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COMPARATIVE ANTIPARASITIC EFFECTIVENESS OF THE COMBINED DRUG AND NIFULIN IN THE TREATMENT OF BLASTOCYSTOSIS AND HISTOMONIASIS IN TURKEYS

Bogach M. V. [1], Rachynskyi A. S. [1], Dunaiev Yu. K. [1], Bohach O. M. [2]

[1] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: bogach_nv@ukr.net
[2] Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine, Lviv, Ukraine

Summary. For decades, effective drugs have been used to prevent and treat protozoosis in Europe, the United States, and other countries. However, these drugs were subsequently withdrawn from use for consumer safety reasons. Since then, research has focused on finding alternative approaches, ranging from new chemotherapeutic agents to plant-based compounds. Using phytogenic substances in turkey feed is considered promising because they are effective and act as antioxidants. This study aimed to compare the effectiveness of domestic antiprotozoal drugs and a newly developed drug containing plant components against protozoosis in turkeys. The developed combined drug, which includes the antiprotozoal substances metronidazole and furazolidone, as well as the plant components garlic powder (*Allium sativum*) and tansy (*Tanacetum vulgare*), demonstrated a rapid and sustained reduction in mean infection intensity in turkeys, particularly on days 7–14 of treatment. The combined drug's effectiveness exceeded that of Nifulin, evidenced by a significant reduction in pathogen numbers and complete *Blastocystis* spp. elimination by day 14. By day 7, the intensity of the infection decreased by more than 78%. By day 14, the infection was completely eradicated (prevalence — 0%, effectiveness — 100%). When Nifulin was used, however, 21.4% of turkeys remained infected. The drug also provided higher effectiveness at all stages of histomoniasis treatment. On day 14, only 7.1% of turkeys were infected, compared to 14.3% in the Nifulin group. The respective effectiveness was 92.9% and 78.6%.

Keywords: antiprotozoal drugs, metronidazole, furazolidone, *Allium sativum*, *Tanacetum vulgare*

Introduction. The gastrointestinal tract is the body's largest exposed surface and is constantly exposed to various potentially harmful substances. It acts as a selective barrier between poultry tissues and their internal environment (Yegani and Korver, 2008).

Gastrointestinal parasites are a major problem in poultry farming because they cause significant economic losses due to reduced productivity, poor feed conversion, poor weight gain, reduced egg production, loss of appetite, diarrhea, intestinal obstruction, exhaustion, anemia, paralysis, plumage disorders, and even death (Jegede et al., 2015; Divyamery et al., 2016; Balarabe et al., 2017).

The most common etiological agents affecting the gastrointestinal tract of farm poultry are helminths (e.g., nematodes, cestodes, and trematodes) and protozoa. These agents can cause significant disturbances in physiological functions and reduce the overall productivity of the flock (Bayzid et al., 2023; Bogach et al., 2024).

Histomonas meleagridis is a protozoan parasite that primarily affects turkeys and chickens, causing a disease known as histomoniasis. The severity of the disease varies by host species. In turkeys, it often leads to high mortality, whereas in chickens, mortality is usually much lower (Mitra et al., 2018).

According to Hauck and Hafez (2013), mortality in turkeys can reach 100%, as demonstrated in several experimental studies.

The pathogenesis of histomoniasis begins with the parasite colonizing the cecum, which leads to severe inflammation and necrosis. After destroying intestinal tissue, the parasite can enter blood vessels and reach the liver via the portal vein. Consequently, areas of inflammation and destruction can occur in the liver. In the final stage, the disease becomes systemic, and the parasite spreads to various organs of the host (Grabensteiner et al., 2006).

Among drugs with antibiotic and antiprotozoal activity, nitroimidazoles were the only substances that were completely effective against *H. meleagridis*. During the studies, nine drugs were tested both *in vitro* and *in vivo* for their activity against *H. meleagridis*. Nitroimidazoles — dimethridazole, metronidazole, ornidazole, and tinidazole — inhibited parasite growth *in vitro* at concentrations of 10 μg/ml and above. In contrast, paromomycin sulfate and carbadox showed weak efficacy only at high concentrations. Quinolinol, mebendazole, diloxanide furoate, and albendazole did not demonstrate proven efficacy *in vitro* (Hu and McDougald, 2004; Hauck, Lotfi and Hafez, 2010).

For decades, histomoniasis could be effectively controlled with potent preventive and treatment drugs; however, these chemicals were subsequently withdrawn from use in Europe, the United States, and other countries due to consumer protection concerns (CEC, 1995; McDougald, 2005; McDougald et al., 2020).

Effective chemotherapeutic agents, such as nifursol and nitroimidazoles, are no longer permitted in the United States or the European Union. Additionally, the European Union banned arsenic compounds (Baynes et al., 2016).

The treatment of experimentally infected turkeys with *H. meleagridis* using paromomycin at doses of 400 mg/kg or 200 mg/kg in feed or 420 mg/l in water significantly reduced mortality and decreased the severity of cecal and liver lesions (Bleyen et al., 2009).

Blastocystis has been found in many animal species, including mammals, birds, and amphibians (Parkar et al., 2010; Bohach et al., 2023; Bogach, Paliy, and Bohach, 2023).

There is still debate about the advisability of treatment due to limited knowledge about this parasite, as the infection may be opportunistic in nature (Biedermann et al., 2002).

The discontinuation of effective chemotherapeutic drugs in recent decades due to concerns about consumer safety has led to frequent disease outbreaks, threatening the welfare of poultry and causing significant economic losses to the poultry industry. Recent studies have examined various approaches to combating the disease, including alternative chemotherapeutic agents and plant-derived compounds (Liebhart et al., 2017).

In many countries, government agencies regulate the use of drugs for poultry raised for food to eliminate or minimize side effects and ensure consumer protection (FDA, 2025).

Following the European Union's ban on growth-promoting antibiotics, research on methods to improve intestinal health has intensified. Many researchers have described the positive effects of plant compounds on poultry health, and the practical application of these compounds shows promise in poultry farming. Research has proven that adding plant-based feed additives protects birds from environmental threats that lead to intestinal barrier dysfunction. Phytogenic feed additives can improve the overall structure of the intestinal mucosa and the intestinal barrier's function at the molecular level (Latek et al., 2022; Bozkurt and Tüzün, 2020).

Alternative strategies for controlling protozoan infections are being developed to reduce the use of veterinary drugs. Examples of these alternative therapeutic options include natural treatments such as prebiotics and probiotics, plant and fungal extracts, and essential oils. While natural compounds typically do not directly combat parasites, they affect the microflora of the gastrointestinal tract and strengthen the immune system (Abd El-Hack et al., 2022).

Using phytogenic substances in turkeys' diets is promising because they are effective and can act as antioxidants (Bozkurt and Tüzün, 2020).

Essential oils are considered promising preventive and therapeutic agents against a number of flagellate parasites of poultry. In particular, the antiprotozoal activity of essential oils obtained from fresh leaves of *Cinnamomum aromaticum*, pericarp of *Citrus limon*, and bulbs of *Allium sativum* was studied. *In vitro* experiments have established their effect on *Tetratrichomonas gallinarum* and *H. meleagridis*. The results of the study indicate that the effective concentrations of essential oils required to inhibit *T. gallinarum* and *H. meleagridis* differ significantly (Zenner et al., 2003).

The use of herbal preparation containing extracts of cinnamon, garlic, lemon, and rosemary reduced the mortality of turkeys infected with *H. meleagridis* to 20%, while in the control infected group it was 50% (Hafez and Hauck, 2006).

In the context of modern research on natural antiprotozoal agents, 43 plant substances and extracts (aqueous, ethanolic, and heptanic) from 18 types of food and feed industry organic waste were studied. Their activity was tested *in vitro* against *H. meleagridis*, *T. gallinarum*, and *Blastocystis* spp., with the highest efficacy shown by ethanol extracts of thyme, serenoa, grape seeds, and pumpkin. These results indicate the potential of these extracts for preventing and treating parasitic infections in poultry (Grabensteiner et al., 2008).

Despite *Blastocystis*' widespread distribution as an intestinal parasite, the issue of its pathogenicity and effective treatment remains controversial. Metronidazole is the most common therapy, but cases of ineffectiveness and drug resistance have been reported. Paromomycin and trimethoprim-sulfamethoxazole are being considered as alternative treatments (Roberts et al., 2014).

The study **aimed** to compare the effectiveness of domestic antiprotozoal drugs and a developed drug with herbal ingredients for treating protozoosis in turkeys.

Materials and methods. Studies were conducted in the vivarium of the Odesa Research Station of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' to determine the therapeutic effectiveness of antiprotozoal drugs for mixed blastocystosis and histomoniasis in turkeys. The drugs' effectiveness was evaluated using forty-two 45-day-old 'Big-6' breed turkeys that were spontaneously infected with *Blastocystis* spp. and *H. meleagridis*. Two experimental and control groups were formed from these turkeys (n = 14).

The research was conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and under Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine).

A microscopic examination was performed to detect *Blastocystis* spp. and *H. meleagridis* in turkeys. Fresh fecal samples were applied to clean, degreased microscope slides and prepared as native smears. The smears were fixed with ethanol for five to ten minutes and stained using the Romanowski–Giemsa method. Then, the slides were examined under a microscope at

630× magnification (90× objective, 7× eyepiece). In the stained smears, the nuclei and flagella of the histomonads appeared red while the cytoplasm appeared blue, allowing for clear visualization of the pathogen.

45-day-old turkeys from the first experimental group were administered an antiprotozoal drug complex (ORS NSC 'IECVM', experimental sample) in powder form at a dose of 2 g/kg of feed for seven days for therapeutic purposes. The turkeys in the second experimental group were given Nifulin (powder) (OLKAR) at a dose of 1 g/kg of feed for seven days for therapeutic purposes. The turkeys in the control group were infected and did not receive any drugs. The dynamics of turkey infection with blastocysts and histomonads, as well as the effectiveness of the drugs, were determined on the third, seventh, and fourteenth days.

The intensity of *Blastocystis* spp. infection was assessed based on the number of parasites observed in a microscope field of view and the presence of clinical manifestations. Low intensity was defined as fewer than five parasites in a field of view and a subclinical course with minor symptoms. Moderate intensity was defined as five to ten parasites and moderately pronounced symptoms, including digestive disorders. High intensity was defined as more than ten parasites and symptoms such as diarrhea, weight loss, and exhaustion, which pose a risk of death to the bird (Tan, 2008).

The intensity of *H. meleagridis* infection was determined using a scale that approximates the number of trophozoites in a microscope field of view: low (1–2 trophozoites; asymptomatic or mild symptoms); moderate (3–10 trophozoites; diarrhea, depression); and high (> 10 trophozoites; pronounced clinical signs, risk of bird death) (McDougald, 2005; Clark and Kimminau, 2017).

A statistical analysis of the data was performed using a free trial version of Minitab 19 software from Minitab, Inc. The arithmetic mean (M) and the standard error of the mean (m) were calculated. The probability of a difference between the means of two series of variations was assessed using the reliability criterion (td), Student's t distribution tables, and the nonparametric Van der Waerden method.

Results. A complex drug containing the antiprotozoal substances metronidazole and furazolidone, as well as the plant components garlic powder (*Allium sativum*) and tansy (*Tanacetum vulgare*), has developed at the Laboratory of Epizootology, Parasitology, Animal Disease Monitoring, and Providing of the Odesa Research Station of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'. This drug is intended to treat protozoal diseases in poultry, particularly cryptosporidiosis, histomoniasis, eimeriosis, tetratrichomoniasis, and blastocystosis.

Due to the ban on traditional drugs for the treatment of histomoniasis, there is growing interest in alternative remedies. Carvacrol, cassia oil, a mixture of thyme and rosemary essential oils, and *Quillaja saponaria* saponin exhibit pronounced antiprotozoal activity against *H. meleagridis* (minimal inhibitory concentration (MIC) 0.25–0.50 µg/ml) and various isolates of *Blastocystis* spp. (Grabensteiner, Arshad, and Hess, 2007).

Before treatment, the intensity of *Blastocystis* spp. infection in 45-day-old turkeys ranged from 7.2 ± 0.1 to 7.6 ± 0.2 cysts per field of view (FOV), while the intensity of *H. meleagridis* infection ranged from 8.9 ± 0.2 to 9.1 ± 0.2 trophozoites per FOV (Table 1).

Table 1 — Intensity of *Blastocystis* spp. and *H. meleagridis* infection during treatment of 45-day-old turkeys (n = 14, M ± m)

Day	Pathogen	Intensity, number of pathogens in the field of view of the microscope		
		Groups of turkeys treated with		Control
		Developed drug	Nifulin	
Before treatment	*Blastocystis* spp.	7.2 ± 0.1	7.6 ± 0.2	7.6 ± 0.1
	H. meleagridis	9.1 ± 0.2	8.9 ± 0.2	9.0 ± 0.1
3	*Blastocystis* spp.	6.1 ± 0.1***	7.2 ± 0.1*	7.9 ± 0.2
	H. meleagridis	8.2 ± 0.1***	8.1 ± 0.2*	9.1 ± 0.1
7	*Blastocystis* spp.	–	5.1 ± 0.1***	8.2 ± 0.1
	H. meleagridis	1.1 ± 0.1***	3.9 ± 0.1***	9.8 ± 0.2
14	*Blastocystis* spp.	–	2.1 ± 0.1***	8.5 ± 0.1
	H. meleagridis	–	2.0 ± 0.1***	10.1 ± 0.1

Note:s * — $p < 0.05$, *** — $p < 0.001$ — compared to day before treatment.

Three days after the administration of the complex drug, the intensity of *Blastocystis* spp. infection decreased significantly ($p < 0.001$) by 15.3%, while the intensity of *H. meleagridis* infection decreased by 9.9%. By the seventh day, *Blastocystis* cysts were undetectable in fecal samples, while the number of *H. meleagridis* trophozoites was 1.1 ± 0.1 in the FOV ($p < 0.001$), indicating a low level of infection (fewer than five parasites) and a subclinical course. By day 14 after treatment, neither *Blastocystis* spp. cysts nor *H. meleagridis* trophozoites were detected in the experimental turkeys, indicating the pathogens were eliminated by the drug.

After the use of Nifulin, on the third day, a significant ($p < 0.05$) decrease in the number of *Blastocystis* spp. cysts by 5.3% and *H. meleagridis* trophozoites by 9.0% ($p < 0.05$) was observed. On the 7th day, the intensity of infection decreased significantly ($p < 0.001$) by 32.9% for *Blastocystis* spp. and by 56.2% ($p < 0.001$) for *H. meleagridis*. After 14 days, the number of *Blastocystis* spp. decreased significantly ($p < 0.001$) by 72.4% to 2.1 ± 0.1 cysts in FOV, indicating a low level of invasion (less than 5 parasites, subclinical course). During this period, the intensity of *H. meleagridis* infection reliably decreased ($p < 0.001$) by 77.5% to 2.0 ± 0.1 trophozoites in FOV. Meanwhile, turkeys exhibited mild clinical signs, including slight depression, moderate decrease in appetite, and slight growth retardation.

In the control group of untreated turkeys, the intensity of *Blastocystis* spp. infection gradually increased from an average of 7.6 ± 0.1 to 8.5 ± 0.1 cysts in the FOV. This level of infection corresponds to the average range of 5–10 parasites in FOV and was clinically manifested by moderate symptoms, including digestive disorders. Concurrently, the number of *H. meleagridis* trophozoites increased from 9.0 ± 0.1 to 10.1 ± 0.1 in FOV, indicating a high level of infection (more than 10 trophozoites in FOV). The turkeys exhibited pronounced clinical signs, including diarrhea, weakness, and weight loss.

Thus, 14 days after treatment, the degree of reduction in infection intensity for the developed complex drug was 100% for both *Blastocystis* spp. and *H. meleagridis*, indicating the complete elimination of the pathogens from turkeys' intestines. In the group that received Nifulin, the reduction in infection intensity was 72.4% against *Blastocystis* spp. and 77.5% against *H. meleagridis*, indicating noticeable but incomplete suppression of the invasion.

According to Yakoob et al. (2011), metronidazole at a concentration of 0.01 mg/mL reduced the number of *B. hominis* cysts in the microscope field of view to approximately 12 cysts, and at 0.1 mg/ml, the number decreased to approximately 1–2 cysts. Garlic had an even more pronounced effect, decreasing the number of cysts in the field of view from 30 to 1 at 0.01 mg/ml and practically eliminating them (0–0.1 cysts) at 0.1 mg/ml. White cumin and black pepper reduced the level of infection to approximately 10 cysts in FOV at 0.1 mg/ml. Ginger reduced the number of *B. hominis* cysts to approximately 13 in FOV, indicating moderate activity.

After the birds in the first experimental group were treated on day 3, *Blastocystis* spp. cysts were detected in nine turkeys and *H. meleagridis* trophozoites in eight turkeys (Table 2).

Table 2 — Effectiveness of drugs against blastocystosis and histomoniasis in 45-day-old turkeys (n = 14)

Groups	Pathogen	Prevalence, %			Effectiveness of the drug, %		
		Day					
		3	7	14	3	7	14
I experimental	*Blastocystis* spp.	64.3	–	–	35.7	100.0	100.0
	H. meleagridis	57.1	14.3	–	42.9	85.7	100.0
II experimental	*Blastocystis* spp.	78.6	42.9	21.4	21.4	57.1	78.6
	H. meleagridis	71.4	35.7	14.3	28.6	64.3	78.6
Control	*Blastocystis* spp.	100.0	100.0	100.0	–	–	–
	H. meleagridis	100.0	100.0	100.0	–	–	–

By day 7, *Blastocystis* spp. were no longer present in the experimental turkeys, suggesting that the drug was 100% effective against this pathogen. Meanwhile, the drug was 85.7% effective against *H. meleagridis*. By the 14th day of the study, the drug was 100% effective against both pathogens, indicating complete eradication of these protozoan infections in turkeys.

In the second experimental group, *Blastocystis* spp. cysts were detected in 11 turkeys, and *H. meleagridis* trophozoites were detected in 10 turkeys after the use of Nifulin on the third day. By the seventh day, the prevalence of both pathogens had decreased by 35.7%, and the drug's effectiveness was 57.1% for *Blastocystis* spp. and 64.3% for *H. meleagridis*. By the 14th day, *Blastocystis* spp. was detected in three birds and *H. meleagridis* in two, indicating effectiveness rates of 64.3% and 78.6%, respectively. Throughout the study period, the control group of turkeys constantly excreted *Blastocystis* spp. cysts and *H. meleagridis* trophozoites.

In vitro studies have shown that extracts from Thai medicinal plants traditionally used to treat diarrhea exhibit varying degrees of activity against *B. hominis*. Dichloromethane and methanol extracts from *Brucea javanica* seeds, as well as methanol extract from *Quercus infectoria* nut branches, demonstrated the highest effect at a dose of 2,000 μg/ml. These extracts destroyed 82%, 75%, and 67% of isolates, respectively, and inhibited their growth by 94%, 100%, and 76%, respectively. By comparison, metronidazole provided 97% eradication and complete inhibition of *Blastocystis* at concentrations of 1.25–20 μg/ml (Sawangjaroen and Sawangjaroen, 2005).

We evaluated the efficacy of benzimidazole derivatives (albendazole and fenbendazole) in treating and preventing histomoniasis in turkeys. Metronidazole produced greater body weight gain and reduced cecum and liver damage compared to the control group. However, albendazole (100 mg/kg) and fenbendazole (10 mg/kg) were ineffective as therapeutic agents when administered orally for five days, as confirmed during the evaluation of their prophylactic effect (Hegngi et al., 1999). One hour after Ag NPs were applied, the number of *B. hominis* cysts decreased by 20.7%. With metronidazole saturated with Ag NPs, the decrease was 28.2%, and with metronidazole alone, the decrease was 18.9%. Three hours later, the decrease was 71.7%, 79.7%, and 62.7%, respectively, indicating the higher efficacy of combining Ag NPs with metronidazole (Younis et al., 2020).

Conclusions. The developed combined drug demonstrated higher therapeutic efficacy against a mixed infection of turkeys with *Blastocystis* spp. and *H. meleagridis* than Nifulin did. By the 7th day of treatment, there was a 78% reduction in *Blastocystis* spp. infection intensity, and by the 14th day, the pathogen was completely eliminated (prevalence — 0%, effectiveness — 100%). The drug decreased the level of histomoniasis infection more significantly, reducing the number of infected turkeys to 7.1% (effectiveness — 92.9%), which exceeds the effectiveness of Nifulin (effectiveness — 78.6%). This high effectiveness is due to the combination of chemotherapeutic components and phytotherapy, which boosts immunity and enhances the overall effect.

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2025 © Bogach M. V. 0000-0002-2763-3663, Rachynskyi A. S. 0009-0008-1600-0298, Dunaiev Yu. K. 0000-0001-7378-430X, Bohach O. M. 0000-0001-5487-7033