Direct anthelmintic effects of *Cereus jamacaru* (Cactaceae) on trichostrongylid nematodes of sheep: *in vivo* studies

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ABSTRACT

Following claims of anthelmintic activity of *Cereus jamacaru* DC (Cactaceae) by a commercial farmer, *in vivo* studies were conducted to determine the possible direct anthelmintic effects of the plant on ovine gastrointestinal nematodes. Eighteen sheep were infected with 4000 *Haemonchus contortus* and 6000 *Trichostrongylus colubriformis* larvae given in three divided doses over a period of three days. Once the infections were patent, the sheep were allocated to three groups and were drenched once a week for six weeks with fresh blended *C. jamacaru* plant material at a single (32.3 g/sheep) or double dose (64.6 g/sheep) or they remained as undrenched controls. Faeces were collected from individual animals on the day of treatment and three days thereafter on a weekly basis for seven weeks for faecal egg count. While there were no statistically significant differences in the egg counts between the groups, a double dose of *C. jamacaru* was effective in reducing the egg counts in the sheep by 18-65% over the 49 days of the experiment. Given that all animals remained in good health throughout the course of the experiment, with no adverse events occurring during the study, further experiments using higher doses or administering the plant material for a longer period of time than in the present study would be warranted.

Keywords: *Cereus jamacaru*; *Haemonchus contortus*; Sheep; *Trichostrongylus colubriformis*

1.   Introduction

Infections with helminth parasites of livestock are among the most common and economically important causes of disease in grazing livestock and they have a serious and detrimental impact on the livelihoods of small-scale farmers in the developing world (Perry and Randolph, 1999). Synthetic anthelmintics are the main way of controlling nematode parasites of livestock today, but these drugs may not be readily available or affordable to smallholder farmers, or to remote pastoralist communities in Africa. Anthelmintic resistance has also emerged as a serious concern worldwide (Reynecke et al., 2009). The use of plants as medicines provides a low-cost alternative but scientific validation of the anti-parasitic effects and investigation of possible side-effects of plant products is necessary prior to their adoption as novel methods for parasite control (Githiori et al., 2006).

Traditional South African medicine makes use of a wide variety of plants to treat gastrointestinal disorders such as diarrhoea and intestinal parasites and these practices are particularly prevalent among small-scale farmers in rural areas of the country (Luseba and Van der Merwe, 2006). The aim of the current study was to test *Cereus jamacaru* DC (Cactaceae), commonly known in South Africa as "Queen of the night" cactus, as a possible control measure against worm infection in livestock. Unusually, this investigation stemmed from the claims of a commercial farmer, who suspected an anthelmintic effect in *C. jamacaru* when he saw kudu (*Tragelaphus strepsiceros*) grazing the plant on his farm in Limpopo Province, South Africa (Bosch, 2007). The farmer then started feeding the plant to his cattle and sheep as an anthelmintic, apparently with positive results.

Cereus jamacaru is a tree-like cactus that originated from Brazil and has become a serious invasive species in parts of South Africa. It grows up to 18 m tall, and has segmented stems with a main trunk that may be over 60 cm thick. Reports from Brazil indicate that the plant is used in ethnobotanical practices (Agra et al., 2007; Albuquerque et al., 2007; Araújo et al., 2008). In Egypt, recent studies on native and cultivated plants have shown that *C. jamacaru* possesses reproducible *in vitro* antischistosomal activity (Yousif et al., 2007). *Cereus jamacaru* also has antimicrobial activity (Davet et al., 2009). No studies have examined the anthelmintic efficacy of the plant in livestock, thus the main objective of this study was to assess this efficacy in sheep experimentally infected with *Haemonchus contortus* and *Trichostrongylus colubriformis*.

2. Materials and methods

2.1 Maintenance of experimental animals

The study was conducted with the permission of the Onderstepoort Veterinary Institute (OVI) Animal Ethics Committee (Project: C-Kandu-UP) and the University of Pretoria Animal Research Committee (Protocol V054/08) and in line with the guidelines of these committees. The study itself was conducted at OVI. Eighteen adult male castrated sheep were used and the animals were individually ear-tagged. The sheep were housed in a common pen with concrete floors which were swept clean of faeces on a daily basis to prevent any accidental nematode infection. A commercial pelleted feed, lucerne hay and free access to water were provided. All the sheep were observed for any signs of ill health at least twice daily, in the morning and the

afternoon. During this adaptation period, one animal developed urolithiasis and was managed surgically through partial penile amputation. At that stage, the animal was maintained in a separate pen but was fed the same diet as the main group of sheep.

On arrival at OVI approximately 12 months before the start of the present study, the animals were dewormed twice with 200 µg/kg moxidectin (Cydectin Injectable, Fort Dodge, South Africa) at an interval of 24 hours. The sheep were maintained in an insect-free facility on concrete floors that were swept clean daily, until the animals were transferred to the facilities for the present experiment.

The faecal nematode egg counts (FECs) of the animals were checked using a modified McMaster method (Van Schalkwyk et al., 1995) eight weeks before the first experimental treatment. One sheep had an FEC of 33 eggs per gram of faeces (epg). Three other sheep were positive on a sensitive egg-flotation technique (Reinecke, 1983). Given that the infections in these four animals were very low, no further anthelmintic treatments were administered. All the animals were then infected six weeks before the start of the experiment with 4000 third-stage larvae (L_3) each of an anthelmintic-susceptible population of *H. contortus* and 6000 L_3 each of an anthelmintic-susceptible population of *T. colubriformis,* administered in three divided doses over a period of three days as low-level, trickle dosing has been shown to be the optimal method for achieving establishment of parasites (Barger et al., 1985; Dobson et al., 1990). The suspension of L_3 of *H. contortus* or *T. colubriformis* was well stirred by swirling the container with the larvae and the required dose was then drawn up in a syringe and deposited at the back of the animal's tongue (Vatta et al., 2009). An amount of water equivalent to the amount of larval suspension was drawn up in the same syringe and administered immediately thereafter. During

the five weeks immediately preceding the start of the experiment, the FECs of the animals were monitored twice a week and the infections were fully patent during the week preceding the start of the experiment (week -1).

2.2   Experimental design

The animals were grouped into control, single dose and double dose groups. The groups were balanced for FECs and live weights based on these values for day -7. This was done as follows. Sheep with similar FECs and live weights were grouped into clusters of three. Thereafter, the three sheep in each cluster were randomly allocated to an experimental group.

The animals were treated orally with a single dose (32.3 g) or double dose (64.6 g), respectively of the test substance, or were not treated. These dosages were extrapolated from the dosage which had been claimed to be effective by the farmer (see Plant preparation). The first treatment was given on day 0 in week 1 and repeated on days 7, 14, 21, 28 and 35 after the first treatment. Animals in the control group received a volume of water equivalent to a single dose of the test substance. Observations of the sheep for any adverse reactions to the cactus were made at hourly intervals for four hours after each administration.

2.3   Plant preparation

Voucher specimens of *C. jamacaru* were identified and deposited in the H.G.W.J. Schweickerdt Herbarium (PRU number 096502) at the University of Pretoria. On the day before administration of the plant material, fresh material was harvested from the farm of Mr. Bosch, the commercial farmer that claimed efficacy of the plant. Thorns were removed from the plant material and the material was kept at room temperature.

Mr. Bosch used 1 m of cactus per 100 sheep. He would remove the thorns and shred the pulp with a penknife into a trough for the sheep to consume. By extrapolation, therefore, one dose corresponded to 1 cm of the plant material; a double dose corresponded to 2 cm. For the first administration, a 25-cm piece was weighed after the centre core had been removed and this piece weighed 808.2 g. A single dose therefore weighed 32.3 g (808.2 g / 25 = 32.3 g) and a double dose 64.6 g. The 25-cm piece was blended in a household kitchen blender and water was added to aid in the blending process. The final volume of cactus and water mixture was divided by 25 to give the volume of one dose. A double dose corresponded to twice this volume. The appropriate dose of blended cactus was drawn up and administered to the animals using a 50 ml syringe. The pasty material was carefully deposited at the back of the tongue of the sheep. After administering the dose, a volume of 50 ml of water was administered to ensure swallowing of the full dose. For all subsequent administrations of the cactus, an initial amount of 808.2 g of material was used and the procedure described here was repeated to prepare the individual doses.

2.4   Measurements

Faecal samples (10-15 g) were collected directly from the rectum of each animal twice a week, on the day of treatment and three days later for seven weeks. The samples were subjected to FEC using a modified McMaster technique that employed the use of Visser Filters (Van Schalkwyk et al., 1995). Briefly 4 g of faeces were washed through a set of three tube sieves (Instavet[®], South Africa) which fitted into one another with pore sizes of 110 μm (inner sieve), 70 μm (middle sieve) and 25 μm (outer sieve), respectively. The faecal material was placed in the inner sieve and washed through the three sieves with water under moderate pressure using a garden hose. Nematode eggs are retained in the outer sieve. After washing, the retained

suspension from the outer sieve was drained into a calibrated container. Four teaspoons of granulated sugar were added to the egg suspension and the filtrate made up to 60ml with water. Nematode eggs were counted in three chambers of a McMaster slide. This system has the ability to detect nematode eggs in a sample to a sensitivity of 33 epg. Faeces remaining after processing for FEC were pooled (days 0 and 7) or pooled per group (days 14, 21, 28, 35, 42 and 49) and cultured for identification of L_3 (Van Wyk et al., 2004).

The packed cell volume (PCV; by the microhaematocrit method - Hansen and Perry, 1994), live weight (Ruddweigh 500 Portable Weighscale, Ruddweigh International Scale Co, Australia) and body condition score (on a scale of 1, thin, to 5, fat; Russell, 1984) of each animal were recorded on the day of treatment.

2.5   *Statistical analysis*

Statistical analyses were performed using GenStat[®] (Payne et al., 2009a). Linear mixed model analysis, also known as restricted maximum likelihood (REML) analysis (Payne et al., 2009b), was applied to the FECs, PCVs, live weights and body condition scores to model the correlation over the 7 weeks of the experiment. The fixed effects were specified as week, group and the week x group interaction. The random effects were specified as sheep and the sheep x group interaction. An autoregressive model of order 1 (AR1) and modelling for unequal variances was found to best model the correlation over weeks. Values for day -7 were included as covariates and, where significant ($P < 0.05$), the modelling proceeded using a covariance structure. Where applicable, the adjusted means and standard errors of the means are presented. FEC were $\log_{10}$ transformed to stabilize the group variances prior to statistical analysis. The

adjusted means and standard errors for the untransformed FEC data are presented, with statistical inferences based upon the transformed data.

The percentage faecal egg count reduction (FECR) was calculated using the arithmetic means and the formula of Coles et al. (1992):

$$FECR = [1-\{T_2/C_2\}] \times 100$$

where C_2 is the mean post-treatment FEC for the control sheep and T_2 is the mean post-treatment FEC for the sheep treated either with a double or a single dose of the plant material.

For the sake of comparison, the formula of Dash et al. (1988) using the arithmetic means was also used to calculate the FECR:

$$FECR = [1-\{T_2/T_1 \times C_1/C_2\}] \times 100$$

where C_1 and C_2 are the mean pre- and post-treatment FECs for the control sheep and T_1 and T_2 are the mean pre- and post-treatment FECs for the sheep treated either with a double or a single dose of the plant material. Since six doses of the plant material were administered, the pre-treatment FEC was considered to be the FEC for the day of treatment in the week preceding the post-treatment FEC. For example, when days 24 and 28 were considered post-treatment FECs, the pre-treatment FEC was day 21.

In this study, an *a priori* cut-off value of 70% reduction in FEC was considered to be a meaningful reduction in FEC.

3.   Results

All animals remained in good health throughout the course of the experiment, with no adverse events occurring during the study. The artificial infections of the sheep resulted in relatively high FECs in the sheep at the time of first treatment (5007 ± 670 epg). The results of larval cultures (Table 1) indicated that *H. contortus* was more predominant than *T. colubriformis* in the group receiving a single dose than in the other groups.

Inclusion of the values for day -7 as covariates was significant for FEC, PCV and live weight ($P < 0.05$). For the FEC data, the group main effect was not significant ($P = 0.368$), providing evidence that the mean FECs between the groups did not differ (Fig. 1). The mean FECs of the groups declined over the period of the experiment and the week main effect on REML analysis was significant ($P < 0.001$), but the week by group interaction was not significant ($P = 0.740$).

The maximum percentages reduction in FEC calculated according to Coles et al. (1992) were 41% at 17 days post-treatment and 65% at 49 days post-treatment in the sheep treated with a single and double dose, respectively (Table 2). When the formula of Dash et al. (1988) was applied, the maximum percentages reduction for the sheep receiving a single dose were 54% for day 45. For the sheep receiving a double dose, the maximum percentage reduction of 41% was noted on day 38. For both methods of calculation, a pattern was noted that the greater percentages reduction were seen for the double-dosed sheep in the last two weeks of the experiment (days 38 to 49).

The overall average PCV was 37.0 ± 0.3 % and the PCVs declined slightly over the course of the experiment (from 38.2 ± 1.1 % to 36.4 ± 1.1 %). The group main effect was not significant (P = 0.422; Fig. 2), but the week main effect was significant (P = 0.001). However, the interaction of week x group was not significant (P = 0.728).

The mean live weight of the sheep was 61.6 ± 0.4 kg on day 0 (Fig. 4). The live weights increased to 65.9 ± 0.4 kg. The week main effect was, however, highly significant (P < 0.001), but the group main effect was not significant (P = 0.497). The week x group interaction was not significant (P = 0.261).

The sheep had an overall mean body condition score of 3.9 ± 0.02 (Fig. 4). The body condition scores of the sheep were lower on day 0 (mean: 3.5 ± 0.1) relative to days 7 to 49 (means: 3.9 ± 0.1 to 4.0 ± 0.1) of the experiment. The week main effect was highly significant (P < 0.001), but the group main effect was not significant (P = 0.974). The interaction of week x group was also not significant (P = 0.762).

4.   Discussion

This study was stimulated by a report of a commercial farmer that *C. jamacaru* was effective as an anthelmintic in his livestock and is apparently the first investigation into the potential anthelmintic efficacy of *C. jamacaru* in livestock. Our results showed no significant differences in FECs between the groups.

While the formula of Coles et al. (1992) is the benchmark for treatment efficacy, in the present study there was large variation in FECs within and between the groups and, as such, the

method of Dash et al. (1988) was also used to determine the percentage reduction in faecal egg count following treatment. This formula seeks to control for some of this variation in FEC, and specifically for changes in FEC in both the treated and untreated animals between the time of treatment and re-sampling, by introducing the pre-treatment FECs into the equation. The use of the two formulae resulted in some variation in results. There appeared to be greater agreement in the results for the double-dosed sheep in the last two weeks of the experiment, which suggests that the plant may have some anthelmintic effect when fed for a longer period of time than was done in the present experiment.

Other considerations are that the dosages used in the present experiment may not have been high enough to have a significant anthelmintic effect on FEC, or the plant may have a better anthelmintic effect against certain parasites than others. In the group given the single dose, *H. contortus* predominated in the faecal cultures while in the group given a double dose, *H. contortus* and *T. colubriformis* occurred in approximately equal numbers in the cultures. In the latter group, a greater, though non-significant efficacy on FEC was seen, suggesting perhaps that there may be greater efficacy against *T. colubriformis*.

The packed cell volumes of the sheep declined marginally over the course of the study, a probable result of the helminth infection, particularly *H. contortus* infection. Because the experimental animals were all adult sheep, the increase in live weights over the experimental period was probably a result of fat deposition. Although the body condition scores increased between day 0 and day 7, they remained more or less constant from days 7 to 49. As such, the increase in live weights over the 7 weeks of the experiment was not reflected in an increase in the body condition scores. However, Thompson and Meyer (1994) state that for a change in one unit of condition, an increase in approximately 13 % of live weight of a ewe at a moderate body

condition (3.0-3.5) is required. Mean increases in live weight in the present study were less than 7 %.

Githiori et al. (2006) reviewed the use of plants for the control of gastrointestinal helminths in livestock and listed 32 plants that have been evaluated *in vivo* for anthelmintic efficacy against gastro-intestinal nematodes in ruminants, 13 of these specifically against *H. contortus*. They stated, however, that in the majority of cases the anthelmintic activity of such plants is lower than that reported for chemical anthelmintics. Much research has been directed towards plants containing condensed tannins, which have been shown to have anthelmintic properties (Ketzis et al., 2006). In the USA, the forage legume, sericea lespedeza [*Lespedeza cuneata* (Dum-Cours.) G. Don], has been shown to have anthelmintic properties against *H. contortus* and these properties have been exploited in different forms, including fresh plant material, hay, pellets and leaf meal (Joshi et al., 2010; Terrill et al., 2007).

Cereus jamacaru has been reported to be used for medicinal practices in north-eastern Brazil. However, any activity that the plant demonstrated may be due to other compounds, perhaps alkaloids or steroids. Araújo et al. (2008) listed *C. jamacaru* as a plant used for wound-healing and/or as an anti-inflammatory agent. Their study was targeted at quantifying tannins and flavonoids in medicinal plants from central Pernambuco State, as these phenolic compounds are known to have anti-inflammatory, anti-fungal, antioxidant and healing properties. However, these authors found that the *C. jamacaru* contained low tannin concentrations.

Agra et al. (2007) reported that in the neighbouring State of Paraíba, the roots of the cactus were used against respiratory and renal diseases and as a diuretic, while the stem pulp was used against gastric ulcers. Albuquerque et al. (2007) listed the cactus as being sold in the oldest

public market in Recife in the Pernambuco State, and the plant was reportedly also used there to treat kidney ailments.

Araújo et al. (2008) discuss that while *C. jamacaru* makes up part of traditional preparations involving many plants, the species may not be the principal element responsible for the attributed medicinal activity. Rather, its importance may be linked to some other biological activity that alters the effects of the other plants in the preparation. The testing of *C. jamacaru* for anthelmintic and other pharmacological activity when combined with other plants with which it is commonly used, also warrants further investigation.

In the study by Yousif et al. (2007), 281 plants were screened for activity against *Schistosoma mansoni* and 72 of these, including *C. jamacaru*, were active. Methanol extracts were used in their study which suggests that investigations into the anthelmintic efficacy of extracts of *C. jamacaru* may also be of benefit.

5. Conclusions

Based on this *in vivo* experiment, *C. jamacaru* was not effective in reducing *H. contortus* and *T. colubriformis* FECs in sheep by 70%, a level considered a *priori* to be a useful level of faecal egg count reduction. Nevertheless, the plant's *in vivo* activity was better when administered as a double dose than as a single dose and an 18-65% non-significant reduction in FECs was noted using the formula of Coles et al. (1992). Because no signs of toxicity were observed, further experiments using higher doses and administering the plant material for a longer period of time than in the present study, or administering extracts of the plant, would be warranted.

Acknowledgements

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Table 1

Faecal larval culture percentages of *Haemonchus contortus* (*H. c.*) and *Trichostrongylus colubriformis* (*T. c.*) for the untreated control sheep and the sheep treated with a single or double dose of *C. jamacaru* on days 0, 7, 14, 21, 28 and 35.

	Control		Single dose		Double dose	
	H. c. (%)	*T. c.* (%)	*H. c.* (%)	*T. c.* (%)	*H. c.* (%)	*T. c.* (%)
Day post initial treatment						
0 [a]	80	20	80	20	80	20
7 [a]	72	28	72	28	72	28
14	67	33	68	32	57	43
21	64	36	85	15	55	45
28	34	66	76	24	21	79
35	53	47	84	16	45	55
42	51	49	90	10	72	28
49	46	54	70	30	60	40
Average [b]	53	47	79	21	52	48

[a] Cultures for day 0 and day 7 post initial treatment were made for faeces pooled for all three groups, not for each individual group. The values for each group reflect the results for the composite culture.

[b] Average values are calculated using the individual group culture results for days 14 to 49 post initial treatment.

Table 2

Arithmetic mean faecal egg counts (FECs) in eggs per gram of faeces (epg) for three groups of sheep ($n = 6$ per group) treated with a single or double dose of *Cereus jamacaru* on days 0, 7, 14, 21, 28 and 35 or not treated (control) and the percentage faecal egg count reduction (FECR) by day post initial treatment (day).

	Control	Single dose			Double dose		
	FEC (epg)	FEC (epg)	FECR (%)		FEC (epg)	FECR (%)	
			C [b]	D [b]		C [b]	D [b]
Day [a]							
0	6028	5139	-	-	3856	-	-
3	3939	2367	40	30	2678	32	-6
7	4853	3440	29	17	2833	42	9
10	3600	2256	37	12	2294	36	-9
14	5194	5767	-11	-57	3044	41	0
17	5955	3510	41	47	3272	45	6
21	3556	4517	-27	-14	2472	30	-19
24	4300	4094	5	25	2667	38	11
28	4367	5383	-23	3	3061	30	-1
31	5611	5578	1	19	4617	18	-17
35	3433	4228	-23	0	2639	23	-10
38	3550	4489	-26	-3	1622	54	41
42	2478	4606	-86	-51	1378	44	28
45	3183	2700	15	54	1406	56	21
49	2889	2800	3	48	1000	65	38

[a] Day post initial treatment.

[b] Coles et al. (1992): FECR (%) = $[1-\{T_2/C_2\}] \times 100$, where T, C and 2 refer to treated, control and post-treatment mean FECs, respectively.

[c] Dash et al. (1988): FECR = $[1-\{T_2/T_1 \times C_1/C_2\}] \times 100$, where T, C, 1 and 2 refer to treated, control and pre- and post-treatment mean FECs, respectively. Pre-treatment FEC is the FEC for the day of treatment in the week preceding the post-treatment FEC.

Fig. 1. Faecal egg counts in eggs per gram of faeces for the untreated control sheep (o) and the sheep treated with a single (▲) or double (■) dose of *Cereus jamacaru*. Adjusted means ± standard errors of the means for untransformed data are presented in the figure, but statistical inferences in the text are based upon $\log_{10}$-transformed data.
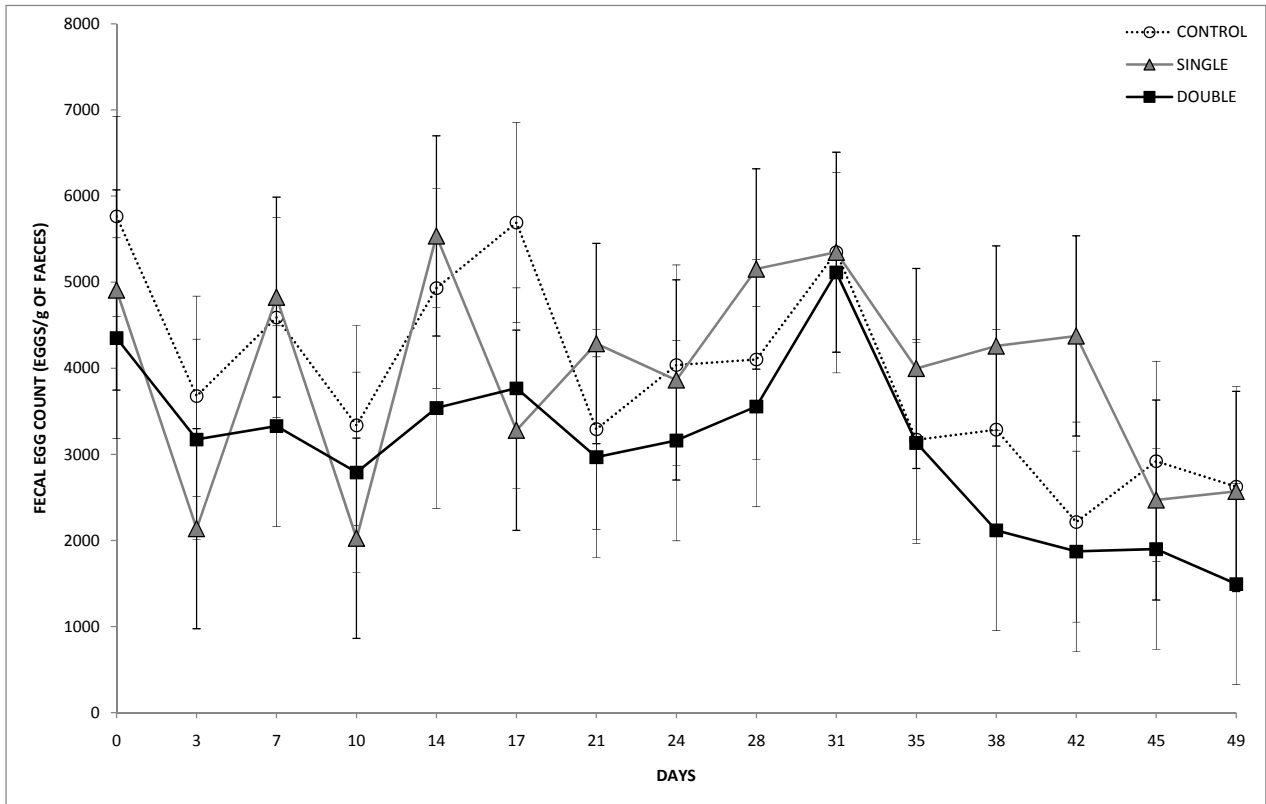
Fig. 2. Packed cell volumes as percentages for the untreated control sheep (o) and the sheep treated with a single (▲) or double (■) dose of *C. jamacaru.* Adjusted means ± standard errors of the means are presented.
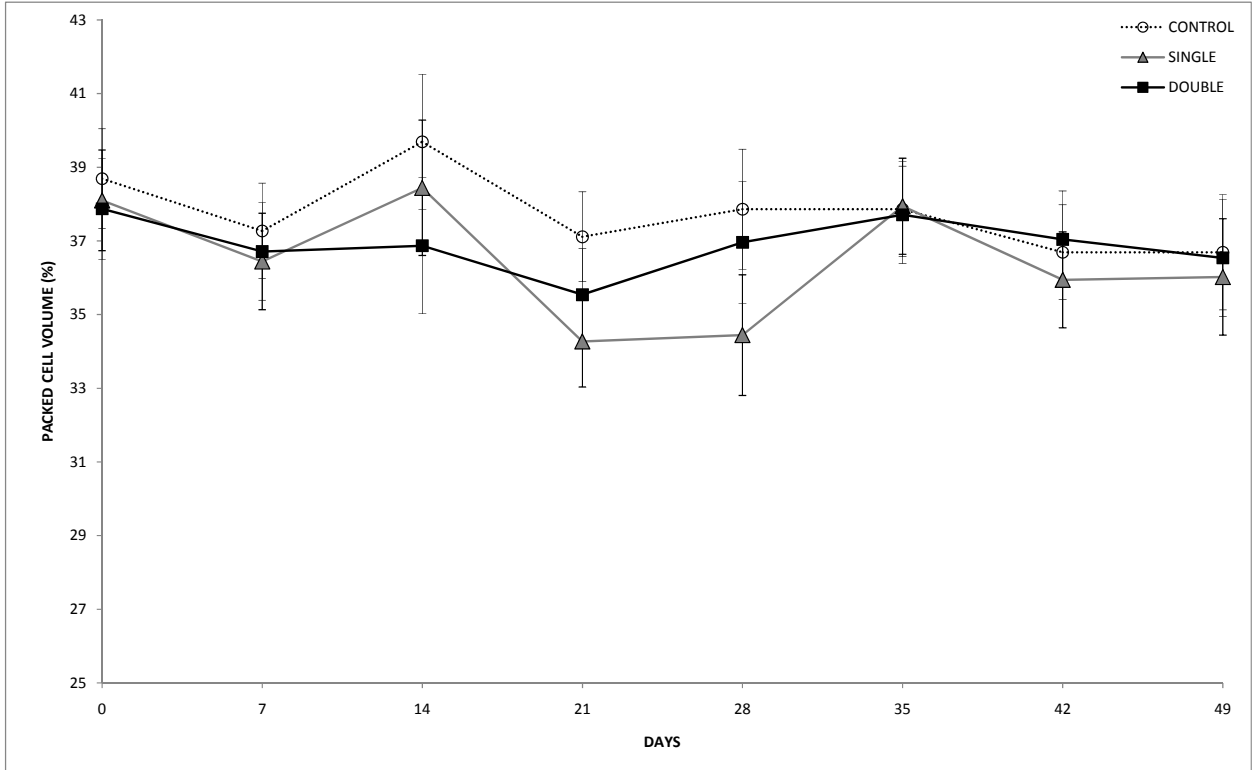
Fig. 3. Live weights in kilograms for the untreated control sheep (♦) and the sheep treated with a single (▲) or double (■) dose of *C. jamacaru.* Adjusted means ± standard errors of the means are presented.
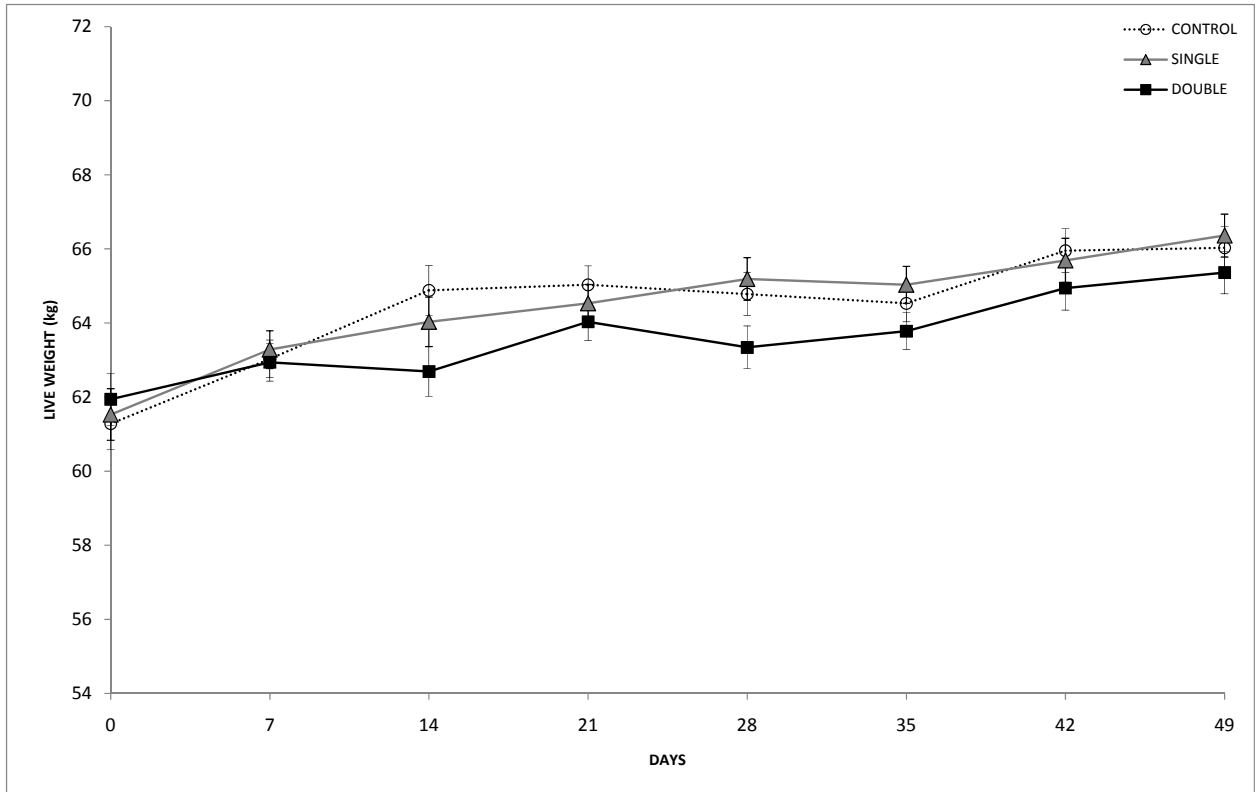
Fig. 4. Body condition scores for the untreated control sheep (♦) and the sheep treated with a single (▲) or double (■) dose of *C. jamacaru.* Means ± standard errors of the means are presented.