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Evaluation of the effectiveness of alternative methods for controlling coccidiosis in broiler chickens: a field trial

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Currently, coccidiostats are the primary and standard method of combating coccidiosis in poultry farms. However, consumer organizations still point to the need to phase out all chemotherapeutic substances from animal nutrition to protect human health. The research material consisted of 720 cocks of the Cobb 500 hybrid, which were divided into three groups (C – coccidiostat/control, V – vaccine, and H – herbals). The following parameters were analyzed: body weight (BW), feed conversion ratio (FCR), mortality, foot pad dermatitis (FPD), European Production Efficiency Factor (EPEF), and the number of oocysts per 1g of feces (OPG). On day 42, the BW of the C group was higher compared with the V and H groups. The C group was also characterized by the best FCR and the highest EPEF. In the V group, oocysts were noted in feces from the 14th day of rearing, and the highest oocysts content was observed on the 21st day of life. In the C and H groups, the highest number of oocysts was recorded in the feces on the 28th day. The investigated alternative methods to coccidiostats showed good antiparasitic potential. Therefore, combining a couple of anticoccidiosis methods in preventive programs may be the best solution in broiler chickens production.

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In 2014-2017, poultry meat production in Poland increased by almost 30%. In 2017, this country represented 16 percent of EU poultry production. Nowadays, Poland is the largest poultry producer in the EU. According to the EC, Poland's share in EU poultry production in 2021 was 19.1% [National Poultry Council].

The increase in consumer awareness of poultry keeping and nutrition, as well as egg and meat production technology, has forced broiler breeding companies to constantly improve the quality of hybrids while maintaining high welfare. Throughout the production cycle, one of the primary factors influencing the rearing of broiler chickens is their health.

Coccidiosis is caused by protozoa of the genus *Eimeria* (called coccidia), which show high host specificity. There are 7 species of this parasite in chickens (*E. acervulina, E. maxima, E. tenella, E. mitis, E. praecox, E. brunetti, and E. necatrix*). Birds of all ages are susceptible to infection, although young birds are by far the most [Mazurkiewicz 2005].

Infection is provoked by ingestion of the invasive parasite forms, i.e., sporulated oocysts. Pathogenicity, or the ability to cause disease, varies between *Eimeria* species. These intracellular parasites destroy the enterocytes of the intestinal epithelium during development. The most pathogenic are *E. tenella, E. necatrix*, and *E. brunetti*. After entering the body, as a result of the action of hydrochloric acid in the stomach and then digestive juice in the duodenum, sporozoites are released, which penetrate the epithelial cells of the intestinal villi. This process occurs in sections of the intestines appropriate to the *Eimeria* species with which the bird has been infected. The severity of symptoms depends primarily on the dose of parasites taken [Trees 2008]. Damage to the intestinal mucosa leads to impaired digestion and food absorption, thus to impaired development and growth of the entire organism, and even death.

Large resources are spent on prevention and control of coccidiosis [Cisman *et al.* 2020]. According to various sources, annual losses due to coccidiosis are estimated to be about 1 to over 10 billion pounds worldwide, and expenditures on treatment constitute about 30% of this amount [Williams 1999, Blake *et al.* 2020]. In Poland, some studies have indicated that this value can reach over 25 million euros [Szeleszczuk 2005].

Coccidiosis is often associated with low zoohygienic standards on farms and insufficient biosecurity. However, each broiler chicken farm is potentially at risk of coccidia infestation [Janiszewski *et al.* 2017]. Over the years of fighting coccidiosis, many methods have been developed to monitor and reduce its occurrence. However, none of them has been reported to completely eradicate coccidiosis. The first to appear were coccidiostats, which have been used since the 1940s. Currently, it is the primary and standard method of combating coccidiosis in poultry farms. Nevertheless, consumer organizations still point to the need to phase out all synthetic chemotherapeutic substances from production animal diet to protect human health. Hence, it seems that

coccidiostats will eventually be prohibited. Thus, the search for alternative methods of combating coccidiosis has begun. These include the introduction of bird vaccinations to the "anticoccidial" program [Peek and Landman 2011]. The use of live vaccines, attenuated or nonattenuated, to control coccidiosis in broiler breeders or laying hens is well established and effective. However, their use in broiler chickens is gaining acceptance extremely slowly, due partly to economic considerations but also to the perceptions of adverse effects of vaccination on early chicken growth, especially for nonattenuated vaccines [Williams 2002]. Another method to fight against coccidiosis is the use of herbal additives (herbal extracts) in feed or drinking water. Many authors [Allen *et al.* 1997, Naidoo *et al.* 2008, Tewari and Maharana 2011, Quiroz-Castañeda and Dantán-González 2015] have reported the beneficial effects of this type of additives in the prevention of coccidiosis. Barbour *et al.* [2015], using herb supplementation in the nutrition of broiler chickens, which contained eucalyptus oil, similar to the present study, reported a significant improvement in the feed conversion ratio, reduced mortality, and a decrease in the number of intestinal oocysts of coccidia. In addition, an *in vivo* study on domestic pigeons has indicated that thymol can minimize the negative effects of infection of these birds with *Eimeria* sp. parasites [Arafa *et al.* 2020]. In this study, a reduction in the severity of clinical symptoms, fewer oocysts, and improvement in the body weight gain, among others, were observed in the group of pigeons receiving thymol compared with the infected and untreated group. Reisinger *et al.* [2011], using a herbal supplement containing oregano, anise, and citrus oils in the feed of broiler chickens infected with *Eimeria* parasites, have observed positive changes in the digestive system of birds (including elongation of intestinal villi). Thus, they have concluded that using the abovementioned additives to feed can improve the protective barrier against coccidia in the digestive system. Most studies on coccidiosis in poultry are conducted under experimental conditions, and the birds are infected in a controlled manner with a specific species and dose of *Eimeria* sp. Unfortunately, this does not reflect farm conditions, where chickens are randomly exposed to different species of this protozoan. Besides, the appearance of new vaccines and herbal additives requires further research.

The present study aimed to assess the effectiveness of vaccine and herbal supplements in contrast to coccidiostat in reducing infestation with *Eimeria* sp. parasites in broiler chickens maintained under commercial conditions. The effectiveness of these methods was evaluated based on the selected production parameters, the health of chicken legs, and the intensity of protozoan infestation.

Material and methods

Ethical statement

According to Directive 2010/63/EU and Polish law (Dz. U. 2015 poz. 266), animal procedures used in this study did not need to be approved by the Local Animal Ethics Committee.

Birds, housing, nutrition, and experimental design

A total of 720 sexed broiler chickens (cocks) of the Cobb 500 hybrid purchased from the Rychwał Hatchery (Poland) were used in this study. The experiment was carried out under commercial farm conditions. That is why the experiment design did not involve experimental challenges with *Eimeria* but exposed birds to naturally existing oocysts in the poultry house. One-day-old chicks were randomized into three groups of 12 replicates each (20 birds in each replicate) and kept in 1 × 1.5 m (1.5 m^2) pens, with a stocking density of about 13 birds/m^2. The pens were equipped with a hand-filled round feeder with a capacity of 20 kg, and two nipple drinkers were supplied with drinking water automatically. All the chickens were kept in the same building on wheat straw litter until 42 days of age. The microclimate and lighting conditions used were in line with the recommendations of Cobb Germany [Cobb 500 Management Guide 2012]. The feed mixture and drinking water were administered *ad libitum*. The composition and nutritional value of the mixtures and premixes used are presented in Table 1.

Experimental factors

In each group, a different method was used to reduce the infestation of *Eimeria* sp. parasites:

– Group C (Control) – Coccidiostat (Sacox®-Huvepharma) at a dose of 70 mg/kg of compound feed. The active substance was the ionophore antibiotic salinomycin. The preparation was administered orally from 0 to 34 of days of age, together with the feed mixture prepared based on the premix containing the coccidiostat.
– Group V – Attenuated vaccine (Hipracox Broilers®-Hipra) at a dose of ≈0.007 ml/bird, which included five *Eimeria* species: *Eimeria acervulina*, 300-390 oocysts per dose; *Eimeria mitis*, 300-390 oocysts per dose; *Eimeria maxima*, 200-260 oocysts per dose; *Eimeria tenella*, 250-325 oocysts per dose; and *Eimeria praecox*, 300-390 oocysts per dose. The vaccine was sprayed on the day of hatching before being placed in the pens.
– Group H – Herbal additive (Adicoxsol PF®-Adifeed) was administered to drinking water at a dose of 1.5 ml/kg BW for three consecutive days in each week of rearing (1-3, 8-10, 15-17, 22-24, 29-31, 36-38 days of rearing). It contained a complete set of natural ingredients, including phytoncides and substances with bactericidal, protozoal, and fungicidal properties of plant origin. The herbal additive consisted of glycerin, sorbitol, and herbal extracts (51.75%) such as thymol (0.5%), eucalyptus oil (0.5%), anise oil (0.5%), eugenol (0.5%), garlic oil (0.5%), and mustard oil (0.5%).

Analyzed parameters

During the experiment (rearing), the following traits were examined in each group:

Table 1. Composition and nutrient contents of the diets

Ingredients	0-10 d.		11-34 d.		35-42 d.
	Group C	Group V and H	Group C	Group V and H	Group C, V and H
Wheat 12.5%	52.0	52.0	56.0	56.0	62.6
Soybean meal 46%	31.5	31.5	27.0	27.0	20.0
Corn 7.5%	10.0	10.0	10.0	10.0	10.0
Soybean oil	2.8	2.8	3.5	3.5	4.5
Premix* without Salinomicyn 2.5%	0.0	2.5	0.0	2.5	2.5
Premix** with Salinomicyn 2.5%	2.5	0.0	2.5	0.0	0.0
Limestone	1.2	1.2	1.0	1.0	0.4
Calculated composition					
Crude protein (%)	22.44	22.44	20.81	20.81	18.32
Metabolisable Energy (kcal/kg)	2973.8	2973.8	3064.5	3064.5	3211.1
Crude fat (%)	4.68	4.68	5.37	5.37	6.34
Crude fibre (%)	2.42	2.42	2.37	2.37	2.39
Dry matter (%)	87.36	87.36	87.37	87.37	87.34
Crude ash (%)	5.42	5.42	5.08	5.08	4.35
NaCl (%)	0.32	0.32	0.17	0.17	0.28
Na (%)	0.16	0.16	0.14	0.14	0.14
Ca (%)	0.87	0.87	0.83	0.83	0.72
P (%)	0.62	0.62	0.59	0.59	0.47
P – available (%)	0.46	0.46	0.44	0.44	0.34
Lysine (%)	1.38	1.38	1.25	1.25	1.04
Methionine (%)	0.63	0.63	0.55	0.55	0.50
Methionine + Cysteine (%)	1.0	1.0	0.91	0.91	0.82
Threonine (%)	0.85	0.85	0.79	0.79	0.68
Tryptophan (%)	0.27	0.27	0.25	0.25	0.22
Linoleic acid (%)	2.17	2.17	2.53	2.53	3.05

*The premix composition (all groups, 0-34 days of rearing): Crude ash – 58.5%; Sodium – 5.2%; Calcium – 13.1%; Total phosphorus – 7.3%; Lysine – 10.0%; Methionine -10.0%; Threonine – 3.0%; Vitamin A – 480,000 IU/kg; Vitamin D_3 – 160,000 IU/kg; Vitamin E – 1,400 mg/kg; Vitamin K_3 – 120 mg/kg; Vitamin B_1 – 120 mg/kg; Vitamin B_2 – 280 mg/kg; Niacinamide – 1,800 mg/kg; Pantothenic acid – 500 mg/kg; Vitamin B_6 – 200 mg/kg; Vitamin B_{12} – 1,000 mg/kg; Biotin – 8,000 mg/kg; Choline chloride – 28,000 mg/kg; Folic acid – 60 mg/kg; Iron – 2,000 mg/kg; Manganese – 4,000 mg/kg; Copper – 600 mg/kg; Zinc – 4,000 mg/kg; Iodine – 36 mg/kg; Selenium – 12 mg/kg; Beta-xylanase – 100,000 EPU/kg; Phytase – 24,000 FTU/kg; BHA – 5.5 mg/kg; BHT – 60 mg/kg; Ethoxyquin – 11 mg/kg; Salinomycin – 2,800 mg/kg (only in group C – control). The premix composition (all groups, 35-42 days of rearing): Crude ash – 64.0%; Sodium – 5.2%; Calcium – 10.6%; Total phosphorus – 3.5%; Lysine – 9.0%; Methionine – 9.0%; Threonine – 2.5%; Vitamin A – 400,000 IU/kg; Vitamin D_3 – 120,000 IU/kg; Vitamin E – 1,200 mg/kg; Vitamin K_3 – 80 mg/kg; Vitamin B_1 – 80 mg/kg; Vitamin B_2 – 160 mg/kg; Niacinamide – 1,200 mg/kg; Pantothenic acid – 400 mg/kg; Vitamin B_6 – 80 mg/kg; Vitamin B_{12} – 800 mg/kg; Biotin – 6,000 mg/kg; Choline chloride – 24,000 mg/kg; Folic acid – 40 mg/kg; Iron – 2,000 mg/kg; Manganese – 3,200 mg/kg; Copper – 600 mg/kg; Zinc – 3,200 mg/kg; Iodine – 32 mg/kg; Selenium – 12 mg/kg; Beta-xylanase – 100,000 EPU/kg; Phytase – 24,000 FTU/kg; BHA – 5.5 mg/kg; BHT – 60 mg/kg; Ethoxyquin – 11 mg/kg.

– The BW (g) of all birds was measured individually on 0, 7th, 14th, 21st, 28th, 35th, and 42nd day using WT-1101 (Tarczyn) electronic balance with an accuracy of 2 g.
– The FCR (kg/kg bw) was determined for the following rearing periods: 0-7, 0-14, 0-21, 0-28, 0-35, and 0-42 days. This index was calculated for each pen (replicate) according to the following formula:

FCR = (penned feed consumed over the period (kg))/
(total weight of birds in the pen over the period (kg)).

– Mortality (%) was recorded daily. The dead animals were removed, and new chicks were not added to the pen.
– FPD (points) in each chicken was scored weekly (0d; 7d, 14d, 21d, 28d, 35d and 42d) on a 3-point "Swedish scale" [Ekstrand *et al.* 1997] where:
  1 point-clean skin of the sole, without any changes or dirt,
  2 points-dirty skin of the sole, with slight inflammatory fields and visible damage to the epidermis, and
  3 points-the skin of the sole is foul, with a large area of inflammation entering the toes.
– EPEF (points) was calculated for each pen in all groups according to the following formula:

EPEF = (liveability (%) × average BW (kg))/(rearing duration (days) × FCR (kg)).

– The *Eimeria* invasion intensity (number of oocysts per 1 g of fecal samples) was calculated using the McMaster coproscopic method [Kochanowski *et al.* 2013, Vadlejch *et al.* 2013]. The research material consisted of fresh fecal samples from each pen. Sampling for testing was made five times, i.e., on the 7th, 14th, 21st, 28th, and 35th day of life. The weight of each aggregate sample was approximately 100 g. After stirring for about 5 min, 2 g of fecal samples was removed from the bulk sample and covered with 28 ml of saturated NaCl solution. After homogenization using agitation, the suspension was poured through a sterile gauze to obtain a liquid without major residues of undigested food. After remixing, the rest was applied to a classic McMaster chamber consisting of two parts (a and b) with a volume of 0.5 ml each. This chamber contained two 10 × 10 mm mesh fields, and the volume of the liquid under the field was 0.15 ml. The suspension chamber was placed under a microscope and observed using a 10× magnification objective 5 min after filling the chamber. Only oocysts in the grid area were counted.

The calculations were made according to the following formula:

$$OPG = A + B \times 50,$$

where OPG is the number of oocysts in 1 g of fecal samples, A is the number of oocysts under the mesh of part a, and B is the number of oocysts under the mesh of part b.

To confirm the presence of oocysts in the pooled samples, the flotation method was used. The tube was filled with the remainder of the suspension and covered with a coverslip. After 10 min, the slide was viewed under a light microscope.

Statistical analysis

The SAS® statistical package (v. 9.2) [SAS 2011] was used to perform calculations. For all analyzed features, mean values and standard error of the mean (SEM) were

calculated. Analysis of variance (ANOVA) was used to compare the mean values of the traits between the experimental groups. Tukey's test was chosen to verify the significance ($p \leq 0.05$) of the differences between the mean values.

Results and discussion

Body weight

Initially, the average BW of the birds in the experiment (day 0) was 44.9 g (P=0.994, Tab. 2). In the following weeks of rearing, it differed significantly between

Table 2. Body weight of broilers (g) in the following weeks of experiment

Age (day)	Coccidiostat		Vaccine		Herbs		*P* value
	mean	SEM	mean	SEM	mean	SEM	
0	44.9[a]	0.06	45.0[a]	0.06	44.9[a]	0.06	0.994
7	187.4[a]	0.46	158.3[b]	0.37	165.0[c]	0.38	<.0001
14	452.2[a]	0.66	417.4[b]	0.65	432.3[c]	0.67	<.0001
21	818.4[a]	1.08	772.8[b]	0.63	750.8[c]	1.25	<.0001
28	1318.9[a]	1.13	1220.7[b]	0.75	1133.6[c]	1.72	<.0001
35	1868.7[a]	0.61	1800.2[b]	0.74	1618.5[c]	2.72	<.0001
42	2399.9[a]	2.19	2294.1[b]	4.05	2216.8[c]	2.49	<.0001

[abc]In rows means bearing different superscripts differ significantly at P≤0.05.

the analyzed groups. The highest BW was observed in the C group. On day 42 of rearing, the BW of the control group was higher, by 105.8 g and 183.1 g, respectively, compared with the V and H groups. The lowest BW was observed in the H group. However, it is worth mentioning that on days 7 and 14 of rearing, the BW of the H group was higher than that of the V group. Pop *et al.* [2019] obtained different results and did not observe differences in the body weight gain between chickens receiving the coccidiostat (amprolium) and the phytogenic supplements. Sánchez-Hernández *et al.* [2019] reported similar results. They observed no differences in the final BW between chickens administered a coccidiostat (salinomycin) and herbals (Peptasan®). Gordillo Jaramillo *et al.* [2021] showed no statistically significant differences in the BW between broiler chickens receiving a coccidiostat and those receiving herbal additives for feed (a combination of oregano and citrus essential oils). However, they observed a tendency for a lower BW in the latter. Behnamifar *et al.* [2019] compared, inter alia, the effectiveness of vaccinating birds and the use of coccidiostats. Similar to the present study, they reported that the administration of the ionophore antibiotic (salinomycin) had a better effect on BW on the 28th and 42nd days of rearing. The difference in BW between the V group and the control group was statistically confirmed (146 g and 125 g, respectively). In addition, Gaweł *et al.* [2005] observed a higher BW at 42 days of age in broiler chickens given a coccidiostat compared with vaccinated ones (2190 g vs. 2150 g). It should be emphasized that with the increase in the level of the Sacox® coccidiostat in the feed, the growth of hens and their final

weight decrease, as reported by, among others, Rychen *et al.* [2017], who compared 0, 50, 60, and 90 mg/kg doses.

Feed Conversion Ratio

The control group showed a significantly lower FCR value in the following periods: 0-7, 0-14, 0-21, 0-28, and 0-35 of rearing, by 0.10 kg/kg compared with the V and H groups (Tab. 3). It can be assumed that this could be because after

Table 3. Feed conversion ratio - FCR (kg/kg bw) in the following time periods

Age (day)	Coccidiostat		Vaccine		Herbs		*P* value
	mean	SEM	mean	SEM	mean	SEM	
0-7	1.009^{c}	0.001	1.072^{a}	0.002	1.066^{b}	0.001	<.0001
0-14	1.276^{c}	0.003	1.381^{a}	0.003	1.351^{b}	0.002	<.0001
0-21	1.385^{b}	0.003	1.477^{a}	0.002	1.471^{a}	0.003	<.0001
0-28	1.603^{c}	0.003	1.684^{b}	0.003	1.696^{a}	0.003	<.0001
0-35	1.728^{c}	0.003	1.779^{b}	0.002	1.845^{a}	0.008	<.0001
0-42	1.905^{b}	0.007	1.913^{b}	0.010	2.051^{a}	0.009	<.0001

^{abc}In rows means bearing different superscripts differ significantly at P≤0.05.

vaccination, the intestinal epithelium of the chickens was damaged and immunity decreased, resulting in worse production parameters, including a lower BW and a higher FCR in this group. This effect was to be expected. It is a natural physiological and immunological reaction of organism after administering the vaccine. Different results were reported, among others, by Behnamifar *et al.* [2019], who did not observe statistically significant differences in the FCR between vaccinated chickens and those that received a coccidiostat for feed. Gautier [2019] compared the production parameters of vaccinated broiler chickens and those administered coccidiostat throughout their rearing. Their results showed that vaccination against coccidiosis did not significantly affect BW and feed consumption but reduced the FCR. In the present study, on 42d of age, the V group showed similar FCR values to group C. Reversing the effects in favor of the V group in comparison with H group above 3 weeks of age most likely can be associated with fewer oocysts in the fecal samples of these birds. Küçükyilmaz *et al.* [2012] reported similar results. They observed in their experiments on broiler chickens that vaccinated birds showed better feed conversion than those provided a phytogenic supplement to the complete diet (1.939 vs. 2.003 kg/kg), and these differences were statistically confirmed.

Mortality

The average mortality of chickens during the entire rearing period in all studied groups was relatively low, with an average value of 2.36% (Tab. 4). In fact, no deaths were observed from the 21st day onward. These results are consistent with those of Gaweł *et al.* [2005], Inam-UI *et al.* [2011], and Küçükyilmaz *et al.* [2012], who also found no statistically significant differences in mortality of chickens between the groups receiving coccidiostats, herbs, and vaccines.

Table 4. Mortality of broilers (%) in the following time periods

Period (days)	Coccidiostat		Vaccine		Herbs		*P* value
	mean	SEM	mean	SEM	mean	SEM	
0-7	1.67	0.71	0.83	0.56	2.08	0.74	0.309
0-14	2.50	0.75	1.66	0.71	2.08	0.74	0.738
0-21	2.50	0.75	2.08	0.74	2.50	0.75	0.901
0-28	2.50	0.75	2.08	0.74	2.50	0.75	0.901
0-35	2.50	0.75	2.08	0.74	2.50	0.75	0.901
0-42	2.50	0.75	2.08	0.74	2.50	0.75	0.901

No significant differences.

Foot Pad Dermatitis

No statistically significant differences (P=0.220-0.942) in the levels of FPD were observed between the studied groups (Tab. 5). This finding suggested that each of the methods used was so effective that it did not lead to the appearance of clinical symptoms of coccidiosis. Coccidiosis may significantly affect the digestive system at various stages of its clinical advancement and thus, indirectly, the condition of the litter (diarrhea). On the other hand, the influence of the litter condition on the occurrence of FPD has been confirmed in many scientific studies [Ekstrand *et al.* 1997; Dowsland 2008, Meluzzi and Sirri 2009]. The lack of results in the literature on the impact of the applied preventive methods against the infestation of *Eimeria* parasites on the frequency and strength of FPD makes an in-depth analysis of the results obtained in this study difficult.

Table 5. The prevalence and severity of footpad dermatitis - FPD in broilers (pts)

Age (day)	Coccidiostat		Vaccine		Herbs		*P* value
	mean	SEM	mean	SEM	mean	SEM	
7	0.06	0.01	0.11	0.01	0.11	0.02	0.220
14	0.14	0.02	0.14	0.02	0.20	0.03	0.581
21	0.64	0.03	0.64	0.03	0.67	0.04	0.823
28	0.79	0.04	0.80	0.04	0.94	0.04	0.741
35	1.30	0.03	1.30	0.03	1.20	0.03	0.828
42	1.47	0.03	1.47	0.03	1.50	0.03	0.942

No significant differences.

The European Production Efficiency Factor

The C group showed the highest EPEF, followed by the V group and the H group (Fig. 1). The differences between the individual groups were confirmed statistically (P≤0.05). The EPEF is an important economic indicator in broiler production. However, it is difficult to find a direct influence of this chemotherapeutic agent on the EPEF. It is instead due to its effect on the BW of chickens (higher) and FCR (lower), with the mortality being not different between the C group, and the others. Mousavinasab *et al.* [2022] presented similar results, who compared the efficacy of a coccidiostat

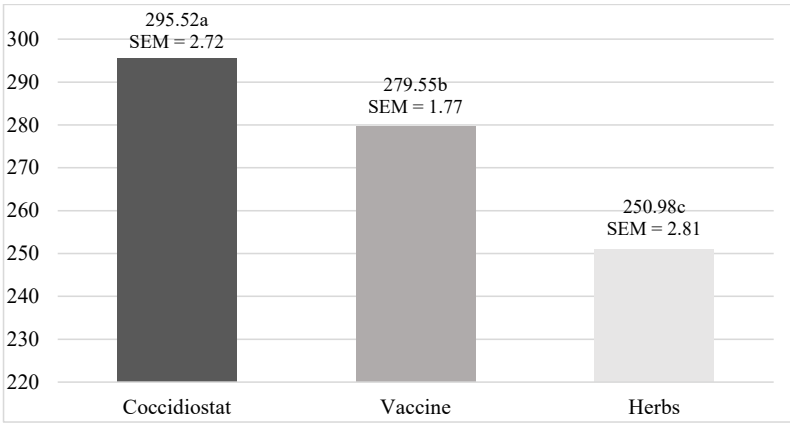

Fig. 1. The European Production Efficiency Factor (pts).

(amprolium) with those of herbal feed additives. They observed the highest EPEF in chickens receiving the coccidiostat. On the other hand, Arczewska-Włosek and Świątkiewicz [2012] showed no significant differences in the EPEF between chickens receiving a coccidiostat (diclazuril) and a phytogenic additive in the feed.

The Eimeria invasion - McMaster coproscopic method

The results concerning the number of *Eimeria* sp. oocysts in the fecal samples of individual groups are presented in Table 6. In the C and H groups, no oocysts were observed in the samples collected on the 7d and 14d of rearing. However, in the V group, oocysts were observed in the fecal samples from the 14th day of rearing, and the highest number of oocysts was observed on the 21st day of life. In the C and H groups, the highest number of oocysts was recorded in the fecal samples collected on the 28th day of rearing. One week later (35 days of age), the lowest number of oocysts was recorded in the fecal samples of the V group. The difference ($P \leq .0001$) between this group and the rest was 5.54×10^3 on average. No oocysts were detected in feces collected on the 7th day of birds' age, also in the vaccinated group. This could be due to the periodicity of oocyst multiplication in the organism of birds, which results from

Table 6. Number ($\times 10^3$) of *Eimeria* sp. oocysts in 1 gram of feces

Age (day)	Coccidiostat		Vaccine		Herbs		*P* value
	mean	SEM	mean	SEM	mean	SEM	
7	0.00	0.00	0.00	0.00	0.00	0.00	-
14	0.00^b	0.00	0.98^a	0.05	0.00^b	0.00	<.0001
21	30.76^b	0.74	71.96^a	2.71	74.56^a	2.67	<.0001
28	85.76^a	2.11	49.70^c	0.89	74.78^b	5.60	<.0001
35	11.97^a	0.59	5.01^c	0.17	9.13^b	0.97	<.0001

^{abc}In rows means bearing different superscripts differ significantly at $P \leq 0.05$.

the specific life cycle of *Eimeria* [Burrell *et al.* 2020]. It doesn't change the fact that oocysts appeared the earliest in the fecal samples from the V group. It is undoubtedly due to the simultaneous administration of a specific dose of vaccine oocysts to this group, already on the 1st day of rearing. However, the C and H groups were exposed to a random invasive infestation of *Eimeria* spp. in the environment of the poultry house. On the other hand, the course of the invasion in each of the analyzed groups was similar. After the highest values of this trait were recorded in all groups, the number of oocysts in the fecal samples decreased, indicating the gradual development of immunity. In the H group, the number of oocysts initially reduced slightly, but differences between this group and control on days 28 and 35 were statistically confirmed. Undoubtedly, this was influenced by the effect of biologically active compounds in the herbal extract. Adicoxsol PF® is a complete set of natural ingredients (phytoncides and phytoalexins) that support the immune system. They enhance their activity against bacterial and protozoal infections. Phytoncides increase, among others, the number of Tγδ and NK lymphocytes. Particularly effective action of the Adicoxsol PF® is observed in preventing and controlling infections with anaerobic bacteria, e.g., *Bacteroides*, *Clostridium*, and protozoa, e.g., *Eimeria* [Cichocka *et al.* 2018].

The decrease of oocytes was most pronounced in the V group, where the lowest number of oocysts was recorded in the fecal samples collected on days 28 and 35. As the attenuated (weakened virulence) strains of coccidia used in the vaccine maintain a high ability to stimulate an immune response [Shirley *et al.* 2005], vaccinated chickens had a chance to develop immunity against parasites faster. Long and Rowell [1975] reported similar invasion characteristics in their studies. In an experiment on broiler chickens, Behnamifar *et al.* [2019] observed a lower number of oocysts in the fecal samples of birds receiving a coccidiostat (salinomycin) compared with those vaccinated against *Eimeria* sp. In the present study, such a situation was observed only on the 21st day of rearing. Later (on days 28 and 35), fewer oocysts were observed in the fecal samples of chickens vaccinated against these pathogenic protozoa. Gaweł *et al.* [2005] presented similar observations. They compared the effectiveness of using a vaccine (Immucox C1) and a coccidiostat administered to the feed (Clinacox 1%). In the 3rd, 5th, and 6th weeks of rearing, a statistically significantly lower OPG (mean number of oocysts $\times 10^3$) was observed in the vaccinated group, and the difference was 0.03, 0.10 and 0.34 $\times 10^3$, respectively.

Based on the obtained results, it can be concluded that the best performance was achieved by the group receiving the ionophore coccidiostat in the feed mixture. This situation, in addition to the apparent antiprotozoal effect of salinomycin, most likely indicates a simultaneous mitigating impact on the complications usually associated with the invasion of protozoa of the *Eimeria* genus, which most often include bacterial enteritis and, in extreme cases, necrotic enteritis. Vaccination may become the most effective alternative to coccidiostats, especially considering the increasing drug resistance of parasites to antibiotic substances in the environment and the efforts of proconsumer organizations to withdraw medicinal preparations from animal products

as much as possible. Ultimately, the antiparasitic potential of the investigated alternative methods to coccidiostats shows that they can be used in combinations in preventive programs, which needs to be investigated further.

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