Supplementary Material

Nano-encapsulation of Halofuginone Hydrobromide Enhances the Anticoccidial Activity Against *Eimeria tenella* in Chickens

Mengjuan Lin[a,b,#], Xinhao Song[a,b,#], Runan Zuo[a,b], Yuling Zheng[a,b], Shiheng Hu[a,b], Shasha Gao[a,b], Lu Chen[a,b], Yuan Zhu[a,b], Xiaolin Xu[a,b], Moxin Liu[a,b], Junren Zhang[a,b], Shanxiang Jiang[a,b], Dawei Guo[a,b,*]

[a] Engineering Center of Innovative Veterinary Drugs, Center for Veterinary Drug Research and Evaluation, Nanjing Agricultural University, 1 Weigang, Nanjing 210095, China

[b] MOE Joint International Research Laboratory of Animal Health and Food Safety, College of Veterinary Medicine, Nanjing Agricultural University, 1 Weigang, Nanjing 210095, China

[#] These authors equally contributed to this paper.

* Dr. Dawei Guo is the corresponding author.

Correspondence:

Dawei Guo, Ph.D., Associate Professor

Engineering Center of Innovative Veterinary Drugs,

Nanjing Agricultural University,

1 Weigang, Nanjing 210095, PR China

Email: gdawei0123@njau.edu.cn

Tel: +86-25-84396215

Fax: +86-25-84398669

Supplementary Data

Table S1 Weight gain of chickens in each experimental group

Group	Mean initial weight (g)	Mean final weight (g)	Weight gain rate (%)	Relative weight gain rate (%)
1	82.46±9.78	122.99±17.27	49.16	100.00
2	77.65±11.81	103.24±18.78	33.04	67.22
3	81.02±9.87	123.35±15.20	52.24	106.27
4	82.85±9.32	122.56±17.72	47.93	97.51
5	82.7±9.47	127.16±15.25	53.77	109.38
6	79.82±12.65	120.61±35.13	53.05	107.92

Note: 1. Negative control group; 2. Infected and non-administered group; 3. Infected and treated with 3 mg/kg HF premix group; 4. Infected and treated with 3 mg/L HTPM group; 5. Infected and treated with 1.5 mg/L HTPM group; 6. Infected and treated with 0.75 mg/L HTPM group.

Table S2 Conversion standard for value of cecal contents after *E.tenella* infection

Number of oocysts per gram of cecal contents ($\times 10^6$)	Oocyst value
0.0~0.1	0
0.11~1.0	5
1.10~1.9	10
2.0~5.9	20
6.0~10.9	30
≥11.0	40

Table S3 Characterization of as-prepared HTPM

Indicators	HD (nm)	PDI	ZP (mV)	EE (%)	DL (%)	Solubility (mg/mL)
HTPM	12.65±0.089	0.274±0.008	8.03±0.242 mV	71.1 ± 0.85	14.04 ± 0.46	34.4 ± 1.68

Table S4 The K_E values of HTPM at 4 ℃ and 25 ℃

Centrifugal time (min)	4 ℃	25 ℃
0	-	-
5	0.049±0.021	0.056±0.018
10	0.034±0.028	0.050±0.011
20	0.041±0.009	0.046±0.010
30	0.074±0.014	0.083±0.044

Table S5 Grade estimation of coccidiostats according to ACI values

ACI	＞180	160-180	120-160	＜120
Grade of coccidiostats	Highly effective	Moderately effective	Inefficient	Invalid

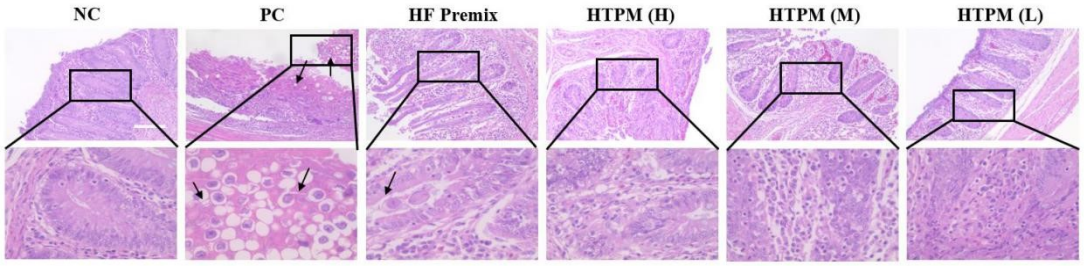

Fig. S1 Histopathological observation of cecum in NC, PC, HF premix (3 mg/kg), HTPM (H) (3 mg/L), HTPM (M) (1.5 mg/L) and HTPM (L) (0.75 mg/L) groups, respectively. Scale bar = 50 μm.