54ab	3.18 $\pm$ 0.51bc	ND	125.8 $\pm$ 15.4ac
	J21	3.2 $\pm$ 0.82a	2.7 $\pm$ 0.66ab	2.47 $\pm$ 0.45ab	0.37 $\pm$ 0.09a	105.9 $\pm$ 8.63ab
Negative Control	J0	2.3 $\pm$ 0.97a	2.9 $\pm$ 0.51ab	3.27 $\pm$ 0.64bc	ND	132.1 $\pm$ 30.62bc
	J21	3.3 $\pm$ 0.33a	2.1 $\pm$ 0.37a	2.76 $\pm$ 0.70ac	0.5 $\pm$ 0.23a	96.9 $\pm$ 8.7a
Positive Control	J0	2.2 $\pm$ 0.36a	2.9 $\pm$ 1.05ab	3.19 $\pm$ 0.29bc	ND	119.08 $\pm$ 14.5ac
	J21	3.4 $\pm$ 0.55a	2.1 $\pm$ 0.24a	2.79 $\pm$ 0.27ac	0.4 $\pm$ 0.1a	105.1 $\pm$ 8.8ab
P		0.01	0.02	0.0007	0.17	0.00006
Reference values		0.8-2.4 D, 3.5-3.80 E	1.68 $\pm$ 1.3 E	3.04 $\pm$ 0.07A	0.5 $\pm$ 0.19 E	130.2 $\pm$ 38.9 E

A: [21], D: [9]; E: [26]; P: Probability, a, b, c: difference between the columns, ND: Not Dosed; CeratoA: dose 14g/kg body weight; CeratoB: dose 7g/Kg body weight.

## 4. Discussion

In vivo tests on mossi sheep artificially infested with *H. contortus* L3 larvae and treated with whole plant powder of *C. sesamoïdes* revealed a high Fecal Egg Count Reduction (FECR). The FECR rate reached at J21 were 87.08% and 74.91% respectively for the dose of 14 and 7g/kg body weight. Our results are identical to the results obtained on Djallonké sheep treated with *Spondias mombin* leaf powder with an FECR rate of 60.41% [1]. Our results corroborate those of [30] who obtained a fecal excretion reduction rate of 85 and 88% with the respective doses of *Newbouldia laevis* leaf powder of 3.2g/kg body weight and 4.8g/kg body weight. Different extracts of *Calotropis procera* as well as his flower powder at doses of 1 to 3g/kg body weight resulted in high FEC reduction rate of several sheep strongyles in Pakistan [17]. Contrary to our results, the dried leaves of *C. procera* administered as a supplement to sheep at 100 and 200 g per day resulted in a decrease FECR rate only in the first days (D3 and D7) and thereafter FEC was elevated until D28 [20]. Both doses of whole plant powder *C. sesamoïdes* did not result in an elevated average daily gain (ADG) in treated animals and statistical analyses showed no significant difference between treated and controls batches. Similarly, to our study, *Vitex thomasi* bark powder administered to naturally infested goats did not result in a significant difference in weight evolution during 126 days of follow-up [29]. Aqueous extracts of *Cratylia mollis* administered to goats naturally infested with NGIs did not result in a significant difference in ADG of the animals after 30 days of follow-up which corroborates to our study although the species of animals are different with our study [23]. The whole plant powder of *C. sesamoïdes* caused an improvement in hematocrit level between D0 (27.66) and D21 (30.5) at the dose of 14g/kg body weight while the mean hematocrit level of the batch treated with 7g/kg body weight decreased slightly between D0 (27.33) and D21 (26.66) but no significant difference was revealed. In conjunction with the hematocrit analysis, a slight decrease in FAMACHA score was noted in treated animals at D21 compared to the score recorded at D0. [24] did not obtain a significant change in hematocrit level in Djallonké sheep treated with single and combined doses of *Zanthoxylum zanthoxyloides* and *Newbouldia laevis* after 34 days similarly to our results. Sheep artificially infested with *H. contortus* in Sudan and then treated with aqueous extracts of *B. aegyptiaca* seeds at 9g/kg body weight and aqueous extracts of whole plant of *Artemisia herba alba* at 3g/kg body weight resulted in a change in hematocrit as in our present study [32]. In contrast to our study the other one obtained a statistically significant difference. The number of animals per batch (4) and/or the age of the animals (4-5 months) could explain the difference in statistical significance with our study. Animals treated with *C. sesamoïdes* whole plant powder did not show any sign of toxicity (salivation, skin reaction, diarrhea) during the

experiment. Also, the biochemical blood tests, in particular transaminases, creatinine, as well as the various energy and mineral parameters carried out on D0 and D21 are all within the usual reference values, indicating good hepatic, renal and energy metabolism function.

## 5. Conclusion

The anthelmintic efficacy of *C. sesamoïdes* whole plant powder is proven through this study. Indeed, the different doses fixed for this study generated a high FECR rate in animals artificially infested by *H. contortus*. However, treated animals did not achieve a high average daily gain but the average hematocrit level improved during treatment. No signs of toxicity were observed in the animals and the biochemical blood parameters did not reveal liver and kidney damage. *C. sesamoïdes* could be used as an alternative to chemical treatment of small ruminants' GIN.

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