Title: Effects of anthelmintic treatment and feed supplementation on parasite infections and morbidity parameters in Cambodian cattle

Authors: Veronique Dermauw, Sothy Meas, Bunthon Chea, Thierry Onkelinx, San Sorn, Davun Holl, Johannes Charlier, Jozef Vercruysse, Pierre Dorny

PII: S0304-4017(17)30028-6
DOI: http://dx.doi.org/doi:10.1016/j.vetpar.2017.01.018
Reference: VETPAR 8239

To appear in: *Veterinary Parasitology*

Received date: 27-10-2016
Revised date: 12-1-2017
Accepted date: 20-1-2017

Please cite this article as: Dermauw, Veronique, Meas, Sothy, Chea, Bunthon, Onkelinx, Thierry, Sorn, San, Holl, Davun, Charlier, Johannes, Vercruysse, Jozef, Dorny, Pierre, Effects of anthelmintic treatment and feed supplementation on parasite infections and morbidity parameters in Cambodian cattle.Veterinary Parasitology http://dx.doi.org/10.1016/j.vetpar.2017.01.018

Effects of anthelmintic treatment and feed supplementation on parasite infections and morbidity parameters in Cambodian cattle

Veronique Dermauw[a,1], Sothy Meas[b], Bunthon Chea[b], Thierry Onkelinx[c], San Sorn[d], Davun Holl[d], Johannes Charlier[e,*], Jozef Vercruysse[e], Pierre Dorny[a,e]

[a]Department of Biomedical Sciences, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen, Belgium, vdermauw@itg.be, pdorny@itg.be,

[b]Division of Research and Extension, Royal University of Agriculture, Phnom Penh, Cambodia, sothymeaskh@gmail.com, bunthonchea@yahoo.com

[c]Research Institute for Nature and Forest (INBO), Kliniekstraat 25, B-1070 Anderlecht, Belgium, thierry.onkelinx@inbo.be

[d]Department of Animal Production and Health, Ministry of Agriculture, Forestry and Fisheries, Phnom Penh, Cambodia, sorn.san@gmail.com, davun2016@gmail.com,

[e]Department of Virology, Parasitology and Immunology, Salisburylaan 133, B-9820 Merelbeke, Belgium, Jozef.Vercruysse@ugent.be

*Current address: Avia-GIS, Risschotlei 33, 2980 Zoersel, Belgium, jcharlier@avia-gis.com

[1]Corresponding author, email: vdermauw@itg.be, telephone: +32(0)32476447

Highlights

- The Cambodian humid tropical climate is conducive for helminth infections in cattle
- Helminthiasis causes high morbidity especially in cattle under nutritional stress
- Village cattle may benefit from anthelmintic treatment and/or protein supplementation
- A short term effect of anthelmintic treatment on parasitological parameters was observed
- The added value of protein supplementation was unclear from the current study

Abstract

Helminth infections are the cause of morbidity in Cambodian cattle but other factors such as nutritional deficiencies and concurrent diseases may enhance the effects of parasites. The present study aimed to investigate the impact of anthelmintic treatment, feed supplementation, or both on gastrointestinal strongyle (GIS) and trematode infections as well as on morbidity parameters in Cambodian village cattle. At the beginning of the dry season, cattle populations in six villages were randomly assigned to a group: (A) receiving anthelmintic treatment (ivermectin + clorsulon) at week 0; (P) feed pellet supplementation during week 0 to 13 or both (AP). On five visits (week 0 to 29), faecal and blood samples were obtained for parasitological examination and haematocrit determination, respectively. Body condition (BCS), hind quarter fouling (HQFS), diarrhoea (DS), and conjunctiva colour (FAMACHA$^{©}$) were scored and heart girth circumference was determined. To investigate the impact of treatment over time (week 0 to 29), a mixed model was used with treatment, time, and their interaction as fixed effects, and animal and village as random factors. At baseline, the proportion of GIS positive animals was high (67.9%), whereas trematode infections were low (*Paramphistomum*: 8.8%; *Fasciola*: 2.6%). Very thin to emaciated cattle (BCS 1-2) were more prevalent (11.4%) and FAMACHA$^{©}$ scores of ≤ 3 or below (65.8%) less prevalent than in an earlier study in the region. A Time × Treatment interaction was present for faecal egg counts (FEC) of GIS, GIS prevalence (both *p*

<0.0001), PCV ($p = 0.0034$), DS ($p = 0.0086$) and HQFS ($p = 0.0241$). For GIS FEC, treatment groups differed at a specific time point, with levels of treatment group P being higher than in A at week 6 ($p = 0.0054$). For *Paramphistomum* prevalence as well as FAMACHA© scoring, heart girth and BCS, the interaction between treatment and time was not significant, yet, time in itself had a significant impact on all ($p < 0.0001$). The beneficial effects of protein supplementation were unclear from the current study.

Keywords: cattle, Cambodia, gastrointestinal nematodes, *Fasciola*, nutrition, anthelmintic treatment, morbidity

1. Introduction

Infections with gastrointestinal nematodes and trematodes are detrimental for cattle health and production (Loyacano et al., 2002; Vercruysse and Claerebout, 2001). Infected animals most commonly suffer from anorexia (Coop and Holmes, 1996), concomitant ill-thrift (Loyacano et al., 2002; Vercruysse and Claerebout, 2001) and diarrhoea (Jones et al., 2009), whilst others also suffer from anaemia (Díaz et al., 2006; Lotfollahzadeh et al., 2002; Van Aken et al., 1997), These symptoms obviously affect farmers' profits and have an economic impact (Magaya et al., 2000). Therefore, the early yet cost-effective treatment and thus identification of animals potentially benefitting from this treatment is paramount. In most cases, traditional parasitological analyses are used as decision-tools for treatment. However, some studies have advocated the use of morbidity parameters related to these infections (*e.g.*, body condition and faecal consistency scoring) as quick and easy methods to detect infected animals (Vercruysse and Claerebout, 2001).

In Cambodia, as in many tropical countries, cattle production is low due to poor nutrition and management as well as the presence of endemic diseases. Important diseases for cattle in the country are foot-and-mouth-disease, haemorrhagic septicaemia and blackleg (*Clostridium chauvoei*) in addition to both external and internal parasites (Young et al., 2014). Climatic conditions favour year-round development and transmission of infective parasite stages (Tum et al., 2007). Not surprisingly, infections with gastrointestinal nematodes and trematodes are thus highly prevalent in the country (Dorny et al., 2015, 2011; Sothoeun et al., 2006). An abattoir study in Kandal province revealed 35.9% to 42.9% of livers being damaged because of immature liver flukes during the dry season (Sothoeun et al., 2006), whereas Tum et al. (2007) found *Fasciola* infections to range between 0 and 85% in 11 provinces in the country. In a large scale study on bovine helminth infections in the Pursat and Kampong Speu provinces, Dorny et al. (2011) reported 37-52% of sampled cattle being positive for gastrointestinal nematodes, most commonly for *Cooperia* spp. Infections with *Fasciola* and *Paramphistomum* were detected in 5-20% and 45-95% of cattle, respectively (Dorny et al., 2011). In the same study, a lower BCS was found to be associated with the presence of gastrointestinal nematodes and *Fasciola* whereas a soft faecal consistency was associated with *Paramphistomum* infections (Dorny et al., 2011).

Next to helminth infections, nutritional stress and extensive farm practices also affect the general condition of animals and morbidity parameters. In a survey conducted in several agro-ecological zones in Cambodia, lack of good quality feed resources was mentioned as one of the major constraints among cattle owners (Samkol et al., 2015). Banana stem, rice straw and bran were commonly used as supplementary feeds but contained low levels of protein (5.6%, 8.3% and 4.0%, respectively) (Samkol et al., 2015). Nematode infected ruminants have higher protein requirements, caused by anorexia, the predominant effect of helminth infections (Coop and

Holmes, 1996) and by increased endogenous losses in the gastrointestinal tract. In sheep protein supplementation does not influence the establishment rate of nematode infections, however, it enhances immunity acquisition, and reduces the pathophysiological consequences of the infection (Coop and Holmes, 1996).

Few studies have investigated the potential benefits of protein supplementation and anthelmintic treatments in cattle in a tropical environment. In a study in Zimbabwe, protein supplementation and anthelmintic treatment in cattle resulted in higher weight gains than in cattle receiving an anthelmintic treatment only. Between those groups, no significant differences could be observed in faecal worm egg counts and haematocrit (Magaya et al., 2000).

The aim of this study was to compare the long-term effects of anthelmintic treatment and protein supplementation on infection levels of gastrointestinal nematodes and trematodes as well as on morbidity parameters under practical circumstances: namely in traditionally reared cattle in six villages in Cambodia.

2. Materials and Methods

2.1.Study setting & area

In South-West Cambodia, six villages in two provinces (Pursat and Kampong Chhnang) were enrolled in a longitudinal study. The selected villages had similar mixed crop-animal farming and management systems, representative for the region, as described by Dorny et al. (2011). , During the rainy season (May until October), cattle are mainly tethered and fed using cut-and-carry methods. Conversely, in the dry season (November until April), communal grazing is mainly practised during the day (Dorny et al., 2011), while overnight, cattle are gathered and receive supplemental feeds, mainly rice straw. As animals were observed to suffer most from nutritional stress at the end of the dry season (Dorny et al. 2011; Sothoeun et al., 2006), the current study opted to cover the entire dry period: it started in November 2010 (start dry season) and lasted until June 2011 (early rainy season).

2.2.Village selection & treatment allocation

Within each province, cattle populations were randomly assigned to one of the following treatments per village: i) individual dietary protein supplementation (P) according to the manufacturers’ instructions with commercially available feed pellets (21% crude protein) (CP Cambodia Co, LTD, Kandal Province, Cambodia) during 13 weeks subsequent to trial initiation (week (wk) 0 to 13); ii) an injection with the anthelmintic (A) Ivomec F ® (ivermectin at 200 µg/kg BW and clorsulon at 2 mg/kg BW, Merial Animal Health, Brussels, Belgium), at the start of the experiment (wk 0); or iii) both (AP). All animals of six months or older received the same treatment in each village. The project and treatment procedures were introduced by means of information sessions in the villages and veterinary assistants regularly visited all villages.

2.3.Sampling & scoring

Each village was visited five times, at six to nine weeks intervals (in wk 0, 6-7, 13, 21-22 and 28-29). At each visit, all available cattle of at least six months old were sampled for blood and faeces. Faecal samples were rectally obtained and kept cool until analysis within 48h after collection, whereas blood was obtained from an auricular capillary into micro-haematocrit tubes. Additionally, a body condition score (BCS) was given using a one-to-five scale (Wildman et al., 1982), describing an emaciated (1) to a very good condition (5) (Wildman et al., 1982). Scores were also given for the presence of diarrhoea: diarrhoea score (DS), using a one-to-three scale (normal (1) to watery (3) faeces), and hind quarter fouling score (HQFS), using a one-to-three scale (clean (1) to dirty (3) hindquarters). The FAMACHA© eye colour chart was used to describe conjunctiva colour on a one-to-five scale, from red (1) to white (5) (Malan et al., 2001). The hearth girth circumference of the animals was also measured, as a proxy for body weight (Lesosky et al., 2012).

2.4.Sample analysis

The haematocrit was determined in blood samples by means of the micro-haematocrit method and expressed in packed cell volume (PCV). The McMaster method was used on faecal samples to determine individual nematode eggs counts (faecal egg counts, FEC) with a sensitivity of 50 eggs per gram (EPG) of faeces (Thienpont et al., 1986). The presence of trematode eggs in faeces was assessed in animals above two years old using a qualitative sedimentation flotation (zinc chloride) method (Charlier et al., 2008).

2.5.Statistical analysis

Descriptive statistics were used to summarize the data. A mixed model with the same base structure was applied to each response variable was applied to examine the effect of treatment over time. The fixed part of the full model contained treatment (β_m), time (β_t) and their interaction (β_{mt}). We specified time as a factor, hence the model made no assumptions on the pattern over time. The random part of the full model contained two random intercepts: the animal effect (b_a) and the village effect (b_v). All random intercepts were assumed to be independent and identically distributed and follow a zero mean normal distribution with variances σ_a^2 and σ_v^2. The sum of the fixed and random parts yielded η_{mtva}: the expected value on the link scale for animal a in village v with treatment m at time t.

Continuous variables (PCV, heart girth circumference) followed Gaussian distribution with identity link. Count variables (GIS) followed a negative binomial distribution with log link, whereas binary variables (*Paramphistomum*, *Fasciola*) followed a binomial distribution with logit-link. For ordinal response variables (FAMACHA$^©$, HQFS, DS, BCS), cumulative link mixed models were used.

Based on the full model, we sequentially tested three hypotheses:.

1. $H_0: \beta mt = 0$: no evidence for a treatment-time interaction
2. $H_0: \beta m = 0$: no evidence for a treatment effect
3. $H_0: \beta t = 0$: no evidence for a time effect

The sequence was stopped in case the null hypothesis was rejected at the $p < 0.05$ level. All hypotheses were evaluated using a likelihood ratio test between nested models. In case a significant treatment-time interaction was found, a post-hoc pairwise comparison among treatments was done at each time point.

Due to a very low frequency of extreme values, some categorical variables were recoded for modelling purposes (i.e., the inclusion of all values would lead to an unstable model). FAMACHA$^©$ scoring was relabelled as follows: “Optimal to acceptable” (FAMACHA$^©$ = 1 or 2), “Borderline” (FAMACHA$^©$ = 3), and “Dangerous to fatal” (FAMACHA$^©$ = 4 or 5). Hind quarter fouling scores were relabelled as follows: “Clean” (HFQS = 1) and “Moderate to severe fouling” (HQFS = 2 or 3), and the diarrhoea scores as follows: “Normal faeces” (DS = 1), and “Loose to watery faeces” (DS = 2 or 3). Finally, the BCS was relabelled as follows: “Emaciated to poor condition” (BCS = 1 or 2), “Borderline to good condition” (BCS = 3 or 4) and “Very good condition” (BCS = 5). The prevalence of *Fasciola* was too low to be modelled in all groups and was thus excluded from further analysis.

All analyses were performed in R 3.2.5 (R Core Team, 2016) with the glmmADMB package (Fournier et al., 2012; Skaug et al., 2012) to fit all models except the cumulative link models fit with the ordinal package (Christensen, 2015). The datasets were prepared with the dplyr package (Wickham and Francois, 2016) and plotted with the ggplot2 package (Wickham, 2009).

3. Results

Characteristics of cattle enrolled in the trial as well as prevalences of gastrointestinal strongyles (GIS), *Paramphistomum* and *Fasciola* per treatment group at wk 0 (start dry season) and wk 29 (early rainy season) are presented in Table 1. The prevalence of GIS decreased over the experimental period in all treatment groups. The *Paramphistomum* prevalence increased between wk 0 and wk 29 in treatment groups A and AP, whereas such an increase seemed to be absent in the P group. The prevalence of *Fasciola* decreased or remained at a low level in all groups. An overall summary of FEC of GIS and morbidity parameters per treatment at wk 0 and wk 29 is given in Table 2. Average FECs decreased over the experimental period in all treatment groups. Levels of PCV remained fairly constant in all treatment groups, while the average FAMACHA$^{©}$ score decreased between the beginning and end of the experiment. Average HQFS and DS decreased, indicating the presence of drier faeces. The heart girth circumferences as well as BCS increased over the experimental period, indicating a weight gain.

Table 3 presents the significance of terms in the tested models. The most complex models were maintained for FEC of GIS, GIS prevalence, PCV, DS and HQFS. For these variables, a significant Time × Treatment interaction was thus observed. Levels of GIS FEC in treatment group P were higher than in other groups at wk 6 (*p*-value post-hoc treatment A vs. P = 0.0054; *p*-value post-hoc treatment AP vs. P = 0.0695) (Fig. 1), but decreased over the experimental period. For A and AP groups, GIS FEC levels decreased more sharply between wk 0 and wk 6, but started to increase again afterwards. The same holds for the GIS prevalence (both *p*-value Time × Treatment: <0.0001) (Fig. 2). Levels of PCV fluctuated throughout the experimental period, but did not significantly differ between treatments at a certain sampling point (Fig. 3) (*p*-value Time × Treatment: 0.0034). DS decreased over time in groups A and AP (Fig. 4), whereas DS in group P increased with a peak in wk 21 and then decreased thereafter (*p*-value Time × Treatment: 0.0086). HQFS sharply decreased in all groups, especially in groups A and AP, between wk 6 and 13. In the same groups, HQFS slowly increased again thereafter, whereas P group HQFS remained fairly constant (Fig. 5) (*p*-value Time × Treatment: 0.0241) (*p*-values all post-hoc for Time × Treatment: > 0.0500).

For *Paramphistomum* prevalence, FAMACHA© scoring, heart girth circumference and BCS, the model without inclusion of Time × Treatment, or Treatment terms was the best fit for the data available. Indeed, *Paramphistomum* infections remained fairly constant until they peaked in wk 21 (start of rainy season) and then decreased again in all groups (Fig. 6). For all groups, FAMACHA© scores (Fig. 7) quickly decreased from wk 0 to wk 6, to remain fairly constant thereafter. Heart girth circumferences (Fig. 8) increased throughout the study. Finally, BCS increased over time in all groups, with a clear peak in wk 13 (Fig. 9) (all *p*-value Time: <0.0001).

4. Discussion

4.1. Baseline infection and morbidity status

At the start of the study (wk 0, November), the proportion of animals positive for GIS was higher than in earlier work (GIS: 44% in young animals, 37% in adult cattle) in the same area at the same time point (Dorny et al., 2011). In contrast, the proportion of *Fasciola* positive animals was lower than in the studies of Dorny et al. (2011) (5-20%) and Sothoeun et al. (2006) (12%), but similar than reported by Tum et al. (2007) in the same provinces (Pursat: 6%; Kampong Chhnang: 3%). Levels of *Paramphistomum* infections were lower (45-95%) than in the study of Dorny et al. (2011). Seasonal variations affect the levels of *Fasciola* and *Paramphistomum* infections in cattle, with levels expected to increase during the dry season (Dorny et al., 2011; Tum et al., 2007). Overall, cattle seemed to experience a greater challenge by nematode than trematode infections in this study compared to earlier studies (Dorny et al., 2011; Sothoeun et al., 2006).

While average baseline BCSs were similar, the frequency of very thin to emaciated cattle (BCS 1-2) was higher than in a previous study (BCS 1 or 2: 7.6%) (Dorny et al., 2011). Such poor BCSs, which are expected to even deteriorate during the dry season (Dorny et al., 2011), leave little reserves to withstand harsh climatic conditions. On the contrary, baseline ranges of PCV were within normal levels reported for cattle (24-46%) (Aiello and Moses, 2016) and only two animals had PCV levels below 24%, indicating anaemia. It is unclear whether this threshold is also applicable for Cambodian cattle types, consisting mainly of local zebu and taurine crossbreeds (Caramelli, 2006). Nevertheless, anaemia levels according to this threshold (1%) were slightly lower compared to those in Cambodian cattle reported earlier (<3% cattle with PCV <24%) (Dorny et al., 2011) and much lower than those in a study in West-Africa (25% cattle with PCV <25%) (Grace et al., 2007) and Zambia (19% cattle with PCV <24%) (Marcotty et al., 2008). Obviously, haemoparasites play a role in the occurrence of low PCV levels. In two villages in Kampong Chhnang and Pursat, Alassane (2011) found very high levels of *Anaplasma* spp. infections in adult cattle (91.3-100.0%) as well as high levels of *Babesia bovis* (12.5-39.1%)

and *Theileria theileri* (8.7-18.8%); however, the clinical importance of these findings was not assessed. In the current study, we did not investigate the presence of haemoparasites.

As a fast and non-invasive haematocrit evaluation tool, the FAMACHA© eye colour chart was developed in order to facilitate treatment decisions in sheep infected with the haematophagous *Haemonchus contortus* (Malan et al., 2001). Its use in cattle to detected anaemia in relation to nematode infections has been limited to few studies. In West-Africa, FAMACHA© scores of ≥ 4 had a sensitivity of 92.4% and specificity of 30.3% to detect anaemia (Grace et al., 2007). In the current study, average baseline FAMACHA© scores were higher, and the frequency of FAMACHA© scores of 3 or below (optimal (1) to borderline (3) levels) was lower than in earlier work (FAMACHA© 2 or 3: 74.5%) in the area (Dorny et al., 2011). Thus, despite having similar average PCV levels, FAMACHA© scores were higher in the current study in comparison with earlier work (Dorny et al., 2011). The reasons for this disparity are unknown but could be due to misinterpretation of the FAMACHA© scores or to the incorrect exposure of the ocular mucosa (Maia et al., 2015). Average baseline DSs were similar, yet the prevalence of loose or watery faeces was lower than in the previous study (57.3%) (Dorny et al., 2011).

4.2.Impact of treatment

The lack of an untreated control group can be considered as a limitation of this study. The inclusion of such a control group was deemed unethical in the rural Cambodian circumstances. Furthermore, we cannot exclude the presence of other pathogens interfering with the treatment effects.

4.2.1. Parasitic infections

In the Zimbabwean study of Magaya et al. (2000), fenbendazole bolus-treated cattle had lower FECs than cattle without anthelmintic treatment at the end of the study (after 6 months), while no difference could be observed between bolus-treated cattle with or without protein supplementation. The same was true for the current study: at wk 6, cattle treated with anthelmintics (A) had lower FECs than those supplemented with concentrates (P); no differences could be observed between groups treated with anthelmintics with (AP) or without concentrate supplementation (P). Not surprisingly, the supplementation of protein supplements alone was not effective in decreasing FECs of GIS. However, at the end of the study, no differences in FECs could be observed between any of the treatments. The study of Magaya et al. (2000) was designed slightly different than the current study, namely, animals were treated twice with a slow release bolus, and were supplemented with cotton seed meal throughout the study. The effect of the treatment in the current study, *i.e.*, a single ivermectin + clorsulon injection at wk 0, supplementation with concentrates from wk 0 to wk 13, or both, was thus expected to be less dramatic in comparison to the Magaya et al. (2000) study.

Despite seemingly clear visual patterns over time and a significant Time × Treatment interaction, no significant differences between treatments could be observed at specific time points for GIS prevalence. Furthermore, no interaction between treatment and time could be observed for *Paramphistomum* infections, which is not unexpected because of the limited efficacy of clorsulon against these parasites (Courtney et al., 1985). The cause of the peak in *Paramphistomum* infections at the end of April (wk 21, towards the end of the dry season) and fall afterwards is unclear, as in previous work a more continuous increase towards the end of the dry season was observed (Dorny et al., 2011).

4.2.2. Morbidity parameters

Although significant Time × Treatment interactions for PCV, DS and HQFS were found, no significant differences between treatments could be observed at specific time points. For PCV, this is in contrast with the study of Magaya et al. (2000), where PCV levels were higher in the anthelmintic treatment + protein supplementation group versus protein supplementation and control groups. As mentioned before, the anthelmintic coverage in the study of Magaya et al (2000) was extended as compared to our study. Also, the parasite challenge and fauna in cattle in Cambodia and Zimbabwe is likely to be different, making comparisons speculative. In our study, an increase in the occurrence of loose or watery faeces was observed at wk 21 in group P, but not in groups A and AP, as measured by a DS of 2 or 3. Surprisingly, the occurrence of mild to severe HQFS did not follow the same pattern as the DS. This indicates that the use of HQFS as an alternative for DS should be done with care. FAMACHA© scores as well as BCS and heart girth circumferences did not seem to be affected by treatment.

Overall, the lack of treatment effect on the investigated morbidity parameters over the time period studied seems to indicate that there was no long-term added value of one treatment versus the others for the general condition of cattle. However, the morbidity parameters improved over the course of the dry season in all treatment groups, in contrast to earlier work (Dorny et al., 2011). This could be an indication of a beneficial effect of the interventions used versus no treatment at all, which should be further explored. As an alternative to supplementation with concentrates and/or providing a strategic anthelmintic treatment at the start of the dry season, an education and intervention programme, consisting of actions with regard to nutrition and parasitic infections could be proposed to Cambodian cattle farmers. Indeed, programmes including training of farmers on identifying parasitic diseases in cattle and on forage cultivation and forage plot development caused promising improvement in smallholder cattle productivity as well as on farmer knowledge (Nampanya et al., 2012; Young et al., 2014).

In conclusion, in the current study we aimed to investigate the impact of anthelmintic treatment and/or protein supplementation on nematode and trematode infections as well as on morbidity parameters in six villages in West-Cambodia. While treatment groups reacted differently over time for GIS counts and prevalence, PCV, DS and HQFS, few differences at specific time points could be identified. Results of this study indicate that no clear benefit could be expected from protein supplementation during thirteen weeks with, or without a single anthelmintic treatment versus the anthelmintic treatment alone on the long-term (29 wk) level of parasite infections and morbidity status. However, in contrast to previous work, BCS and heart girths did increase over the course of the dry season in all treatment groups, indicating that these treatments had a positive impact on the general condition of cattle in the region.

Acknowledgements

The present study was funded by the Flemish Interuniversity Council (VLIR), as a component of the Cambodian Parasitology project framework, implemented at the Royal University of Agriculture (RUA) and the National Veterinary Research Institute (NaVRI) in Phnom Penh. The authors are grateful for the kind cooperation of local cattle owners, and acknowledge the RUA and NaVRI technical staff and students for their much appreciated assistance during field work and laboratory analysis.

References

Aiello, S.E., Moses, M.A., 2016. The Merck Veterinary Manual. 11th Edition. Merck & Co., Inc., Kenilworth, N.J., U.S.A

Alassane, A., 2011. Détection des hémoparasites dans des échantillons de bovins provenant du Cambodge. MSc thesis. Institute of Tropical Medicine, Antwerp, Belgium, 28pp.

Caramelli, D., 2006. The origins of domesticated cattle. Hum. Evol. 21, 107–122.

Charlier, J., De Meulemeester, L., Claerebout, E., Williams, D., Vercruysse, J., 2008. Qualitative and quantitative evaluation of coprological and serological techniques for the diagnosis of fasciolosis in cattle. Vet. Parasitol. 153, 44–51.

Christensen, R., 2015. ordinal—Regression Models for Ordinal Data.R package version 2015.6-28. http://www.cran.r-project.org/package=ordinal/

Coop, R.L., Holmes, P.H., 1996. Nutrition and parasite interaction. Int. J. Parasitol. 26, 951–962.

Courtney, C., Shearer, J., Plue, R., 1985. Efficacy and safety of clorsulon used concurrently with ivermectin for control of *Fasciola hepatica* in Florida beef cattle. Am. J. Vet. Res. 46, 1245–1246.

Díaz, P., Lomba, C., Pedreira, J., Arias, M., Sánchez-Andrade, R., Suárez, J.L., Díez-Baños, P., Morrondo, P., Paz-Silva, A., 2006. Analysis of the IgG antibody response against Paramphistomidae trematoda in naturally infected cattle. Application to serological surveys. Vet. Parasitol. 140, 281–288.

Dorny, P., Devleesschauwer, B., Stoliaroff, V., Sothy, M., Chea, R., Chea, B., Sourloing, H., Samuth, S., Kong, S., Nguong, K., Sorn, S., Holl, D., Vercruysse, J., 2015. Prevalence and associated risk factors of *Toxocara vitulorum* infections in buffalo and cattle calves in three provinces of central Cambodia. Korean J. Parasitol. 53, 197–200.

Dorny, P., Stoliaroff, V., Charlier, J., Meas, S., Sorn, S., Chea, B., Holl, D., Van Aken, D., Vercruysse, J., 2011. Infections with gastrointestinal nematodes, *Fasciola* and *Paramphistomum* in cattle in Cambodia and their association with morbidity parameters. Vet. Parasitol. 175, 293–299.

Fournier, D.A., Skaug, H.J., Ancheta, J., Ianelli, J., Magnusson, A., Maunder, M.N., Nielsen, A., Sibert, J., 2012. AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. Optim. Methods Softw. 27, 233–249.

Grace, D., Himstedt, H., Sidibe, I., Randolph, T., Clausen, P.H., 2007. Comparing FAMACHA$^{©}$ eye color chart and Hemoglobin Color Scale tests for detecting anemia and improving treatment of bovine trypanosomosis in West Africa. Vet. Parasitol. 147, 26–39.

Jones, J., Mitchell, E., Redman, E., Gilleard, J., 2009. *Toxocara vitulorum* infection in a cattle herd in the UK. Vet. Rec. 164, 171–172.

Lesosky, M., Dumas, S., Conradie, I., Handel, I.G., Jennings, A., Thumbi, S., Toye, P., de Clare Bronsvoort, B.M., 2012. A live weight-heart girth relationship for accurate dosing of east African shorthorn zebu cattle. Trop. Anim. Health Prod. 45, 311-316.

Lotfollahzadeh, S., Mohri, M., Rangjbar Bahadori, S., Mokhber Dezfouly, M., Tajik, P., 2002. The relationship between normocytic, hypochromic anaemia and iron concentration together with hepatic enzyme activities in cattle infected with *Fasciola hepatica*. J. Helminthol. 82, 85–88.

Loyacano, A., Williams, J., Gurie, J., DeRosa, A., 2002. Effect of gastrointestinal nematode and liver fluke infections on weight gain and reproductive performance of beef heifers. Vet. Parasitol. 107, 227–234.

Magaya, A., Mukaratirwa, S., Willingham, A.L., Kyvsgaard, N., Thamsborg, S., 2000. Effects of anthelmintic treatment and feed supplementation on grazing Tuli weaner steers naturally infected with gastrointestinal nematodes. J. S. Afr. Vet. Assoc. 71, 31–37.

Maia, D., Rosalinski-Moraes, F., de Torres-Acosta, J.F., Cintra, M.C.R., Sotomaior, C.S., 2015. FAMACHA$^{©}$ system assessment by previously trained sheep and goat farmers in Brazil. Vet. Parasitol. 209, 202–209.

Malan, F.S., Van Wyk, J.A., Wessels, C.D., 2001. Clinical evaluation of anaemia in sheep: early trials. Onderstepoort J. Vet. Res. 68, 165–74.

Marcotty, T., Simukoko, H., Berkvens, D., Vercruysse, J., Praet, N., Van den Bossche, P., 2008. Evaluating the use of packed cell volume as an indicator of trypanosomal infections in cattle in eastern Zambia. Prev. Vet. Med. 87, 288–300.

Nampanya, S., Suon, S., Rast, L., Windsor, P.A., 2012. Improvement in smallholder farmer knowledge of cattle production, health and biosecurity in southern Cambodia between 2008 and 2010. Transbound. Emerg. Dis. 59, 117–127.

R Core Team, 2016. R: A language and environment for statistical computing. http://www.r-project.org/

Samkol, P., Sath, K., Patel, M., Windsor, P.A., Holtenius, K., 2015. Survey of smallholder beef cattle production systems in different agro-ecological zones of Cambodia. Trop. Anim. Health Prod. 1299–1306.

Skaug, H., Fournier, D., Nielsen, A., Magnusson, A., Bolker, B., 2012. glmmADMB - Generalized linear mixed models using AD model builder. R package version 0.7, 2012. http://glmmadmb.r-forge.r-project.org

Sothoeun, S., Davun, H., Copeman, B., 2006. Abattoir study on *Fasciola gigantica* in Cambodian cattle. Trop. Anim. Health Prod. 38, 113–115.

Thienpont, D., Rochette, F., Vanparijs, O., 1986. Diagnosing helminthiasis by coprological examination. Janssen Research Foundation, Beerse, Belgium.

Tum, S., Puotinen, M.L., Skerratt, L.F., Chan, B., Sothoeun, S., 2007. Validation of a geographic information system model for mapping the risk of fasciolosis in cattle and buffaloes in Cambodia. Vet. Parasitol. 143, 364–367.

Van Aken, D., Vercruysse, J., Dargantes, A.P., Lagapa, J.T., Raes, S., Shaw, D.J., 1997. Pathophysiological aspects of *Mecistocirrus digitatus* (Nematoda: Trichostrongylidae) infection in calves. Vet. Parasitol. 69, 255–263.

Vercruysse, J., Claerebout, E., 2001. Treatment vs non-treatment of helminth infections in cattle: Defining the threshold. Vet. Parasitol. 98, 195–214.

Wickham, H., 2009. ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York.

Wickham, H., Francois, R., 2016. Dplyr: A grammar of data manipulation. R package version 0.5.0. https://github.com/hadley/dplyr

Wildman, E., Jones, G., Wagner, P., Boman, R., 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65, 495–501.

Young, J.R., Rast, L., Suon, S., Bush, R.D., Henry, L.A., Windsor, P.A., 2014. The impact of best practice health and husbandry interventions on smallholder cattle productivity in southern Cambodia. Anim. Prod. Sci. 54, 629–637.

Figure captions

Fig. 1. Geometric mean faecal eggs counts (FEC) of gastro-intestinal strongyles (GIS), expressed as eggs per gram (EPG) in cattle treated with anthelmintics (A) at wk 0, supplemented with concentrates (P) from wk 0 to wk 13, or both (AP) in six villages in Cambodia. Wk 0: start of the study, wk 29: end of the study. Data points represent fixed effects of the base model with treatment, time, and their interaction as fixed effects, and animal and village as random factors.

Fig. 2. Prevalence of gastro-intestinal strongyles (GIS) in cattle treated with anthelmintics (A) at wk 0, supplemented with concentrates (P) from wk 0 to wk 13, or both (AP) in six villages in Cambodia. Wk 0: start of the study, wk 29: end of the study. Data points represent fixed effects of the base model with treatment, time, and their interaction as fixed effects, and animal and village as random factors.

Fig. 3. Packed cell volume (PCV) in cattle treated with anthelmintics (A) at wk 0, supplemented with concentrates (P) from wk 0 to wk 13, or both (AP) in six villages in Cambodia. Wk 0: start of the study, wk 29: end of the study. Data points represent fixed effects of the base model with treatment, time, and their interaction as fixed effects, and animal and village as random factors.

Fig. 4. The prevalence of loose or watery feces[1] in cattle treated with anthelmintics (A) at wk 0, supplemented with concentrates (P) from wk 0 to wk 13, or both (AP) in six villages in Cambodia. Wk 0: start of the study, wk 29: end of the study. Data points represent fixed effects

of the base model with treatment, time, and their interaction as fixed effects, and animal and village as random factors. [1]Equals diarrhoea scores (DS) of 2 or 3.

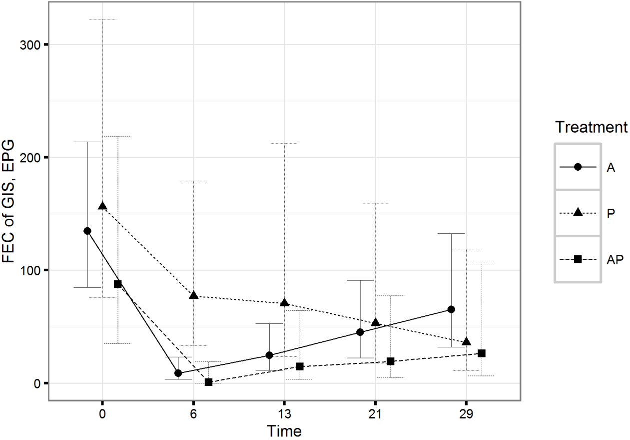
Fig. 5. Prevalence of mild or severe hind quarter fouling[1] in cattle treated with anthelmintics (A) at wk 0, supplemented with concentrates (P) from wk 0 to wk 13, or both (AP) in six villages in Cambodia. Wk 0: start of the study, wk 29: end of the study. Data points represent fixed effects of the base model with treatment, time, and their interaction as fixed effects, and animal and village as random factors. [1]Equals hind quarter fouling scores (HQFS) of 2 or 3.

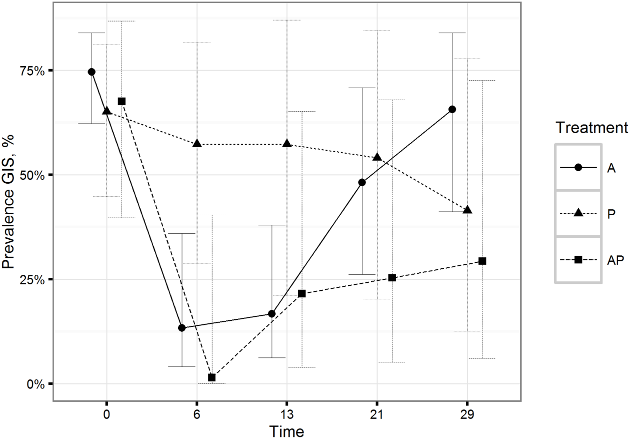
Fig. 6. Prevalence of *Paramphistomum* in cattle treated with anthelmintics (A) at wk 0, supplemented with concentrates (P) from wk 0 to wk 13, or both (AP) in six villages in Cambodia. Wk 0: start of the study, wk 29: end of the study. Data points represent fixed effects of the base model with treatment, time, and their interaction as fixed effects, and animal and village as random factors.

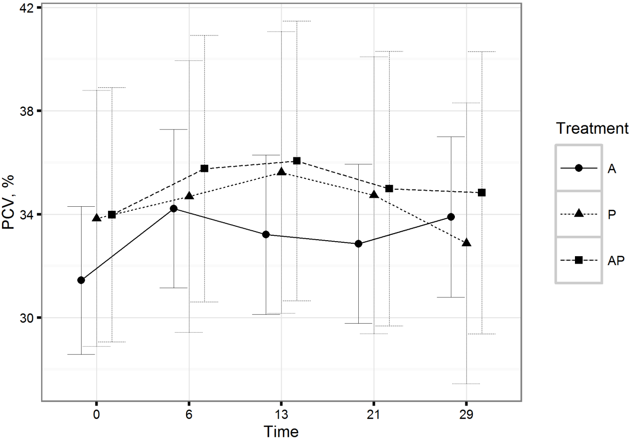
Fig. 7. FAMACHA© scores in cattle treated with anthelmintics (A) at wk 0, supplemented with concentrates (P) from wk 0 to wk 13, or both (AP) in six villages in Cambodia. Wk 0: start of the study, wk 29: end of the study. Data points represent fixed effects of the base model with treatment, time, and their interaction as fixed effects, and animal and village as random factors. "Optimal to acceptable": FAMACHA© = 1 or 2, "Borderline": FAMACHA© = 3, "Dangerous to fatal": FAMACHA© = 4 or 5.

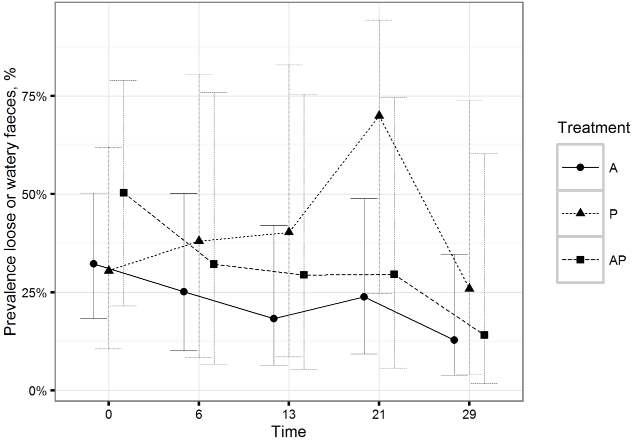
Fig. 8. Heart girth circumferences of cattle treated with anthelmintics (A) at wk 0, supplemented with concentrates (P) from wk 0 to wk 13, or both (AP) in six villages in Cambodia. Wk 0: start of the study, wk 29: end of the study. Data points represent fixed effects of the base model with treatment, time, and their interaction as fixed effects, and animal and village as random factors.

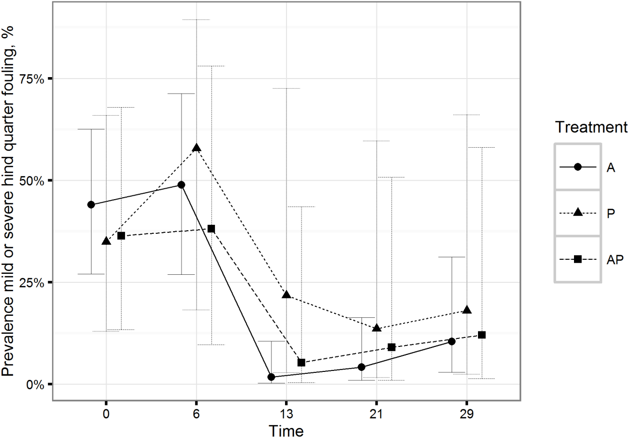
Fig. 9. Body condition scores (BCS) in cattle treated with anthelmintics (A) at wk 0, supplemented with concentrates (P) from wk 0 to wk 13, or both (AP) in six villages in Cambodia. Wk 0: start of the study, wk 29: end of the study. Data points represent fixed effects of the base model with treatment, time, and their interaction as fixed effects, and animal and village as random factors. "Emaciated to poor": BCS = 1 or 2, "Borderline to good condition": BCS = 3 or 4, "Very good": BCS = 5.

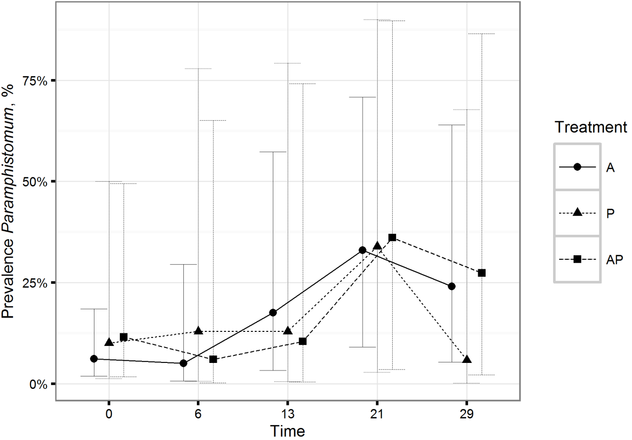

FEC of GIS, EPG
300
200
100
0
0
6
13
21
29
Time
Treatment
A
P
AP

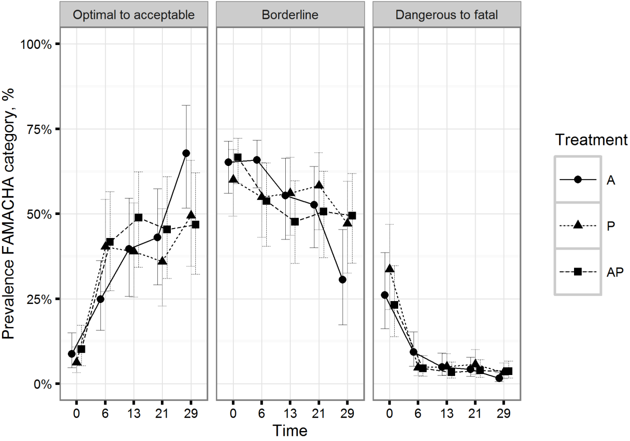

Prevalence GIS, %
75%
50%
25%
0%
0
6
13
21
29
Time
Treatment
A
P
AP


42
38
34
30
PCV, %
0
6
13
21
29
Time
Treatment
A
P
AP

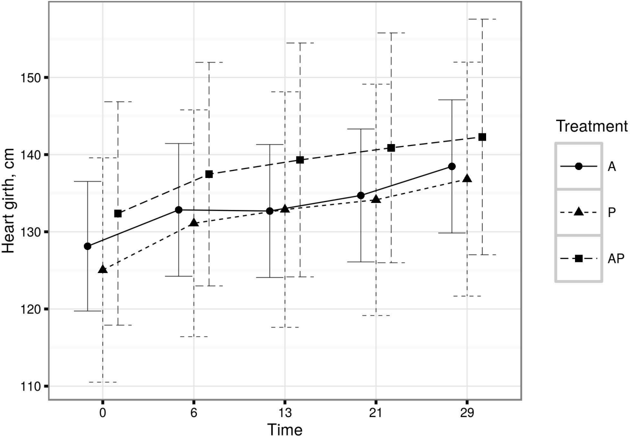

Prevalence loose or watery faeces, %
75%
50%
25%
0%
0
6
13
21
29
Time
Treatment
A
P
AP

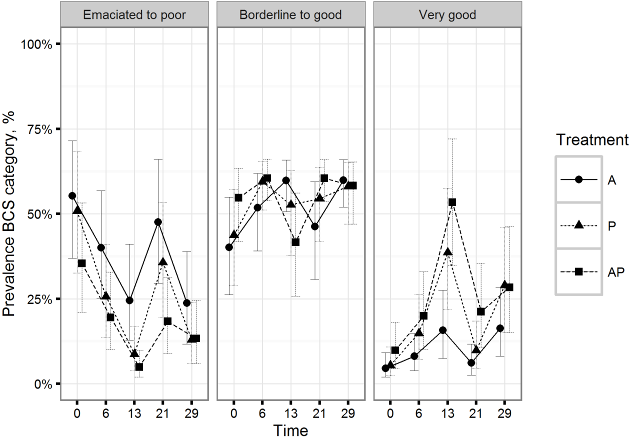

Prevalence mild or severe hind quarter fouling, %
75%
50%
25%
0%
0
6
13
21
29
Time
Treatment
A
P
AP


Prevalence *Paramphistomum*, %
75%
50%
25%
0%
0
6
13
21
29
Time
Treatment
A
P
AP



Optimal to acceptable
Borderline
Dangerous to fatal
Prevalence FAMACHA category, %
100%
75%
50%
25%
0%
0
6
13
21
29
Time
Treatment
A
P
AP


Heart girth, cm
150
140
130
120
110
0
6
13
21
29
Time
Treatment
A
P
AP


Emaciated to poor
Borderline to good
Very good
100%
75%
50%
25%
0%
Prevalence BCS category, %
0
6
13
21
29
Time
Treatment
A
P
AP

Tables

Table 1. Characteristics of cattle enrolled in the trial and prevalence of gastrointestinal strongyle and trematode infections in these cattle in six villages in Cambodia.

Variable[2]	Time[3]	Treatment[1]		
		A	P	AP
		(*n* = 70)	(*n* = 61)	(*n* = 62)
Adult (%)	wk 0-29	70.0	47.5	69.4
Male (%)	wk 0-29	30.0	49.2	33.9
GIS (%)	0wwk 0	72.9	63.9	66.1
	29wwk 29	64.7	42.3	30.8
Paramphistomum (%)	wk 0	10.2	17.2	16.3
	wk 29	31.4	12.0	31.6
Fasciola (%)	wk 0	6.1	0.0	4.7
	wk 29	0.0	0.0	2.6

[1] A: treatment with anthelmintics at wk 0, P: supplementation with concentrates from wk 0 to wk 13, AP: treatment + supplementation

[2] GIS = gastrointestinal strongyles

[3] wk 0: start of the experiment; wk 29: end of the experiment

Table 2. Descriptive statistics of faecal egg counts of gastrointestinal strongyles as well as morbidity parameters in cattle in six villages in Cambodia.

		Treatment[1]					
		A (*n* = 70)		P (*n* = 61)		AP (*n* = 62)	
Variable[2]	Time[3]	Mean	Min-max	Mean	Min-max	Mean	Min-max
GIS (FEC)	wk 0	148.6	0-700	249.2	0-2350	108.9	0-900
	wk 29	74.5	0-300	51.0	0-550	33.7	0-300
PCV (%)	wk 0	31.4	15-41	33.9	24-46	34.0	23-44
	wk 29	33.5	25-45	32.9	23-43	34.5	25-46
FAMACHA©	wk 0	3.2	2-4	3.3	2-5	3.1	2-5
	wk 29	2.4	2-3	2.5	2-4	2.6	2-4
HQFS	wk 0	1.5	1-3	1.4	1-3	1.5	1-3
	wk 29	1.2	1-2	1.3	1-2	1.2	1-3
DS	wk 0	1.4	1-3	1.4	1-3	1.6	1-3
	wk 29	1.2	1-3	1.3	1-2	1.2	1-2
Heart girth circumference (cm)	wk 0	128.1	80-160	124.7	80-160	132.4	90-180
	wk 29	138.0	110-168	136.7	108-160	143.1	122-185
BCS	wk 0	3.4	1-5	3.6	2-5	3.7	1-5
	wk 29	3.9	2-5	4.1	2-5	4.0	2-5

[1] A: treatment with anthelmintics at wk 0, P: supplementation with concentrates from wk 0 to wk 13, AP: treatment + supplementation

[2] GIS = gastrointestinal strongyles, FEC = faecal egg counts, PCV = packed cell volume, HQFS = hind quarter fouling score, DS = diarrhoea score, BCS = body condition score

[3] wk 0: start of the experiment; wk 29: end of the experiment

Table 3. Significance of terms in the mixed models investigating the effect of treatment over time in cattle in six villages in Cambodia.

	Approximate *p*-value of model terms[1]		
Response[2]	Time × Treatment[3]	Treatment	Time
GIS (FEC)	<0.0001	-	-
GIS	<0.0001	-	-
Paramphistomum	0.2466	0.8519	<0.0001
PCV	0.0034	-	-
FAMACHA©	0.1213	0.5894	<0.0001
BCS	0.4546	0.0698	<0.0001
Heart girth circumference	0.3474	0.5599	<0.0001
DS	0.0086	-	-
HQFS	0.0241	-	-

[1] Approximate *p*-values were obtained through likelihood ratio testing. Missing *p*-values indicate that the main effect is untested because it is part of a significant interaction.

[2] GIS = gastrointestinal stronglyes, FEC = faecal egg counts, PCV = packed cell volume, BCS = body condition score, DS = diarrhoea score, HQFS = hind quarter fouling score

[3] Treatment with anthelmintics at wk 0, supplementation with concentrates from wk 0 to wk 13, or both