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Article

Effects of purge potato (*operculina macrocarpa*) in multinutritional blocks on sheep naturally infected with gastrointestinal helminth

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Resumo: Objetivo desse estudo foi avaliar os efeitos da batata de purga (*O. macrocarpa*) oferecida em blocos multinutricionais sobre ovinos naturalmente infectados por helmintos gastrintestinais. O trabalho foi desenvolvido de acordo com um delineamento inteiramente casualizado, com quatro tratamentos e seis repetições de um animal. Os tratamentos foram: T1 = apenas BMs; T2 = BMs + batata de purga (1g/Kg/peso vivo); T3 = BMs + batata de purga (0,5g/Kg/pv) e T4 = BMs + anti-helmíntico químico. Nos tratamentos com batata de purga 1g/Kg/pv, 0,5g/Kg/pv e químico o OPG diminuiu aos sete dias com 767, 516, 267 e eficácia de 53, 68, e 84% respectivamente. Os valores médios das hemácias, hemoglobina, hematócrito e leucócitos foram menores para o tratamento apenas com BMs, porém se mantiveram dentro dos valores de referência. Para globulina, proteínas totais, creatinina os valores médios ficaram discretamente abaixo da referência para ovinos. Conclui-se que a *O. macrocarpa* adicionada aos blocos multinutricionais nas doses e período estudado não provocaram respostas fisiorgânicas características de toxicidade.

Palavras-chave: bioquímica, *Operculina macrocarpa,* parâmetros hematológicos, toxicidade.

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Introduction

Infection with gastrointestinal nematodes in northeastern Brazil affects animals in many ways, including: retarded growth; weight loss; reduced food consumption, digestion, and absorption; and high mortality rates (BIZIMENYERA et al., 2006).

Food supplementation is used to correct deficiencies of grazing animals and reduce the damage caused by parasitism. Some examples are agroindustrial byproducts, urea-molasses mixtures, and multi-nutritional blocks (MBs). The latter consist of a solid mixture of several ingredients containing proteins, energy, and minerals, meeting the nutritional needs of animals in critical periods of nutrient scarcity (BEN SALEM & NEFZAOUI, 2003; MARTÍNEZ-MARTÍNEZ et al., 2012).

Due to the growing problem of the development of resistance to anti-parasitic agents, besides residues in products of animal origin, research has been focusing on the anti-parasitic properties of condensed tannins (CT) as a strategic alternative to control gastrointestinal parasitism in ruminants (ATHANASIADOU et al., 2001). Tannins are plant secondary metabolites, being a complex group of polyphenolic compounds. These metabolites are linked to plant defense mechanisms and are divided according to their chemical structure and properties into two groups: hydrolysable and condensed tannins. The latter are harmful and toxic to ruminants (ATHANASIADOU et al., 2001; HERVÁS et al., 2003).

Research has addressed several plant species with a possible anthelmintic effect in ruminants (COSTA, 2006; CENCI, 2007). Purge potato (*Operculina macrocarpa* (L) Urb.), common in northeastern Brazil, has stood out with its proven effect in several studies (ALMEIDA et al., 2007; SILVA et al., 2010). This study evaluates the effects of purge potato (*O. macrocarpa*) in multi-nutritional blocks on sheep naturally infected with gastrointestinal helminths.

Materials and methods

The experiment was conducted at the "Lameirão" farm of the Federal University of Campina Grande (UFCG), located at coordinates 7º02'58.3" S and 37°29'32.5" W, in Santa Teresinha city, Paraíba State, Brazil. The experimental period was from November 2013 to January 2014.

Multi-nutritional blocks (MBs) were prepared at the Paraíba State Agricultural Research Corporation (EMEPA-PB), using the following ingredients for the respective treatments: T1 - 25% molasses, 5% livestock urea, 28% crushed corn, 20% soybean bran, 5% common salt, 4% mineral salt, 10% hydrated lime, and 3% limestone; T2 - 25% molasses, 5% livestock urea, 1% crushed corn, 20% soybean bran, 5% common salt, 4% mineral salt, 10% hydrated lime, and 30% *O. macrocarpa* bran; T3 - 25% molasses, 5% livestock urea, 16% crushed corn, 20% soybean bran, 5% common salt, 4% mineral salt, 10% hydrated lime, and 15% *O. macrocarpa* bran; T4 - equal to T1. After being weighed on a digital scale, the ingredients were mixed in a concrete mixer and placed in a 7-ton hydraulic press for up to 1 minute. Then, the mixture was removed from the press and stored at room temperature for 48 hours before consumption.

The experimental period lasted for 60 days (November 2013 to January 2014), preceded by 14 days for management adaptation. The animals remained in a semi-extensive regime, and were maintained in a secondary succession of Caatinga enriched with Buffel grass, in four 0.6-ha paddocks randomly distributed by treatment. At the end of the day, the animals returned from the paddocks to the sheepfold where they remained in individual stalls, with a block available for each animal, and water *ad libitum* from 5 P.M. to 5 A.M.

Twenty-four sheep (Santa Inês sheep and crossbred sheep: ½ Santa Inês X, ½ Dorper) were identified and distributed equally in four treatments of 6 animals each (replicates). These animals were intact males naturally infected with gastrointestinal nematodes, aged approximately 6 months and with average live weight of 24.60 ± 0.91 Kg / lbs.

Treatment 1 (T1) consisted of only MBs (positive control - PC); if necessary, the animals would receive a rescue dose with chemical anthelmintic, preventing deaths from parasitism. Treatments 2 (T2) and 3 (T3) added purge potato (*O. macrocarpa*) bran *in natura* in the doses of 1g/Kg/lw and 0.5g/Kg/lw, respectively, according to a previous study on acute and sub-chronic toxicity in mice (unpublished data); Treatment 4 (T4) included MBs and, separately, a chemical anthelmintic (Cydectin®, 1% moxidectin, Fort Dodge, Brazil) applied subcutaneously in a single dose of 1 mL / 50 Kg (negative control - NC).

Individual stool samples were collected directly from the rectal ampoule, being processed for parasitological analysis (GORDON & WHITLOCK, 1939) at the Laboratory of Parasitic Diseases of Domestic Animals at UFCG. The first collection was performed before exposure (day zero), followed by collections every seven days in the first month and after 44 and 60 days to determine the reduction in the number of eggs per gram of feces (EPG) of parasites of the family Trichostrongylidae. The arithmetic means of EPG for each treated group (EPGt) were calculated and compared with the means of the control group (EPGc). Reduction in egg count per gram of feces (RECF) was determined using the formula RECF = [1- (EPGt / EPGc)] x 100, described by COLES et al. (1992).

Blood was collected for hematological examinations on day zero, followed by collections every seven days in the first month, and at the end of the experiment, after 60 days. The blood was placed in individual tubes containing ethylenediaminetetraacetic acid (EDTA), which were kept in Styrofoam boxes and covered with ice until processing. The following hematological parameters were evaluated (JAIN, 1993): red blood cell count, hematocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leukocytes, neutrophils, eosinophils, lymphocytes, and total plasma protein (TPP).

For serum biochemistry analysis, blood was collected from / in identified tubes, with further evaluation of the following biochemical parameters: aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), albumin, globulin, total protein (TP), urea, and creatinine (KANEKO et al., 2008). The evaluations were performed immediately after collection in a commercial veterinary laboratory, following the protocol of specific commercial kits (Labtest® Diagnóstica S.A., Lagoa Santa city, Minas Gerais State, Brazil), with reading in a semiautomatic device (BIO-200® Products Laboratories Ltda, São Paulo State, Brazil).

At the end of the experiment, two animals (33.3%) from each treatment were chosen with the highest mean EPG. These animals were necropsied, having the following viscera collected: heart, lung, kidneys, liver, stomach, large and small intestine (BRASIL, 2004). The viscera were immersed in a 10% buffered formaldehyde fixative solution, being then histopathologically processed and stained with hematoxylin-eosin.

The experimental design was completely randomized, with four treatments and 6 replicates. To reduce disparity, EPG was transformed by the logarithmic formula $Log_{10}(X + 1)$; however, the values are shown in the table as arithmetic means of the untransformed values. The data were submitted to variance analysis (ANOVA) and to Tukey test at 5% significance ($P < 0.05$) using the SAS program (2003).

Results and discussion

In the determination of EPG, treatments interacted with periods; all periods did not differ significantly between treatments ($p > 0.05$). Eggs per gram (EPG) counts decreased after seven days in the treatments BMs + potato (1g/Kg/lw), BMs + potato (0.5g/Kg/lw), and BMs + moxidectin, yielding the values of 767, 516, 267, and an efficacy of 53, 68, and 84%, respectively (Table 1). Considering the baseline EPG value (2017), the treatment BMs + potato (1g/Kg/lw) led to the highest decrease of this variable at 60 days, with an efficacy of 46%. These data are close to the values of ALMEIDA et al. (2007), who administered purge potato bran to goats at a dose of 4.5g/10Kg and found an average reduction of 63.09% and 72.32% in EPG after 30 and 60 days of treatment, respectively. However, the results diverge from SILVA et al. (2010), who orally administered purge potato bran to goats at a dose of 9g/20Kg, finding a reduction of 84 and 70% in EPG on days 7 and 25, respectively.

It is noteworthy that although it was not the objective of this study, the species *Haemonchus contortus* was found in the abomasum, with a prevalence of females in all treatments. The treatment BMs + potato (0.5g/kg/lw) accounted for the highest number of females of this species (3182), and BMs + moxidectin accounted for the lowest number (844). Moreover, species *Trichostrongylus colubriformis* and *Strongyloides papillosus* were found in the small intestine, and *Oesophagostomum columbianum* in the large intestine.

The phytochemical analysis performed on purge potato (*O. macrocarpa*) tubers revealed the presence of condensed tannins - CT (unpublished data). Studies have demonstrated anthelmintic effects of several plants with CT, as observed by SHALDERS et al. (2014). These authors administered pink pepper (*Schinus terebinthifolius*) pie and observed a reduction of up to 35.8% in EPG, which they believed to be the result of CTs acting on fertility and elimination of worms. Furthermore, KAHIYA et al. (2003) observed that the quantity and type of tannin influences the anthelmintic effect.

Table 1 - Mean number of eggs per gram of feces (EPG) and efficacy (RECF) of sheep treated with multi-nutritional blocks (MBs) plus *purge potato (*Operculina macrocarpa*) bran.

Treatment	Period (days) 0	7	14	21	30	44	60
MBs (PC)	1150Aa	1633Aa	1750Aa	1950Aa	1433Aa	2233Aa	3733Aa
	(±723)	(±1282)	(±1407)	(±1449)	(± 715)	(±1966)	(±4301)
MBs+potato*	2133Aa	767Aa	2883Aa	1967Aa	1400Aa	5100Aa	2017Aa
(1g/Kg/lw)	(±1113)	(±383)	(±3223)	(±1987)	(±805)	(±5861)	(±2244)
RECF (%)		53	-65	-1	2	-128	46
MBs+potato	1350Ab	516Ab	1283Ab	1300Ab	1300Ab	1850Aa	9633Aa
(0.5g/Kg/lw)	(±635)	(±319)	(±902)	(±1131)	(±1122)	(±992)	(±6274)
RECF (%)		68	27	33	9	17	-158
MBs+moxid. (CN)	2100Aa	267Ab	667Aa	1100Aa	1017Aa	1467Aa	3617Aa
	(±2499)	(±225)	(±641)	(±1175)	(±948)	(±927)	(±2887)
RECF (%)		84	62	44	29	34	3

(PC) = positive control; (NC) = negative control; moxid. = moxidectin; RECF = reduction in egg count per gram of feces. Different uppercase letters in the column and lowercase letters in the row differ statistically by Tukey test ($P < 0.05$).

In the evaluation of the hematological results, there was no interaction between treatments and periods. For variables MCV, MCH, MCHC, neutrophils, lymphocytes, and TPP, there was no significant difference ($p > 0.05$) between treatments (Table 2). All variables were within the reference values for sheep (JAIN, 1993), with the exception of neutrophils.

Red blood cells were normochromic normocytic, and the treatment with only BMs showed the lowest mean values, differing statistically ($p < 0.05$) from the treatments BMs + potato (0.5g/Kg/lw) and BMs + moxidectin (Table 2). However, all counts remained within the reference values (8.0-16.0 x 10^6/µl) according to JAIN (1993). The same trend was observed for hemoglobin (8-16g/dl) and hematocrit (24-50%). These results demonstrate no impairment in the production of red blood cells, with no regenerative anemia that can be caused by acute or chronic hemorrhage from injuries and parasites in the gastrointestinal tract (THRALL et al., 2006).

These data are similar to those of CENCI et al. (2007), who evaluated the effects of CT in sheep naturally infected with gastrointestinal helminths. After 31 and 49 days of treatment, the authors obtained the values of 31.6 and 32.4% for hematocrit and 14.3 and 10.9 g/dl for hemoglobin, respectively. Notwithstanding, the results diverge from MACEDO (2007), who evaluated the effects of administering Neem leaf (*Azadirachta indica* A. Juss) on the control of helminths in sheep. These authors observed the following values in the group treated with 3g of the extract: hematocrit (37.3%), hemoglobin (9.3 g/dl), MCV (31.3 fL), and MCHC (25.5%). Moreover, they found a progressive reduction in hematocrit, red blood cells, and hemoglobin associated with the evolution of infection, which did not occur in this experiment.

The morphology of leukocytes remained unchanged, with a difference ($p < 0.05$) between the treatment with only BMs and the treatment with BMs + potato (1g/Kg/lw), which, in turn, did not differ from the other treatments (Table 2). The results did not exceed the reference values ($4.0\text{-}12.0 \times 10^3$) of JAIN (1993). In contrast, COSTA et al. (2006) found no significant difference in the total and differential leukocyte counts between the groups treated with Neem (*Azadirachta indica* A. Juss) and the control group.

The treatments did not differ statistically ($p > 0.05$) for neutrophils, with the results for these counts showing a slight increase in relation to the upper reference value (10-50%) of JAIN (1993), characterizing mild neutrophilia (Table 2). Neutrophilia is believed to be due to the inflammatory process in the mucosa, determined by gastrointestinal parasites (BIRGEL et al., 2014). The eosinophil count differed ($P < 0.05$) between the treatments BMs + potato (1g/Kg/lw), BMs + potato (0.5g/Kg/lw), and BMs + moxidectin; however, the results were within the reference values (0-10%) of JAIN (1993).

Table 2 – Hematological parameters of sheep treated with multinutritional blocks (MBs) plus purge potato (*Operculina macrocarpa*) bran for helminth infections.

Hematological Parameter*	Treatment			
	MBs	MBs + potato (1g/Kg/lw)	MBs + potato (0.5g/Kg/lw)	MBs + moxid.
Red cells (x106/µl)	9.6b±0.32	10.6ab±0.27	10.6a±0.23	10.6a±0.21
Hemoglobin (g/dl)	9.6b±0.31	10.6a±0.25	10.7a±0.25	10.7a±0.19
Hematocrit (%)	29.1b±0.95	31.6ab±0.78	32.0a±0.73	31.9a±0.55
MCV (fl)	30.4a±0.28	29.9a±0.24	30.2a±0.24	30.2a±0.27
MCH (pg)	10.1a±0.12	10.05a±.,10	10.1a±0.10	10.1a±0.11
MCHC (%)	33.1a±0.25	33.6a±0.22	33.4a±0.22	33.4a±0.22
Leukocytes (mm3)	6666b±358	8362a±543	7525ab±543	7114ab±396
Neutrophils (%)	50.8a±2.04	54.8a±1.83	51.5a±1.83	52.5a±1.92
Eosinophils (%)	2.1ab±0.45	2.7 a±0.37	1.0b±0.35	1.5b±0.27
Lymphocytes (%)	45.3a±2.33	41.9a±1.86	48.0a±1.86	44.8a±2.06
TPP (g/dl)	7.4a±0.07	7.3a±0.06	7.3a±0.06	9.1a±1.64

Values represent the mean ± standard deviation. MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, TPP = total plasma protein, moxid. = moxidectin. Values with different superscript lowercase letters on the same row differ statistically by the Tukey test ($P < 0.05$). *Compared with the reference values of Jain (1993).

For biochemical parameters, the treatments did not interact with the periods under study, differing significantly for the variables albumin, globulin, TP, and creatinine (Table 3).

The treatment with BMs + potato (1g/Kg/lw) showed the lowest value of AST (Table 3), differing significantly ($P < 0.05$) from the treatments with only BMs and with BMs + potato (0.5g/Kg/lw). All treatments were within the reference values for sheep (60-280 U/L) according to KANEKO et al. (2008). The results corroborate HERVÁS et al. (2003), who treated sheep with quebracho tannin extract and found values of 52.8 and 58.4 U/L for the treatments Q1 (0.5g/Kg/lw) and Q2 (1.5g/Kg/lw), respectively. High AST values and low cholesterol and albumin indicate liver disorders (GONZÁLES et al., 2006).

For GGT, there was a significant difference ($p < 0.05$) between the treatment with BMs + moxidectin and the treatment with only BMs, which, in turn, did not differ from the others (Table 3). The treatments with phytotherapic or chemical anthelmintics showed a slight increase in values in relation to the reference values (20-52 U/L) of KANEKO et al. (2008). However, the values were similar to those found by HERVÁS et al. (2003) when treating sheep: 59.2 and 53.9 U/L for Q1 and Q2, respectively. Only hepatic GGT is normally found in the plasma, increasing in cirrhosis (GONZÁLES et al., 2006), which was not seen in liver histopathology.

The mean value of albumin also increased in relation to the reference values (24-30 g/L) of KANEKO et al. (2008). Albumin concentration is affected by liver function and the availability of proteins in the diet, which probably influenced the results. The inverse was observed for globulin, whose mean value was lower than the reference values (35-57 g/L) of KANEKO et al. (2008). Albumin decreases and globulins increases in liver dysfunction (GONZÁLES et al., 2006), which did not occur in this experiment, since the globulin was lower and the albumin higher (Table 3).

Urea levels differed significantly ($p < 0.05$) between the treatment with only BMs and the treatments with BMs + potato (1g/Kg/lw) and with BMs + moxidectin (Table 3), with higher values in relation to the reference values (2.86 - 7.14 mmol/L) described by KANEKO et al. (2008) for sheep. Notwithstanding, the values corroborate those of HERVÁS et al. (2003), who obtained 8.1 and 8.4 mmol/L for the treatments Q1 and Q2, respectively, in sheep. Urea is synthesized in the liver, whose blood level is an indicator of kidney function. This function can be affected by the nutritional level in ruminants fed with excess protein or urea, which may be increased

(GONZÁLES et al., 2006). This has probably occurred in the present study since MBs were composed of protein and urea sources (5%) in all treatments.

Creatinine was below the lower reference level (1.2-1.9 mg/dl) obtained by KANEKO et al. (2008) in sheep (Table 3); however, there was no significant difference between treatments. Once again, the results corroborate those of HERVÁS et al. (2003), who obtained 0.87 and 0.99 mg/dL for the treatments Q1 and Q2, respectively. Plasma creatinine levels reflect the renal filtration rate, so that high levels indicate kidney deficiency (GONZÁLES et al., 2006), which did not occur in this experiment.

The treatments did not show macroscopic or histopathological lesions of organ toxicity. This corroborates FRUTOS et al. (2000), who used up to 25g of quebracho powder in sheep for 60 days and observed animals without lesions in autopsy and histopathology. Notwithstanding, the results disagree with HERVÁS et al. (2003), who administered 3g of quebracho tannin extract and observed ulcerative lesions and necrosis in the digestive tract.

Table 3 – Biochemical parameters of sheep treated with multi-nutritional blocks (MBs) plus purge potato (*Operculina macrocarpa*) bran for helminth infections.

Biochemical Parameter*	Treatment			
	MBs	MBs + potato (1g/Kg/lw)	MBs + potato (0.5g/Kg/lw)	MBs + moxid.
AST (U/L)	177^{a}±6.6	153^{b}±4.0	192^{a}±5.9	155^{b}±7.8
GGT (U/L)	48.2^{b}±1.4	54.5^{ab}±1.9	53.7^{ab}±2.0	60.7^{a}±2.5
Albumin (g/L)	36^{a}±1	35^{a}±1	35^{a}±1	37^{a}±1
Globulin (g/L)	23^{a}±1	22^{a}±1	21^{a}±2	22^{a}±2
TP (g/L)	59^{a}±1	56^{a}±2	56^{a}±1	59^{a}±1
Urea (mmol/L)	11.9^{a}±0.4	9.4^{c}±0.4	11.5^{a}±0.4	10.4^{b}±0.4
Creatinine (mg/dl)	0.6^{a}±0.01	0.6^{a}±0.02	0.7^{a}±0.01	0.6^{a}±0.02

Values represent the mean ± standard deviation. AST = aspartate aminotransferase, GGT = gamma glutamyl transferase, TP = total protein, moxid. = moxidectin. Values with different

superscript lowercase letters on the same row differ statistically from e Tukey test ($P < 0.05$).
* Compared with the reference values of Kaneko et al. (2008).

Conclusion

The results lead us to conclude that adding purge potato (Operculina macrocarpa) bran to the multi-nutritional blocks in the studied doses and period did not cause physiological responses characteristic of toxicity. Eggs per gram (EPG) decreased considerably at the beginning and at the end of the experiment at the highest dose of the treatment (1g/Kg/lw). This reinforces safety in its use as a phytotherapeutic additive to improve the control of helminth infections in sheep.

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Abstract: This study evaluates the effects of purge potato (*Operculina macrocarpa*) in multi-nutritional blocks (MBs) on sheep naturally infected with gastrointestinal helminths. The experimental design was completely randomized, with four treatments and six replicates per animal. Treatments were: T1 = only MBs; T2 = MBs + purge potato (1g/Kg/lw); T3 = MBs + purge potato (0.5/Kg/lw); and T4 = MBs + chemical anthelmintic. In the treatments with 1g/Kg/lw and 0.5g/Kg/lw purge potato and with chemical anthelmintic, eggs per gram (EPG) counts decreased to 767, 516, and 267 at seven days, with an effectiveness of 53, 68, and 84%, respectively. The mean values of red blood cells, hemoglobin, hematocrit, and leukocytes were lower for the treatment with only MBs; however, they were maintained within the reference values. For globulin, total protein, and creatinine, the mean values were discretely below the reference for sheep. It is concluded that adding *O. macrocarpa* to the multi-nutritional blocks in the studied doses and period did not cause physiological responses characteristic of toxicity.
Keywords: biochemistry, *Operculina macrocarpa*, hematological parameters, toxicity.

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Ethics committee

The animal experimentation protocol was approved by the Animal Use Ethics Committee (CEUA) of the Federal Rural University of the Semiarid (UFERSA) by protocol No. 64/12.

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