Benzimidazoles Pharmacodynamics in Equine Strongyles

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Abstract

Our research aimed to assess the effectiveness of four benzimidazoles: albendazole, fenbendazole, mebendazole and thiabendazole against equine strongyles. The tests were performed between March 2015 and May 2016, on samples collected from 20 horses and 8 donkeys living in Harghita County. *In vivo*, Faecal Egg Count Reduction Test (FECRT) was used to evaluate fenbendazole pharmacodynamics. *In vitro*, Egg hatch assay (EHA) and Larval development assay (LDA) were used to evaluate the effectiveness of albendazole, fenbendazole, mebendazole and thiabendazole. The predominance of small strongyle species was observed, mostly *Cyathostomum* type A. In the horse group, before treatment, the average intensity was 1595.5 EPG, the maximum value being 4000, and extensivity 55%. Tested again at 14 days after treatment, all samples were negative. In the donkey group, before treatment, the total number was 6550 EPG, intensity of 935.7 and extensivity of 87.5%. 14 days after treatment, the average intensity was 150 and the extensivity 50%. In the horse group, EHA proved the efficacy of fenbendazole (0.0192%), albendazole (0.3740%) and thiabendazole (11.62%) and a major risk of inducing adaptive phenomena for mebendazole (Y parameter 1009.92). In the donkey group, all benzimidazoles had limited effectiveness: thiabendazole (73.93%), mebendazole (87.51%), fenbendazole (94.05%), albendazole (111.67%). All benzimidazoles inhibited larval development. For all tested benzimidazoles, the resistance induction predictive comparative risk analysis highlighted the benefit of their use, provided that the treatment protocol allows sufficient contact time.

Keywords: benzimidazoles, equines, resistance, strongyles.

INTRODUCTION

Equine strongyles research and analysis is an important topic due to the very high morbidity (up to 99%) and the serious, sometimes fatal, repercussions of the infection (Cerneia *et al.*, 2010; Cernea *et al.*, 2015; Smith *et al.*, 2015).

Strongyles can alter the animal's behaviour, fertility, physical condition, development, reducing its resistance to other pathogens or even cause death. The large number of species, varying ontogenesis and problems related to diagnosis turn this pathology into a permanent challenge for both veterinarians and equine owners (Cerneia *et al.*, 2010; King *et al.*, 1990, Madeira de Carvalho *et al.*, 2014). In addition, the development of anthelmintics resistance, along

with the high costs required for finding new drugs force the researchers try to find new ways in approaching diagnosis, treatment and prevention of this pathology (Cerneia *et al.*, 2010, Madeira de Carvalho *et al.*, 2003).

Our research aimed to assess the effectiveness of four benzimidazoles: albendazole (ABZ), fenbendazole (FBZ), mebendazole (MBZ) and thiabendazole (TBZ) against equine strongyles.

MATERIAL AND METHODS

The tests were performed between March 2015 and May 2016 on samples obtained from two groups of animals living in Tomești village, Harghita County.

Group 1 was constituted of 20 horses aged 6 months to 16 years and Group 2 was constituted of 8 donkeys aged 7 months to 19 years.

In vivo, we used the Faecal Egg Count Reduction Test (FECRT) in order to evaluate the fenbendazole effectiveness.

In vitro, in order to evaluate the effectiveness of albendazole, fenbendazole, mebendazole and thiabendazole, two tests were used: Egg Hatch Assay (EHA) and Larval Development Assay (LDA).

Six months prior to this study, in the horse group, ivermectin-based products treatments were carried out. No recorded data regarding the anthelmintic treatments of the donkey group is available. For accuracy, faecal samples were collected directly from the rectal ampulla or immediately after defecation. For each of the collected samples, McMaster and Stoll methods and coprocultures were performed.

FECRT aimed to assess the strongyles intensity and extensivity before (BT) and post-treatment (PT), evaluation of fenbendazole effectiveness *in vivo*, statistical analysis of the obtained data and identification of the resistant strongyle species.

For EHA, we used serial dilutions from 500 mcg to 0.98 mcg active ingredient/ml. In order to evaluate the strongyles resistance against benzimidazoles, the following parameters were interrelated: hatching percentage and larval development at the reference concentration, the trendline equation, lethal concentration for 50% (LC50) and minimum inhibitory concentration (MIC). The trend line equation was calculated automatically allowing interpretation of resistance risk level.

RESULTS AND DISCUSSION

The stool tests were based on the morphostructural aspects of stage 3 larvae after 14 days of incubation. In the horse group, a predominance of the small strongyle species, especially those of the species *Cyathostomum* type A was revealed (53.40%) and *Cyathostomum* type D (with a percentage of 25). No species of the *Strongylidae* subfamily were identified, confirming the international data reporting a decreased incidence of these species in horses, along with an increased incidence of small strongyles (Cernea *et al.*, 2010).

In the donkey group, a predominance of small strongyles was observed (*Cyathostomum* type A

29.33%, *Cyathostomum* type D 8%), though along with larger strongyles, mainly *Triodontophorus* spp. (1.33%). Furthermore, a high percentage of *Trichostrongylus axei* L3 larvae was observed (37.33%) (Tab. 1). The differences observed between the two equine species, in terms of digestive strongyle populations, can be explained by owners' choice of excluding the donkeys from anthelmintic treatments. Thereby, due to interspecific contamination, the donkey strongyle species constitute a reservoir, unaltered by the anthelmintic treatments. Consequently, regardless of the deworming treatments applied, the horses can be recontaminated with resistant strongyles derived from the untreated donkeys, through cohabitation.

FECRT was performed using a fenbendazole-based product. Before the treatment (BT) individual stool samples were collected. Afterwards, fenbendazole was administered in a dose of 7,5 mg/kg bw. 14 days post-treatment (PT), individual stool samples were collected once again, determining EPG, the intensity and the extensivity level.

In the horse group, the average intensity BT was 1595.5 EPG, with a maximum of 4000 EPG. The extensivity level BT was 55%. 14 days PT, all stool samples were negative, reflecting the high efficacy of fenbendazole (Tab. 2).

For the donkey group, results were different. The total EPG determined prior to treatment was 6550, with an intensity of 935.7 and an extensivity of 87.5%. The results, 14 days post-treatment, showed an average intensity of 150 EPG and a decline of the extensivity to 50% (Tab. 2). Fenbendazole proved to be less effective compared to the horse group, even if the donkey group has never been treated with anthelmintics. One may speculate that the donkeys were infected with resistant equine strongyles from the horses.

FECRT statistical interpretation presented a different situation between the two groups. Group 1 had a 100 percentage of reduction, upper and lower limits of 95% thrust interval being null.

This result highlights the maximum effectiveness of the fenbendazole anthelmintic treatment and the fact that the strongyle population found in horses was not resistant to fenbendazole.

Statistical analysis of the data obtained in the donkey group BT and PT showed a 90.83

Tab. 1. The infestation degree and the strongyle species found in the two groups

Species	Group 1 - horses			Group 2 - donkeys		
	Total number of identified larvae	Mean	Percent	Total number of identified larvae	Mean	Percent
Cyathostomum A	47	2.35	53.4	22	2.75	29.33
Cyathostomum B	0	0	0	0	0	0
Cyathostomum C	9	0.45	10.2	2	0.25	2.67
Cyathostomum D	22	1.1	25.0	6	0.75	8.00
Cyathostomum E	0	0	0	0	0	0
Cyathostomum F	6	0.3	6.8	5	0.63	6.67
Cyathostomum G	3	0.15	3.4	0	0	0
Cyathostomum H	0	0	0	0	0	0
Gyaloecephalus capitatus	1	0.05	1.1	0	0	0
Oesophagodontus robustus	0	0	0	0	0	0
Poteriostomum	0	0	0	0	0	0
Crateorostomum acuticadatum	0	0	0	0	0	0
Strongylus equines	0	0	0	0	0	0
Strongylus edentatus	0	0	0	0	0	0
Triodontophorus	0	0	0	1	0.13	1.33
Triodontophorus serratus	0	0	0	0	0	0
Strongylus vulgaris	0	0	0	0	0	0
Trichostrongylus axei	0	0	0	28	3.50	37.33
Strongiloides westeri	0	0	0	0	0	0
Free nematodes	10	0.50	11.4	11	1.38	14.67

Tab. 2. The infestation level (EPG) for both groups before treatment (BT) and post-treatment (PT)

Group 1 - horses	EPG BT	EPG PT	Group 2 - donkeys	EPG BT	EPG PT
1	4000	0	1	250	0
2	1400	0	2	1050	0
3	300	0	3	1000	0
4	3700	0	4	200	200
5	0	0	5	2650	100
6	550	0	6	200	100
7	700	0	7	1200	200
8	800	0	8	0	0
9	3400	0	Total	6550 EPG	600 OPG
10	2500	0	Intensity	935.7 EPG	150 OPG
11	100	0	Extensivity	87.50 %	50%
12	100	0			
13	0	0			
14	0	0			
15	0	0			
16	0	0			
17	0	0			
18	0	0			
19	0	0			
20	0	0			
Total	17550 EPG	0			
Intensity	1595.5 EPG	0			
Extensivity	55%	0			

percentage of reduction, lower than the minimum percentage accepted (95%). The confidence interval 95% was 73%. This data showed that the strongyle population found in the donkey group is resistant to fenbendazole.

The Egg Hatch Assay (EHA) for albendazole in horses showed a hatching percentage of 100 at the reference concentration and the LC_{50} 64.32 mcg/ml. The statistical analysis of the trend line equation and parameters a and b showed the correct hatching percentage of 0.3740, much lower than the accepted limit of 50%. The maximal value of Y parameter was -242.42, drawing a negative tendency for the trend line. All data shows the efficacy of albendazole against the strongyle population found in horses (Tab. 3). Similar results were obtained for fenbendazole as well. The LC_{50} was 47.31 mcg/ml and the hatching percentage at the reference concentration was 0.0192. The Y parameter value was way lower than for albendazole, reaching -257.17 and inducing a negative tendency for the trend line equation. This test confirms the results obtained by FECRT proving the fenbendazole efficacy.

EHA showed a totally different situation for mebendazole compared to albendazole and

fenbendazole. The hatching percentage at the reference concentration was 105.10, showing the weak inhibitory capacity of this active ingredient against strongyle eggs. Even if LC_{50} had a negative value of -0.1453 mcg/ml, corroborating the data obtained at the hatching percentage shows us that, in order to obtain a positive value for the LC_{50} , the test should be made with active ingredient concentrations higher than 5 mcg/ml. These results have no practical relevance because of the too high dosage. The trend line equation had a high positive tendency, the maximal value for Y parameter being 1009.92. Integrated analysis of obtained values shows a weak efficacy of mebendazole over strongyle population found in horses and the risk of inducing the adaptive phenomena for this substance.

EHA thiabendazole testing showed the best results, but we have to take in consideration the fact that, because of its capacity to delay egg hatch, this active ingredient was tested only as standard (Cernea *et al.*, 2010). The egg hatch percentage at the reference concentration was 11.62, with LC_{50} of 0.0086 mcg/ml and the trend line equation with a powerful negative tendency (Y parameter = -1,305.1).

Tab. 3. Group 1 EHA results for ABZ and FBZ

Sample	A.i. conc. (mcg/ml)	ABZ				FBZ			
		No. of eggs	L1	Hatching %	Trend line equation	No. of eggs	L1	Hatching %	Trend line equation
1	5.0000	150	0	0.00	-242.42	50	50	50.00	-257.17
2	2.5000	50	0	0.00	-84.25	50	50	50.00	-100.22
3	1.2500	100	0	0.00	-5.16	0	150	100.00	-21.75
4	0.6250	150	0	0.00	34.39	0	150	100.00	17.49
5	0.3125	50	100	66.67	54.16	100	50	33.33	37.11
6	0.1563	0	100	100.00	64.04	100	0	0.00	46.92
7	0.0781	0	50	100.00	68.99	50	0	0.00	51.83
8	0.0391	0	100	100.00	71.46	0	50	100.00	54.28
9	0.0195	50	50	50.00	72.70	0	50	100.00	55.51
10	0.0098	50	50	50.00	73.31	100	0	0.00	56.11
HCL	0.0000	0	100	100.00	73.93	0	200	100.00	56.73
H2O	0.0000	0	50	100.00	0.00	100	0	0.00	0.00
Total		600	600	55.56		550	750	52.78	

Parameters	LC 50 (mcg)	Ref. 0.15 mcg/ ml (%)	Parameters	LC 50 (mcg)	Ref. 0.15 mcg/ ml (%)
a	-63.27	64.3208	a	-62.78	47.3100
b	73.93	0.3740	b	56.73	0.0192

Note: A.i. conc. = active ingredient concentration, LC_{50} = lethal concentration 50%,

Even if the donkey and the horse group live in the same environment, EHA results for the two groups were totally different. This fact can be explained by the lack of anthelmintic treatments in the donkey group, therefore obtaining different pharmacodynamic results.

The albendazole EHA showed a hatching percentage of 111.67 at the reference concentration and LC_{50} -0.54 mcg/ml. The trend line tendency was positive reaching a maximum value of 541.82 (Tab. 5). This results show that albendazole has a reduced efficacy against donkey strongyles, determining a major predictive risk of inducing the resistance phenomena.

Fenbendazole EHA presented 94.05 egg hatching percentage, for the reference concentration, a lot higher than the accepted value of 50%. LC_{50} was 2.43 mcg/ml, the Y parameter having a value of 0.69. Even if the trend line had a downward tendency, it is not negative, showing a substantial risk of strongyles to develop adaptative phenomena against this active ingredient.

For mebendazole, in the donkey group, EHA showed an egg hatching percentage of 87.51 at the reference concentration, LC_{50} being 1.20 mcg/ml. These results showed a reduced efficacy. Even so, compared to albendazole and fenbendazole

tests, the risk of strongyles developing adaptive phenomena is lower. The Y parameter showed a value of -85.15 and the trend line had a negative tendency. For thiabendazole the results were similar, the egg hatching percentage being 73.93 and the LC_{50} -0.085 µg/ml, with a highly negative trend line tendency ($Y = -1,840.17$).

Larval development assay showed similar values in the horse group. MIC value was the same (0.0489 mcg/ml). The sole difference noted regarded the Y parameter. For albendazole, this value was -525.46, for fenbendazole -849.21 and -581.11 for mebendazole and thiabendazole (Tab. 7 and 8). In horses, all LDA results showed a good efficacy of benzimidazoles against strongyle eggs and larvae (Tab. 7 and 8).

For all benzimidazoles, the risk of resistance is negligible, for fenbendazole being the lowest ($Y = -849.21$). Therefore, treatment efficacy would be greater if the therapeutic protocol would consider a sequential administration for 2-3 days or repeating it at 7-14 days. For the donkey group, LDA had similar results as for the horse group. The Y parameter had a value of -1104.10 for albendazole, mebendazole and thiabendazole, and lower for fenbendazole (-1226.83) (Tab. 9 and 10).

Tab. 4. Group 1 EHA results for MBZ and TBZ

Sample no.	A.i. conc. (mcg/ml)	MBZ				TBZ			
		No.of eggs	L1	Hatching %	Trend line equation	No.of eggs	L1	Hatching %	Trend line equation
1	5.0000	150	100	40.00	1,009.92	500	0	0.00	-1,305.1
2	2.5000	0	150	100.00	543.52	500	50	9.09	-626.38
3	1.2500	100	450	81.82	310.32	250	0	0.00	-287.01
4	0.6250	0	100	100.00	193.72	450	0	0.00	-117.33
5	0.3125	0	100	100.00	135.42	400	0	0.00	-32.49
6	0.1563	0	50	100.00	106.28	350	0	0.00	9.92
7	0.0781	0	100	100.00	91.69	250	0	0.00	31.15
8	0.0391	0	50	100.00	84.41	250	50	16.67	41.73
9	0.0195	100	50	33.33	80.76	0	100	100.00	47.06
10	0.0098	0	50	100.00	78.95	0	100	100.00	49.69
HCL	0.0000	100	0	0.00	77.12	0	150	100.00	52.35
H2O	0.0000	50	50	50.00	0.00	50	100	66.67	0.00
Total		500	1250	75.43		3000	550	32.70	
		Parameters	LC 50 (mcg)	Ref. 0.15 mcg/ml (%)		Parameters	LC 50 (mcg)	Ref. 0.15 mcg/ml (%)	
		a	186.56	-0.1453	105.1000	a	-271.49	0.0086	11.62
		b	77.12			b	52.35		

Note: A.i. conc. = active ingredient concentration, LC_{50} = lethal concentration 50%,

Minimum inhibitory concentration for all benzimidazoles was 0.0489 mcg/ml, proving the fact that, prolonging the contact time between exogenous parasitic forms and anthelmintics, a

much higher effectiveness is obtained. At the same time the predictive risk of resistance is greatly diminished, Y parameter showing a highly negative trend line tendency.

Tab. 5. Group 2 EHA results for ABZ and FBZ

Sample no.	A.i. conc. (mcg/ml)	ABZ				FBZ			
		No. of eggs	L1	Hatching %	Trend line equation	No. of eggs	L1	Hatching %	Trend line equation
1	5.0000	150	0	0.00	541.82	0	50	100.00	0.69
2	2.5000	0	50	100.00	320.10	0	100	100.00	48.82
3	1.2500	0	50	100.00	209.23	0	150	100.00	72.88
4	0.6250	0	50	100.00	153.80	0	150	100.00	84.91
5	0.3125	0	100	100.00	126.09	0	50	100.00	90.92
6	0.1563	0	100	100.00	112.23	50	100	66.67	93.93
7	0.0781	0	50	100.00	105.30	0	100	100.00	95.44
8	0.0391	0	150	100.00	101.84	0	100	100.00	96.19
9	0.0195	0	100	100.00	100.10	0	150	100.00	96.56
10	0.0098	0	50	100.00	99.24	0	200	100.00	96.75
HCL	0.0000	0	150	100.00	98.37	0	50	100.00	96.94
H2O	0.0000	50	50	50.00	0.00	0	350	100.00	0.00
Total		200	900	87.50		50	1550	97.22	

Parameters	LC 50 (mcg)	Ref. 0.15 mcg/ ml (%)	Parameters	LC 50 (mcg)	Ref. 0.15 mcg/ ml (%)
a	88.69	-0.5453	a	-19.25	2.4382
b	98.37		b	96.94	94.0555

Note: A.i. conc. = active ingredient concentration, LC 50 = lethal concentration 50,

Tab. 6. Group 2 EHA results for MBZ and TBZ

Sample no.	A.i. conc. (mcg/ml)	MBZ				TBZ			
		No. of eggs	L1	Hatching %	Trend line equation	No. of eggs	L1	Hatching %	Trend line equation
1	5.0000	0	50	100.00	-85.15	50	0	0.00	-1,840.17
2	2.5000	0	50	100.00	3.85	150	0	0.00	-911.00
3	1.2500	0	250	100.00	48.35	50	0	0.00	-446.41
4	0.6250	0	50	100.00	70.60	50	0	0.00	-214.11
5	0.3125	50	0	0.00	81.73	50	0	0.00	-97.97
6	0.1563	0	100	100.00	87.29	50	0	0.00	-39.91
7	0.0781	0	100	100.00	90.07	150	0	0.00	-10.85
8	0.0391	0	100	100.00	91.46	50	0	0.00	3.65
9	0.0195	0	150	100.00	92.16	50	0	0.00	10.93
10	0.0098	0	50	100.00	92.50	50	0	0.00	14.54
HCL	0.0000	0	100	100.00	92.85	0	50	100.00	18.18
H2O	0.0000	0	50	100.00	0.00	0	50	100.00	0.00
Total		50	1050	91.67		700	100	16.67	

Parameters	LC 50 (mcg)	Ref. 0.15 mcg/ ml (%)	Parameters	LC 50 (mcg)	Ref. 0.15 mcg/ ml (%)
a	-35.6	1.2035	a	-371.67	-0.0856
b	92.85		b	18.18	73.9300

Note: A.i. conc. = active ingredient concentration, LC 50 = lethal concentration 50,

CONCLUSIONS

In the horse group, tested stool samples showed the predominance of small strongyles, especially those of *Cyathostomum* type A, *Cyathostomum* type D and *Cyathostomum* type

C. After the fenbendazole treatment, all stool samples were negative, showing the high efficacy of this active ingredient.

In the donkey group, we found *Trichostrongylus axei*, *Cyathostomum* type A, *Cyathostomum* type

Tab 7. Group 1 LDA results for ABZ and FBZ

A.i. conc. (mcg/ml)	Sample no.	ABZ				FBZ			
		Eggs + L1 + L2	L3	Development %	Trend line equation	Eggs + L1 + L2	L3	Development %	Trend line equation
5.0000	1	250	0	0.00	-525.46	650	0	0.00	-849.21
2.5000	2	400	0	0.00	-260.14	150	0	0.00	-420.41
1.2500	3	50	0	0.00	-127.47	350	0	0.00	-206.01
0.6250	4	300	0	0.00	-61.14	450	0	0.00	-98.81
0.3125	5	350	0	0.00	-27.98	350	0	0.00	-45.21
0.1563	6	150	0	0.00	-11.40	300	0	0.00	-18.42
0.0781	7	650	0	0.00	-3.10	450	0	0.00	-5.01
0.0391	8	250	0	0.00	1.04	850	0	0.00	1.68
0.0195	9	100	0	0.00	3.12	250	0	0.00	5.05
0.0098	10	300	0	0.00	4.15	400	0	0.00	6.71
0.0000	Average H ₂ O +HCl	375	150	28.57	5.19	175	150	46.15	8.39
Total		3175	150	2.60		4375	150	4.20	
MIC - mcg		Parameters				Parameters			
0.0489		a				a			
		-106.13				-171.52			
		b				b			
		5.19				8.39			

Note: A.i. conc. = active ingredient concentration, MIC = Minimum inhibitory concentration

Tab 8. Group 1 LDA results for MBZ and TBZ

A.i. conc. (mcg/ml)	Sample no.	MBZ				TBZ			
		Eggs + L1 + L2	L3	Development %	Trend line equation	Eggs + L1 + L2	L3	Development %	Trend line equation
5.0000	1	100	0	0.00	-581.11	400	0	0.00	-581.11
2.5000	2	150	0	0.00	-287.69	450	0	0.00	-287.69
1.2500	3	200	0	0.00	-140.97	650	0	0.00	-140.97
0.6250	4	350	0	0.00	-67.62	250	0	0.00	-67.62
0.3125	5	800	0	0.00	-30.94	250	0	0.00	-30.94
0.1563	6	300	0	0.00	-12.60	300	0	0.00	-12.60
0.0781	7	400	0	0.00	-3.43	100	0	0.00	-3.43
0.0391	8	350	0	0.00	1.15	50	0	0.00	1.15
0.0195	9	150	0	0.00	3.45	200	0	0.00	3.45
0.0098	10	150	0	0.00	4.59	350	0	0.00	4.59
0.0000	Average H ₂ O +HCl	325	150	31.58	5.74	325	150	31.58	5.74
Total		3275	150	2.87		3325	150	2.87	
MIC - mcg		Parameters				Parameters			
0.0489		a				a			
		-117.37				-117.37			
		b				b			
		5.74				5.74			

Note: A.i. conc. = active ingredient concentration, MIC = Minimum inhibitory concentration

D, but also *Triodontophorus* spp. Fenbendazole treatment had a reduced efficacy, the average intensity being 150 EPG and the extensivity 50%.

EHA revealed the efficacy of albendazole, fenbendazole and thiabendazole against the strongyles found in the horse group and the poor efficacy of mebendazole together with the risk of inducing adaptive phenomena. In the

donkey group, EHA showed a low efficacy for all benzimidazoles against the tested strongyles.

LDA revealed that, in both groups, benzimidazoles inhibited larval development, with high efficacy.

Predictive risk analysis showed the fact that new therapeutic protocols that provide a prolonged contact of the benzimidazoles with the parasitic forms should be performed. Anthelmintic

Tab 9. Group 2 LDA results for ABZ and FBZ

A.i. conc. (mcg/ml)	Sample no.	ABZ				FBZ			
		Eggs + L1 + L2	L3	Development %	Trend line equation	Eggs + L1 + L2	L3	Development %	Trend line equation
5.0000	1	50	0	0.00	-1,104.10	50	0	0.00	-1,226.83
2.5000	2	50	0	0.00	-546.60	50	0	0.00	-607.36
1.2500	3	100	0	0.00	-267.85	50	0	0.00	-297.62
0.6250	4	50	0	0.00	-128.48	100	0	0.00	-142.75
0.3125	5	50	0	0.00	-58.79	50	0	0.00	-65.31
0.1563	6	150	0	0.00	-23.95	100	0	0.00	-26.61
0.0781	7	200	0	0.00	-6.52	100	0	0.00	-7.23
0.0391	8	150	0	0.00	2.18	100	0	0.00	2.43
0.0195	9	50	0	0.00	6.55	150	0	0.00	7.29
0.0098	10	150	0	0.00	8.71	200	0	0.00	9.69
0.0000	Average H2O +HCl	100	150	60.00	10.90	75	150	66.67	12.12
	Total	1100	150	5.45		1025	150	6.06	
MIC - mcg		Parameters				Parameters			
0.0489		a				a			
		-223				-247.79			
		b				b			
		10.9				12.12			

Note: A.i. conc. = active ingredient concentration, MIC = Minimum inhibitory concentration

Tab 10. Group 2 LDA results for MBZ and TBZ

A.i. conc. (mcg/ml)	Sample no.	MBZ				TBZ			
		Eggs + L1 + L2	L3	Development %	Trend line equation	Eggs + L1 + L2	L3	Development %	Trend line equation
5.0000	1	100	0	0.00	-1,104.10	250	0	0.00	-1,104.10
2.5000	2	50	0	0.00	-546.60	100	0	0.00	-546.60
1.2500	3	50	0	0.00	-267.85	50	0	0.00	-267.85
0.6250	4	100	0	0.00	-128.48	200	0	0.00	-128.48
0.3125	5	50	0	0.00	-58.79	150	0	0.00	-58.79
0.1563	6	50	0	0.00	-23.95	100	0	0.00	-23.95
0.0781	7	100	0	0.00	-6.52	100	0	0.00	-6.52
0.0391	8	150	0	0.00	2.18	100	0	0.00	2.18
0.0195	9	50	0	0.00	6.55	100	0	0.00	6.55
0.0098	10	100	0	0.00	8.71	200	0	0.00	8.71
0.0000	Average H2O +HCl	100	150	60.00	10.90	100	150	60.00	10.90
	Total	900	150	5.45		1450	150	5.45	
MIC - mcg		Parameters				Parameters			
0.0489		a				a			
		-223				-223			
		b				b			
		10.9				10.9			

Note: A.i. conc. = active ingredient concentration, MIC = Minimum inhibitory concentration

treatments should be performed regularly and verified using both *in vitro* and *in vivo* tests.

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