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Evaluation of plant commercial feed additives for equine cyathostomin control

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ABSTRACT

The increasing emergence of anthelmintic-resistant parasitic isolates prompts us to reassess the management of intestinal strongylosis in horses. Additionally, societal demand is shifting toward reducing the use of chemical treatments, aligning with environmentally-friendly practices and the exploration of natural alternatives. In this context, we provide an initial view of the antiparasitic activity and the effect on immune circulating blood cells of three commercialized plant-based feed additives in ponies. Three treatments, based either on mugwort (*Artemisia vulgaris*), echinacea (*Echinacea purpurea*) or curcumin (*Curcuma longa*) were administrated to 18 (six per treatment) Welsh female ponies naturally infected with cyathostomins to mimic their practical use in farming conditions. Another group of six untreated ponies was used as a control. Fecal egg count (FEC), the larval development percentage and the number of red blood cells, lymphocytes, monocytes, neutrophils, eosinophils and basophils were measured the first and the last day of each treatment, and compared with those characterizing the control group. None of the three treatments showed a significant effect on the studied parameters. Moreover, the efficacy of treatments, measured from the FEC reduction compared to the control group, was weak ($\leq$ 38.6 %). Therefore, these results do not support the practical use of these additives in equine farming, even if the determination of Cohen's d values associated with the three treatments revealed some incidences on FEC and blood immune cell counts, as well as on larval development for mugwort.

1. Introduction

Cyathostomins (small strongyles) are the most prevalent and problematic gastrointestinal nematodes in equines [1,2]. Many horses show no clinical signs of infection (*i.e.* they are asymptomatic carriers), but depending on the helminth load, clinical signs due to cyathostomins may result in colic, diarrhea with significant weight loss and potentially death when large number of encysted larvae emerged from the colonic mucosa (*i.e.* larval cyathostominosis) [3–6]. Cyathostomin control relies on the use of chemical dewormers, a practice that is currently challenged as the massive use of these molecules has selected drug-resistant isolates [2,7–9]. In this context, alternative strategies to the use of chemical anthelmintic are needed. Some studies have investigated the use of bioactive plants as a new strategy for the control of small strongyle infections in horses, with varying degrees of success obtained *in vivo*. Two studies did not report any effect of a sainfoin pellet-based diet on the number of fecal egg count (FEC) nor on larval development [10,11], while another reported a significantly lower average number of eggs in the feces over a 28-day period [12]. A recent study comparing the effect of sainfoin supplementation in the case of either a high-fiber or a high-starch diet in horses, observed a lower increase in FEC in horses fed with the second diet, but not significantly [13]. Recently, a first study investigating the efficacy of chicory "cultivar Puna II" in grazing horses reported a strong efficacy of the plant, leading to a reduction in FEC and a reduction in the development of eggs into larvae [14]. Alongside these varied results, we are witnessing an increasing number of commercial plant feed additives for horses that mention anthelmintic properties or immunity-boosting effects available on the market. This is despite the

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lack of evidence supporting their efficacy against cyathostomins. Trials on naturally infected donkeys fed with a commercialized phytotherapeutic product had slight FEC reduction [15] while another investigation of a plant-based dewormer did not report any effect [16]. Similarly, evaluation of a garlic-based feed additive showed no effect on naturally infected horses [17].

To further expand current knowledge on plant-based feed additives and identify potential candidates for the control of small strongyle infections in equines, we evaluated the direct and indirect activity of three feed additives commercialized for their health benefits. We focused on single-plant products containing either mugwort, echinacea or curcuma commercialized, respectively, to support natural defenses against intestinal parasites, to support immune defenses and for joint or digestion problems in equines. Curcuma is commercialized for pathologies quite unrelated to cyathostomins infection. However, the literature describes curcuma as carrying immunostimulatory properties, which could potentially act indirectly on parasites.

With the objective of providing owners and breeders with more information on plant-based feed additives and their use in controlling cyathostomin infection, we analyzed the effects of these three products in naturally infected horses on their FEC, development of eggs into larvae and circulating blood cell counts, including immune cell populations.

Table 1
Age (years), last anthelmintic treatment and fecal egg count (eggs per gram) for each pony before the start of the experiment.

Treatment group	Age per individual	Age (mean ± sd)[1]	Last treatment date	Anthelmintic	Date of FEC	FEC per individual[2]	FEC (mean EPG [95 % CI])[3]	Stalls
Control	2	3.5 ± 1.76	07/28/2020	Panacur	09/28/2020	1550	600.0 EPG [287.02; 913.98]	7
	5		Before the 10/28/2019	Unknown	09/28/2020	750		1
	2		07/28/2020	Panacur	09/28/2020	500		7
	4		04/08/2020	Panacur	09/28/2020	300		8
	6		02/07/2019	Panacur	09/28/2020	300		9
	2		07/28/2020	Panacur	09/28/2020	200		6
Mugwort	1	3.0 ± 1.41	07/28/2020	Panacur	09/28/2020	1080	540.8 EPG [360.21; 721.45]	4
	4		Before the 10/28/2019	Unknown	09/28/2020	585		5
	2		07/28/2020	Panacur	09/28/2020	550		7
	3		07/28/2020	Panacur	09/28/2020	420		10
	5		Before the 10/28/2019	Unknown	09/28/2020	360		8
	3		07/28/2020	Panacur	09/28/2020	250		6
Echinacea	8	3.5 ± 2.73	03/31/2020	Eraquell	09/28/2020	1300	507.0 EPG [250.53; 763.47])	1
	4		Before the 10/28/2019	Unknown	09/28/2020	615		9
	1		07/28/2020	Panacur	09/28/2020	350		6
	5		10/28/2019	Strongid	09/28/2020	330		2
	1		07/28/2020	Panacur	09/28/2020	250		3
	2		07/28/2020	Panacur	09/28/2020	200		10
Curcumin	3	2.8 ± 1.47	07/28/2020	Panacur	09/28/2020	700	553.3 EPG [483.83; 622.84]	4
	2		07/28/2020	Panacur	09/28/2020	645		7
	4		Before the 10/28/2019	Unknown	09/28/2020	570		2
	2		07/28/2020	Panacur	09/28/2020	550		10
	1		07/28/2020	Panacur	09/28/2020	450		4
	5		Before the 10/28/2019	Unknown	09/28/2020	405		2
P value	/	/	/	/	/	0.900[4]	0.976[5]	/

[1] Arithmetic mean ± standard deviation
[2] Individual FEC data were determined using a modified McMaster technique [18] based on the dilution of 5 g of fecal matter in 70 mL of a saturated NaCl solution (density = 1.18), as reported in a previous study [14]. Eggs were counted using a McMaster numbering cell and an optical microscope (×150 magnification), the minimum detection limit was set at 50 EPG. These FEC were used to balance the four groups.
[3] Arithmetic mean and 95 % confidence interval.
[4] *P value* obtained with ANOVA test with group as fixed effect
[5] *P value* obtained with a linear mixed-effects model of group as fixed effect and horses as random effect

2. Materials and methods

The *in vivo* trial was conducted from October 16th to November 26th, 2020, at the experimental unit of animal physiology at l'Orfrasière (EUPAO) in Nouzilly, Indre-et-Loire, France (DOI of the experimental unit: 10.15454/1.5573896321728955E12).

2.1. Ethics approval

Experiments were carried out according to EU directives and French regulations (Directive 2010/63 / EU, 2010; Rural Code, 2018; Decree n° 2013-118, 2013). The experimental procedure received approval from the French Ministry of Research under protocol number APAFIS#26140-2020062216271790v2. The procedures involving Welsh ponies were evaluated by the Val de Loire ethics committee (CEEA VdL, committee number 19).

2.2. Animal condition

Naturally infected female Welsh ponies (1 to 8 years old, n = 24) with individual FEC higher than 200 eggs per gram (EPG) were used for the study. The number of animals was determined with the aim of testing the efficacy of supplements under classical breeding conditions, *i.e.* with a low number of individuals as found in many farms. Under these conditions, with six animals per group, the study was able to reveal only strong effects, as demonstrated by the measured Cohen's effect size of 1.4 (statistical justification in *2.5 Statistical analysis* section). Animals were allocated into four experimental groups, balanced for their age and their FEC measured 18 days before being enrolled in the study (September 28th, 2020). Due to their young age (≤3 years), fifteen ponies were treated with Panacur® on July 28th, 2020 (Table 1). Other ponies, aged over three years, were not treated during the seven months preceding the study. Their last treatment was Eraquell®, Strongid® or unknown (Table 1). A last anthelmintic treatment (Panacur®) was orally administered to one pony (4 years old) in the control group, which excreted a high number of strongyle eggs (2055 EPG), on 4 August 2020 (Table 1). From 11 days before the start of the experiment, ponies were housed in groups of two or three individuals of similar ages in 11 different stalls (*i.e.* 7 groups of 1 to 3-year-old ponies and 4 groups of 4 to 8-year-old ponies) (Table 1) to get accustomed to their housing conditions and their diet. Animals were fed at the trough with wheat straw and pellets containing wheat straw, oats, wheat bran, dehydrated alfalfa, barley, dried beet pulp, and sugarcane molasses.

2.3. Experimental design

The three different tested feed additives based on dehydrated plant (Table 2) were included in each pony's ration (*i.e.* 600 g of pellets and 5 kg of wheat straw) in two daily doses (morning and evening). Feed additives were mixed with the pellets (300 g in the morning and evening). Ponies were individually tied to the feeder during 30 min to give them enough time to eat their pellet ration. At the end of the 30 min period, an operator ensured that the entire ration was taken. No feed refusals were observed for all the horses during the study. Ponies, whose mean body weight was 275.4 ± 56.0 kg, received 1.8 times the manufacturer's recommendations for 500 kg horses (Table 2). The control group did not receive any feed additive and water was available *ad libitum* to all ponies. In order to analyze the effects of feed additives on parasitic parameters, fecal samples were collected individually from the rectum of each animal on the first and last day of each treatment. At the same time, samples from the control groups were taken at the end of each treatment (*i.e.* at d10, d15 and d30). Blood samples were collected the same day using K3 EDTA tubes (Dutscher 367525A), containing an anticoagulant to measure blood cell counts.

2.4. Fecal and blood sample analysis

Immediately after the collection of feces, individual FEC data were determined using a modified McMaster technique [18] based on the dilution of 5 g of fecal matter in 70 mL of a saturated NaCl solution (density = 1.18), as reported in a previous study [14]. Eggs were counted using an optical microscope (×150 magnification), the minimum detection limit was set at 50 EPG. To reduce bias, all coproscopy was performed by the same person. The fecal egg count reduction (FECR) percentage attributed to each feed additive was measured using a Bayesian hierarchical model as recommended by WAAVP guideline [19] (see 2.5 Statistical analysis).

To evaluate the effects of feed additives on the larval development rate, the remaining fecal matter (70 to 225 g) was incubated individually for each horse for 12 days at +25 °C and 60 % relative humidity, as previously reported [14]. Infective third-stage larvae (L3) of cyathostomins were then collected using a Baermann apparatus after 48 h of sedimentation for each horse and timepoint. The developed larvae (L3) count in each sample was determined from 30 drops of 5 μL from the larval solution under the microscope (×4 magnification). The larval development rate was then calculated for each sample as previously reported [10], as:

$$\left(\frac{\textit{Number of L3 counted}}{\textit{FEC} \times \textit{quantity of fecal matter cultured}}\right) \times 100$$

Total leukocytes (lymphocytes, monocytes, neutrophils, eosinophils, basophils) and erythrocytes were counted using an MS9-5 Haematology Counter® (automated digital hematology analyzer, Melet Schloesing Laboratories), as described by other authors [20].

2.5. Statistical analysis

All data were analyzed using R software v. 4.3.0 [21]. To measure the theoretical Cohen effect size (Cohen's d) of food additives associated to our sample size, the *pwr.t.test* function of the pwr v.1.3-0 package was used [22]. In the case of a paired test, we used a risk of error alpha of 0.05, a power of 0.8 and a "n" number of animals of 6. This value measures the magnitude of the differences between our two groups [23], this means the degree to which the treatment effect is present in the population [24]. Based on the mean, this value is complementary to the statistical significance used to determine whether results are likely to be due to chance. Both are essential to understand the full impact of your work [23]. The mean age between groups was statistically analyzed using an ANOVA with groups as the fixed effect, using the *anova_test* function of the rstatix v.0.7.2 package [21]. The mean FEC between

Table 2
Information about mugwort, echinacea and curcumin food additives.

	Commercial name	Scientific name	Plant part used	Function	Manufacturers recommended dose[1]
Mugworth	Common artemisia	*Artemisia vulgaris*	Whole plant	Horse intestinal parasites	10 g / day (10 days)
Echinacea	Echinacea	*Echinacea purpurea*	Aerial part or root[2]	Horse immune system	15 g / day (15 days)
Curcumin	Curcumin	*Curcuma longa*	Root	Joints and horse digestion	10 g / day (30 days)

[1] These doses were recommended for horses weighing 500 kg at the time of the study. At the time of paper writing, the horse's weight to be considered according to the manufacturer was 550 kg and the recommended dose for curcumin was changed to 20 g per day for 3 weeks.
[2] The manufacturer specifies that the parts used may be the aerial or the root part, depending on the batches received.

groups at d-11 was statistically analyzed using a linear mixed-effects (LME) model with groups as fixed effect, using the *lm* function of the stats v.4.3.2 package [21]. The effect of feed additives on FEC, larval development and blood parameters was analyzed using LME model with group, day and group × day interaction as fixed effect and horses as random effect. In order to minimize type 1 errors, a Bonferroni correction was applied to analyses including multiple fixed effects using *p. adjust* function of the stats v.4.3.2 package [21]. Studied p_values < 0.05 were considered as significant. The observed Cohen effect size of each feed additive on FEC, larval development and blood cell count was measured by dividing the "group" fixed effects estimate by the standard deviation of residuals. For this, the functions *fixef* of lme4 v.1.1-35.1 package [25] was used to extract the "group" fixed effects estimate, and *resid* of the stats v.4.3.2 package [21] to extract residuals, both from the LME model. Cohen's d ≥ 0.8 was considered as a strong effect of the extracts [24]. The fecal egg count reduction (FECR) was measured with a Bayesian hierarchical model, using the *fecr_stan* function from eggCount v. 2.3-2 package [26], including the average FEC of the control group as pre-treatment, the average FEC of the treated group as post-treatment and the correction factor for the McMaster technique.

3. Results

3.1. Plant-based feed additive effect on parasitic parameters and their FECR

No significant statistical differences were observed between the control and treated experimental groups for FEC and larval development (Table 3). However, the Cohen's effect size associated to the three treatments were higher than 1.09 for FEC data (Table 3). Concerning larval development results, the Cohen's d was higher than 0.8 only in the case of mugwort treatment (Table 3). The Bayesian approach provided efficacy values for FECR ranging from 25.2 % to 38.6 %, with the highest results obtained for mugwort (Table 3).

3.2. Plant-based feed additive effect on blood cell count

Over the study period, only the average monocyte, neutrophil and eosinophil counts changed significantly in the control condition. Indeed, the monocyte count was significantly lower at d10 than at d0 (mean ± sd = 5.4 ± 1.49 vs. 6.9 ± 1.59, $P = 0.02$), before becoming higher at d30 (9.1 ± 1.20, $P < 0.01$). Neutrophil count was significantly lower at d10 compared to d0 (21.4 ± 3.36 vs. 27.9 ± 2.31, $P = 0.004$), while eosinophil count was higher (23.2 ± 3.29 vs. 15.6 ± 4.07, $P < 0.01$). However, this decreased, becoming significantly lower at d30 than at d0 (10.8 ± 5.38, $P = 0.01$). As for parasitological parameters, feed additives did not induce any relevant changes in the count of blood cell populations (Fig. 1). However, a time effect was observed, with a higher average number of eosinophils at d10 compared to d0 (21.3 ± 4.12 vs. 14.5 ± 3.69, $P < 0.01$), while the average number of neutrophils decreased (23.2 ± 6.67 vs. 27.2 ± 4.06, $P = 0.04$) (Fig. 1). Moreover, the average number of circulating monocytes at d30 was significantly higher compared to d0 (9.3 ± 1.15 vs. 6.7 ± 1.45, $P < 0.01$) (Fig. 1). Considering the Cohen's effect size of the mugwort treatment, we observed values > 1 for three different cell populations (*i.e.* lymphocyte, eosinophil and basophil) (Table 4). Same results were obtained in the case of echinacea treatment for four different cell populations (*i.e.* red blood cells, lymphocytes, neutrophils and eosinophils) (Table 4). Finally, we obtained Cohen's d value > 0.9 when considering the effect of curcumin on basophil, monocyte and lymphocyte counts, respectively (Table 4).

4. Discussion

This study evaluated the effects of three commercialized feed additives composed either of mugworth, echinacea or curcumin, on immune

Table 3
Fecal egg count (eggs per gram), larval development percentage and fecal egg count reduction percentage of feed additives (6 individuals per group).

	FEC (mean EPG [95 % CI])[1]			
	d0	d10	d15	d30
Control	516 [290.68; 742.65]	983.3 [542.59; 1424.07]	766.7 [501.77; 1031.56]	825.0 [453.77; 1196.23]
Mugwort	816.7 [569.00; 1064.33]	1008.3 [516.61; 1500.05]	/	/
Echinacea	983.3 [586.63; 1380.03]	/	941.7 [582.17; 1301.16]	/
Curcumin	850.0 [570.06; 1129.94]	/	/	775.0 [550.89; 999.11]
P value	0.292/0.108/ 0.244[2]	1.00[3]	1.00[3]	0.97[3]
Cohen's d[4]	/	1.12	1.11	1.09
	Larval development rate (mean % ± s.d)[5]			
	d0	d10	d15	d30
Control	25.5 ± 21.99	42.4 ± 20.18	53.7 ± 27.23	47.0 ± 37.68
Mugwort	38.1 ± 13.16	49.0 ± 23.82	/	/
Echinacea	29.3 ± 16.63	/	34.2 ± 8.21	/
Curcumin	22.4 ± 5.17	/	/	28.9 ± 9.45
P value	0.180/0.696/ 0.746[6]	1.00[3]	0.72[3]	1.00[3]
Cohen's d[4]	/	1.04	0.21	0.15
	FECR			
	Efficacy (%) [95 % CI][7]			
Mugwort	38.6 [14.1; 69.3]			
Echinacea	25.2 [10.2; 46.6]			
Curcumin	32.0 [10.7; 58.2]			

[1] Arithmetic mean and 95 % confidence interval for the FEC. Individual FEC data were determined using a modified McMaster technique [18] based on the dilution of 5 g of fecal matter in 70 mL of a saturated NaCl solution (density = 1.18), as reported in a previous study [14]. Eggs were counted using a McMaster numbering cell and an optical microscope (×150 magnification), the minimum detection limit was set at 50 EPG.

[2] *P value* of the FEC between treatment and the control group at d0 (mugworth, echinacea and curcumin, respectively), obtained with a linear mixed-effects (LME) model of group as fixed effect and horses as random effect at d0

[3] *P value* (Bonferroni correction) of LME model of group, day and group × day interaction as fixed effect, and horses as random effect

[4] Cohen effect size measured by dividing the "group" fixed effects estimate by the standard deviation of residuals of the LME model

[5] Arithmetic mean percentage + standard deviation for the larval development percentage. The larvae were collected after fecal matter (70 g to 225 g) incubation individually for each horse during 12 days at +25 °C and 60 % relative humidity, as previously reported [14]. Infective third-stage larvae (L3) of cyathostomins were then collected using a Baermann apparatus after 48 h of sedimentation for each horse and timepoint. The developed L3 count in each sample was determined from 30 drops of 5 μL of from the larval solution under the microscope (×4 magnification). The larval development rate was then calculated for each sample as previously reported as [10] study, as: $\left(\frac{Number\ of\ L3\ counted}{FEC\ \times\ quantity\ of\ fecal\ matter\ cultured}\right) \times 100$

[6] *P value* of larval development rate of treatment compared control group at d0 (mugworth, echinacea and curcumin, respectively), obtained with a LME model of group as fixed effect and horses as random effect at d0

[7] Efficacy percentage measured with a Bayesian hierarchical model as recommended by WAAVP guideline [19], including the average FEC of the control group as pre-treatment, the average FEC of the treated group as post-treatment, the correction factor for the McMaster technique, and 95 % confidence interval measured on FEC

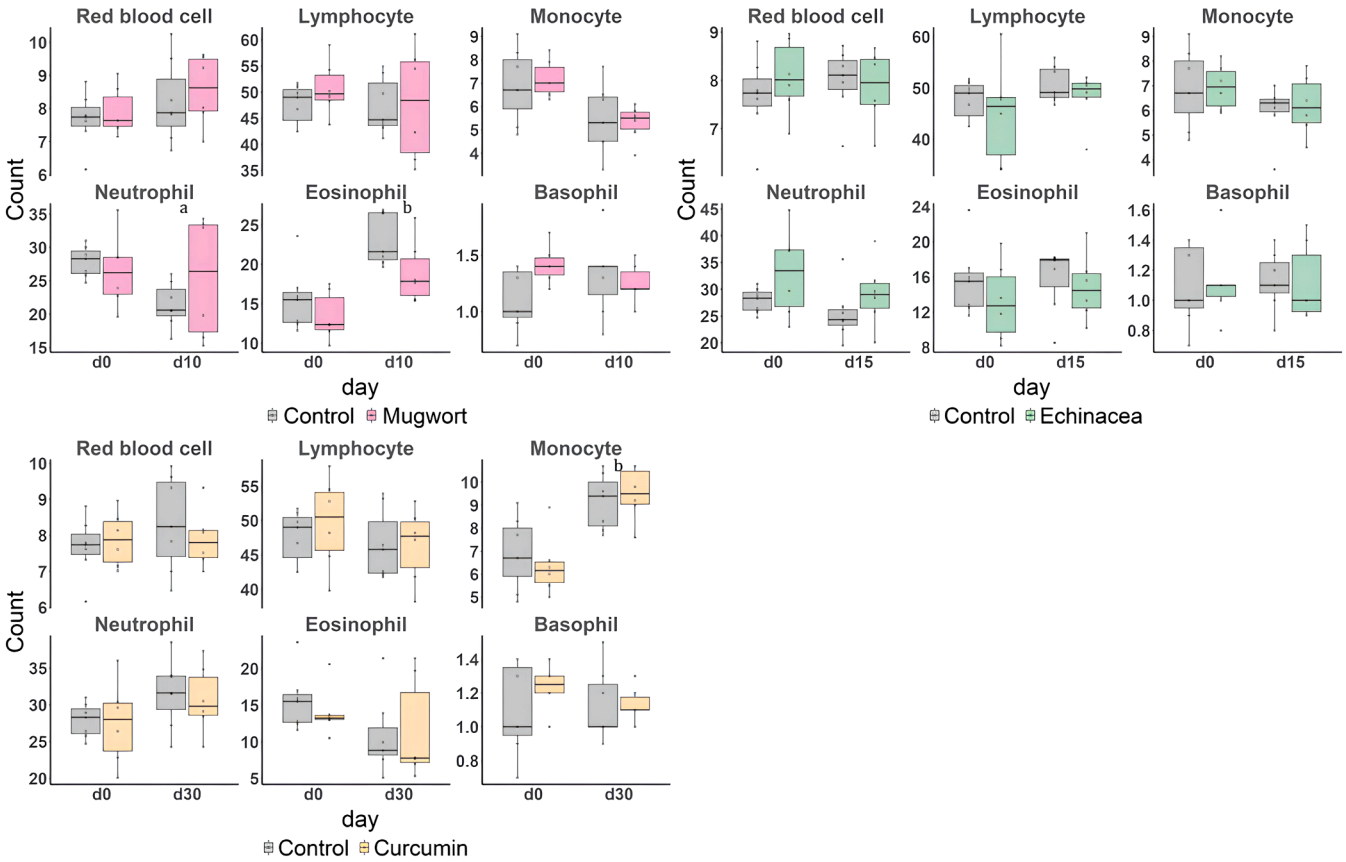

Fig. 1. Count of blood parameters on samples collected at the beginning and the end of treatment for each group (n = 6), control (grey), mugwort (pink), echinacea (green) or curcumin (yellow). Lowercase letters indicate significant differences for a day effect (a: $P = 0.04$, b: $P < 0.01$). Statistics were performed on each treatment with a linear mixed-effects model of group, day and group × day interaction as fixed effect and horses as random effect.

Table 4
Cohen's d measured on the different studied cell population counts for mugwort, echinacea and curcumin treatments.

Cell population	Mugworth	Echinacea	Curcumin
Red blood cells	0.4	1	0.3
Lymphocytes	1.1	1	1.2
Monocytes	0.2	0.04	1.1
Neutrophils	0.4	1.7	0.1
Eosinophils	1.1	1.1	0.4
Basophils	1.7	0.1	0.9

Note: Cohen's d was measured for each treatment (mugworth, echinacea or curcumin) using the linear mixed-effects model of group (control vs. treatment), day and group × day interaction as fixed effect, and horses as random effect. The Cohen size effect was subsequently measured by dividing the "group" fixed effects estimate by the standard deviation of residuals from the statistical model used

circulating cells in ponies and their efficacy to reduce cyathostomins egg excretion and larval development. In the literature, mugwort has been described to exhibit anti-inflammatory activity in Wistar albino rats using the cotton pellet granuloma model [27], or to increase neutrophil count in male rats [28]. Echinacea has been observed to exert both pro- and anti-inflammatory activity. *In vitro, E. purpurea* extracts induced an increase in natural killer function [29], dendritic cell differentiation in human peripheral blood mononuclear cells (PBMC) [30] and pro-inflammatory cytokine counts in immortalized human T lymphocyte cell line [31]. Conversely, a decrease in the TNFα level induced by alkylamides from *E. purpurea* was observed in human whole blood [32] or PBMCs [33]. *In vivo*, the oral *E. purpurea* extract intake increased levels of Th1 cytokines, leukocyte counts, and immunoglobulin levels in C57BL/6N mice [34]. Finally, it has been observed that curcumin exhibits anti-inflammatory activity on murine macrophage cells and decreases the activity of proinflammatory cytokine (*i.e.* IL-6, TNFα) [35]. Similar results have been observed in *in vivo* models such as in mice with cyclophosphamide [36] or in rats with traumatic spinal cord injury [37].

During the study, we observed that the circulating monocyte, neutrophil and eosinophil counts significantly varied in the untreated control group. At d30, the number of monocytes was significantly higher than at the start of the study. This observation may be explained by the fact that monocytes differentiate into M2 macrophages via the Th2 response [38], subsequently participating in parasite expulsion and in the repair of induced damage [39,40]. As macrophages, eosinophils participate in the anti-parasite response [40], which may explain the significantly higher count at d10, compared with d0. However, the count decreased and became significantly lower at d30. The study finished at the end of November, these observations may reflect the start of larval hypobiosis, thus explaining the results of eosinophil count reduction. Alongside the control condition, none of these plant-based food additives, administrated 1.8 times higher than manufacturer's recommendations, induced a significant change of these variations or in blood cell count. However, a Cohen's effect size higher than 0.8 have been measured. Mugwort treatment showed a strong effect on eosinophil and basophil counts; echinacea treatment on red blood cell, neutrophil and eosinophil count, and curcumin on monocyte and basophil counts. We also found values > 0.8 on lymphocyte counts for all treatments. Despite the absence of significant effects, a high Cohen's d was measured. These contrasting results can be explained by the variations in cell counts that can be observed between six horses in the same treatment group, making it more difficult the identification of a significant effect. Furthermore, as described in the literature, both

echinacea and curcumin have been found to modulate cytokine levels *in vitro* across various cell types [41]. Additionally, *in vivo* studies have confirmed this effect specifically for curcumin in rats and mice [41]. Despite the absence of any significant difference in the cell populations studied between the control and the treatments, supplements may have an effect on the immune cell populations studied, possibly by the modulation of cytokine.

As for blood cells counts, we did not observe any significant reduction in cyathostomin fecal egg count (FEC) and larval development consecutive to treatments. The mugwort treatment presented the highest efficacy (*i.e.* 38.3 % vs. 25.4 % and 32.1 % for echinacea and curcumin treatments, respectively). These values, measured according to the WAAVP recommendations, remained low compared to those expected in the case of chemical treatments: 95 % for fenbendazoles, 88 % for pyrantel and 96 % for macrocyclic lactones [19]. Nevertheless, they are in line with those found in the literature for other plant-based food additives (*e.g.* 29.9 %), tested in a group of donkeys of similar size ($n = 8$) [16]. As observed for immune blood cells, all treatments exhibited a Cohen's d higher than 1 for FEC. Concerning larval development, only the mugwort treatment was characterized by a Cohen's $d > 0.8$. These results suggest that these treatments may affect the parasites, but that the small number of animals chosen to reflect the conditions of most equine farm and/or the high variability across groups, as observed for cell count results, did not enable us to highlight significant effects of treatments. Moreover, we can assume that the absence of a significant effect may be due to the fact that certain species of cyathostomins are less sensitive to treatments. This was observed in another *in vivo* study where the anti-parasitic activity of chicory forage (cv. Puna II) was investigated at pasture [14]. As we used a natural infection, resulting from an unknown ecological species composition, it would have been interesting to include a metabarcoding approach to monitor the cyathostomins species composition before and after each treatment.

Based on our results, we can conclude that these three plant-based supplements have a high effect on the data measured, but not enough to observed a significant effect under our conditions.

5. Conclusion

This study provides an initial overview of the potential activity of three plant-based food additives to reduce cyathostomins infections in ponies. Our results indicate that mugwort, echinacea and curcumin treatments have no statistically significant effect on cyathostomins and on the blood immune cell count. However, the Cohen's size effect indicates that mugwort supplement have the largest effect, *i.e.* on FEC, larval development and lymphocyte, eosinophil and basophil counts.

Under our conditions chosen to be close to those of equine owners (*i.e.* small number of animals, natural infestation), our efficacy results do not justify the practical use of these additives even if our study does not reject the existence of their effect on cyathostomins and blood immune cells. Thus, this study may prevent horse owners, breeders, as well as equine veterinarians, from making mistakes in the management of small strongyle infection in equids. Undoubtedly, further studies should be conducted in the future to standardize the correct dosages of plant feed additives against cyathostomins in equids.

Declaration of generative AI in scientific writing

The authors did not use any artificial intelligence assisted technologies in the writing process.

Data and model availability statement

Links to the R scripts and data of the analyses (https://github.com/Joshua-Malsa/Commercial-product-evaluation-paper).

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Ethics in publishing statement

Experiments were carried out according to EU directives and French regulations (Directive 2010/63 / EU, 2010; Rural Code, 2018; Decree n° 2013-118, 2013). The experimental procedure received approval from the French Ministry of Research under protocol number APAFIS#26140-2020062216271790v2. The procedures involving Welsh ponies were evaluated by the Val de Loire ethics committee (CEEA VdL, committee number 19).

CRediT authorship contribution statement

J. Malsa: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **F. Reigner:** Resources, Data curation. **M. Riou:** Writing – original draft, Data curation. **A. Gesbert:** Data curation. **F. Guégnard:** Data curation. **N. Perrot:** Data curation. **D. Serreau:** Data curation. **G. Fleurance:** Writing – original draft, Visualization, Project administration, Funding acquisition, Conceptualization. **G. Sallé:** Writing – original draft, Visualization, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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