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The effect of increased crude protein level and/or dietary supplementation with herbal extract blend on the performance of chickens vaccinated against coccidiosis

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ABSTRACT

The experiment was designed as a 2 × 2 × 2 factorial arrangement with 6 replicate pens per treatment (8 male Ross 308 chicks per pen) conducted from 1 to 42 d of age to evaluate the effect of dietary crude protein level (CP) and herbal extract blend on performance indices, results of slaughter analysis, and oocyst shedding in broilers vaccinated against coccidiosis. Treatments included a lack or single dose of live anticoccidial vaccine (Livacox T®, administered at 1 d of age, VAC), normative (21.6 and 20% in the starter and grower-finisher feeding phase, respectively) or increased (23.6 and 21.6% in the starter and grower-finisher feeding phase, respectively) dietary crude protein level, with or without supplementation with an herbal extract blend (*Echinacea purpurea*, *Salvia officinalis*, *Thymus vulgaris*, *Rosmarinus officinalis, Allium sativum*, *Origanum vulgare;* HE, 0 or 2 g/1 kg of feed). Each dose of vaccine contained 300–500 sporulated oocysts of each *Eimeria acervulina*, *E. maxima* and *E. tenella* which are specific to different sections of the intestinal tract. Wood shavings as a bedding guaranteed vaccine oocyst recirculation. A negative effect of VAC on FI, BWG or FCR ($P < 0.05$) was found in the starter feeding phase and remained noticeable ($P < 0.05$) till the end of the rearing period. There was no significant effect of CP or HE on performance in the starter feeding phase period. The increased CP level decreased fat deposition in the carcass and improved the BWG ($P < 0.05$) of vaccinated birds in the second and entire experimental period, while HE positively influenced the 1–42 FCR ($P < 0.05$) in vaccinated birds. The dietary treatment did not interfere with recirculation of the vaccine's oocysts, which is necessary for building up immunity. It can be concluded that an increased CP level or HE supplementation may be considered as support tools in terms of vaccination.

1. Introduction

Coccidiosis, a widespread intestinal parasitic disease caused by protozoa of the genus *Eimeria*, remains the most important threat to the economical efficacy of poultry production. The annual financial losses due to coccidiosis are estimated at over $3 billion worldwide (Dalloul and Lillehoj, 2006) and €51 million in Poland (Szeleszczuk et al., 2016). There are 7 specific species of *Eimeria* recognized as pathogenic to chickens, i.e. *Eimeria acervulina*, *E. maxima*, *E. tenella*, *E. brunetti*, *E. necatrix*, *E. mitis* and *E. praecox*, with variable degrees of pathogenicity, a specified predilection to infect different regions of the intestines, as well as the ability to produce

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diverse lesions in the epithelium (Sharman et al., 2010; Barbour et al., 2015). All species are characterized by high fecundity and are easily transmitted between chickens, especially in the conditions of high density of birds in intensive production. Parasite proliferation in the intestinal epithelium cells leads to tissue damage resulting in diarrhoea, haemorrhage, digestive disorders, malabsorption, and thus impaired feed utilization and decreased body weight gain, or systemic effects such as blood loss, shock syndrome or even death (Vermeulen et al., 2001; Williams, 2005). Moreover, the damaged gut integrity due to coccidiosis is considered to be the main predisposing factor for necrotic enteritis (Williams, 2005).

The main form of coccidiosis prophylaxis in broiler chickens is continuous in-feed medication with ionophores or synthetic coccidiostats, with the exception of the withdrawal period. Their use, regarded by producers as the most effective tool to combat coccidiosis in intensive rearing systems, is however undesirable for consumers. Immunoprophylaxis with live oocyst vaccination is the most promising alternative to coccidiostats (Chapman et al., 2002). Coccidia are immunogenic and primary infections that stimulate the development of resistance to homologous challenge (Williams, 1998). Thus, live vaccines are used to deliver a small number of sporulated oocysts to develop in the epithelium and to provide contact with antigens of all the parasite's life stages. During their circulation via trickle infection, solid adaptive immunity is built after 2–3 consecutive infections (Sharman et al., 2010). Although there is a lack of information about the scale of current usage of anticoccidial vaccines, in 2005 it was estimated at around 3×10^9 doses within the 3 previous years, for either attenuated or comprising wild-type parasites vaccines (Shirley et al., 2005).

Live vaccines against coccidiosis have been used in broiler breeders, but there has been a widespread averseness to their use in broilers due to reports of transient deterioration of performance parameters during immunity acquisition in comparison to coccidiostats (Danforth, 1998; Williams and Gobbi, 2002). This worsened performance is the result of a state of mild subclinical coccidiosis associated with a reduced absorptive area of the intestinal epithelium, malabsorption and inflammation, as well as secondary enteritis accompanying the replication of the vaccine's oocysts in the intestinal tissue. Moreover, the relatively short lifespan of chickens is not always adequate to compensate for the decreased BWG due to vaccination (Chapman et al., 2002; Lehman et al., 2009; Williams, 1998). As such, a nutritional approach to the transitory loss of performance is crucial, along with appropriate maintenance conditions and biosecurity. Nutrition methods may act beneficially in cocci-vaccinated broilers by various modes of action, i.e. through the satisfaction of the nutritional requirements of vaccinated chickens, a stabilizing effect of feed additives on intestinal microbiota, maintenance of intestinal integrity, improvement in the digestibility and absorption of nutrients, as well as immunostimulation (Arczewska-Włosek and Świątkiewicz, 2014). An increased level of protein in the diet, especially administered directly after vaccination, seems to be a successful strategy in alleviating performance deterioration due to vaccination (Lee et al., 2011).

Another method, complementary to vaccination, is the use of phytogenic feed additives. Our previous studies (Arczewska-Włosek and Świątkiewicz, 2015) showed the favourable effect of an herbal extract blend, including extracts of *Echinacea purpurea*, *Salvia officinalis*, *Thymus vulgaris*, *Allium sativum*, and *Origanum vulgare*, on the stimulation of compensatory growth in terms of clinical coccidiosis, and improved performance in the condition of natural, low exposure to coccidia. Thus, the aim of the current study was to investigate the effects of single and combined nutritional methods, such as a greater than normative dietary crude protein content and supplementation with herbal extracts, as supportive factors limiting the potential negative influence of live anticoccidial vaccine on the performance of broiler chickens.

2. Material and methods

2.1. Birds and experimental design

All of the experimental procedures involving animals were approved by the 2nd Local Ethical Committee on Animal Testing, Cracow, Poland. The study was conducted as a 2 × 2 × 2 factorial arrangement with the following factors: anticoccidial vaccine (0 or 1× dose), dietary crude protein level (normative or increased), and dietary supplementation with herbal extracts blend (0 or 2 g/kg feed). A total of 384 male one-day-old Ross 308 broiler chickens obtained from a local commercial hatchery were randomly allocated to 8 groups with 6 replicates (pens) of 8 birds. All the birds were reared up to 42 d of age and fed with starter (1–21 d) and grower-finisher (22–42 d) maize-soya bean basal diets, free of antibiotic growth promoters and coccidiostats, and formulated to meet or exceed the nutrient requirements of broilers (Smulikowska and Rutkowski, 2005). All diets were fed ad libitum in a mash form. The birds were kept in standard environmental rearing conditions – in floor pens with wood shavings as a bedding material – to ensure the circulation of the vaccine's oocysts. The pens were separated from each other by PCV barriers to reduce the transition of vaccine oocysts.

2.2. Experimental factors

Half of the chicks were vaccinated (VAC) against coccidiosis *per os* at 1 d of age, before being placed in the pens, with commercial live attenuated trivalent vaccine Livacox T (BIOPHARM Research Institute of Biopharmacy and Veterinary Drugs, The Czech Republic). Each dose (0.01 ml) of the vaccine contained 300–500 sporulated oocysts each of *Eimeria acervulina, Eimeria maxima* and *Eimeria tenella*, and were suspended in distilled water up to the volume of 0.25 ml. Birds from the non-vaccinated groups were administered the same volume of distilled water.

The calculated crude protein levels in the experimental starter diets were 21.6 and 23.6% in the groups receiving normative or increased CP, respectively, while the analysed contents of CP were accordingly 21.3 or 23.24% (Table 1). The diets for the grower-finisher feeding phase were formulated to contain 20 or 21.6% of CP, while the analysed CP content was 20.02 or 21.97% (Table 1).

Table 1
Composition of experimental diet for starter (1–21 d) and grower-finisher (22–42 d) feeding phase and analysed nutrient content in basal feed mixtures.

Ingredient	Starter		Grower- finisher	
	Normative CP level [g/kg]	Increased CP level [g/kg]	Normative CP level [g/kg]	Increased CP level [g/kg]
Maize	579.3	505.3	597.5	531.5
Soybean meal	360	420	323	375
Soybean oil	18	32	38	52
Limestone	16	16	16	16
Monocalcium phosphate	14.5	14.5	14	14
Sodium choloride	3	3	3	3
DL-Methionine	2	2	2	2
L-Lysine hydrochloride	1.2	1.2	1.5	1.5
Vitamin-mineral premix*	6	6	5	5
Analyzed nutrient content in basal feed mixtures:				
Dry matter [%]	87.4	87.72	88.38	88.14
Crude ash [%]	6.9	7.61	6.84	7.6
Crude protein [%]	21.33	23.24	20.02	21.97
Crude fat [%]	3.51	3.61	4.33	4.4
Crude fibre [%]	2.42	2.29	2.03	2.1
Phosphorus [g/kg]	7.45	7.33	7.17	7.16
Asp	21.86	25.31	19.4	22.26
Tre	7.7	8.65	7.17	7.91
Ser	10.21	11.23	9.35	10.23
Glu	36.95	41.17	33.36	37.3
Pro	12.11	13.17	11.33	12.02
Gli	8.51	9.67	7.83	8.74
Ala	10.05	11.19	9.36	10.11
Val	9.75	11.06	8.77	9.85
Ile	8.68	10.01	7.83	8.93
Leu	17.49	19.6	16.12	17.54
Tyr	6.73	7.25	5.29	5.86
Fen	11.32	12.6	9.6	10.72
His	6.41	7.25	5.41	6.16
Lis	14.54	16.37	12.03	13.65
Arg	15.68	17.58	12.14	13.64
Cys	3.11	3.27	2.95	3.27
Met	5.11	5.46	4.81	5.44
Trp	2.6	2.82	2.3	2.63

* Each kg of vitamin-mineral premix contained: vitamin A – 2 000000 IU; vitamin D3 – 500000 IU; vitamin E – 7000 IU; vitamin K3 – 600 mg; vitamin B1 – 400 mg; vitamin B2 – 1400 mg; vitamin B6 – 1000 mg; vitamin B12 – 8 mg; Ca-pantotenate – 2000 mg; niacine – 8000 mg; folic acid – 200 mg; biotin- 16 mg; choline chloride – 29480 mg; manganese – 16000 mg; zinc – 12000 mg; iron – 12000 mg; copper – 3000 mg; iodine – 400 mg; selenium – 50 mg.

The herbal extract blend was administered at a dose of 2 g/kg feed (Intermag Sp. z o.o., Olkusz, Poland). 1 kg of blend provided: dry extract from *Echinacea purpurea*, 400 mg; oleoresin *Salvia officinalis*, 27 800 mg; oleoresin *Thymus vulgaris*, 5 000 mg; oil extract from *Rosmarinus officinalis* 2 500 mg; oil from *Allium sativum*, 1 670 mg; and oil from *Origanum vulgare*, 1 000 mg.

2.3. Sample collection

The birds were weighed at the age of 1, 21, and 42 d. The body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and mortality were calculated for d 1–21, 22–42 and 1–42. FCR was calculated as kg feed/kg BWG, and the data were corrected for mortality.

To demonstrate the profile of vaccine oocysts production, the number of oocysts per gram of excreta (OPG) was determined using a McMaster chamber in pooled excreta samples taken from each replicate (pen) at 5, 6, 7, 14, 21, 28, 35 and 41 d post vaccination. To obtain the pooled excreta sample, several faecal samples from different locations in the pen were collected and homogenized for analysis of OPG from each pen (replicate) separately. Despite intense efforts to maintain the hygiene regimen during the entire experimental period, the analysis of OPG indicated the presence of oocysts in some of the samples collected in the non-vaccinated groups from 21 d of age. However, the recorded OPG remained at a low level and mean values in those groups were 3655, 15135, 3950 and 5605 at a 21, 28, 35 and 41 d of age, respectively. In view of this fact, OPG values were compared only in the vaccinated groups. The OPG values were logarithmically transformed [$\log_{10}$ (OPG + 1)] to create a normal distribution.

At the end of the experiment, all of the chickens were weighed, and 8 representative chickens with live body weights close to the group average were chosen from each group, marked with numbers and decapitated. The chickens were plucked, the intestines and crop were removed and the carcasses were stored overnight at 4 °C. The mass of the cooled carcass with the edible giblets (gizzard, liver and heart) was estimated and the carcass yield calculated. The breast muscles, abdominal fat, livers, gizzards, spleens and bursa

Table 2
The effect of experimental factors on the performance data of broiler chickens (n = 6).

Factors									SEM	Significance (P-value)						
VAC	−				+											
CP	N		I		N		I									
HE	−	+	−	+	−	+	−	+		VAC	CP	HE	VACxCP	VACxHE	CPxHE	VACxCPxHE
days 1–21																
BWG, g	608	610	602	603	488	569	533	537	11.6	*	NS	NS	NS	NS	NS	NS
FI, g	960	952	941	947	796	909	859	862	17.1	*	NS	NS	NS	NS	NS	NS
FCR, g feed/g BWG	1.58	1.56	1.56	1.57	1.64	1.6	1.61	1.61	0,005	*	NS	NS	NS	NS	NS	NS
days 22–42																
BWG, g	1737	1752	1667	1663	1637	1678	1644	1783	14.7	NS	NS	NS	*	NS	NS	NS
FI, g	3485	3473	3310	3374	3440	3424	3361	3611	30.5	NS	NS	NS	NS	NS	NS	NS
FCR, g fee/g BWG	2.01	1.98	1.99	2.03	2.1	2.04	2.04	2.02	0.01	*	NS	NS	NS	NS	NS	NS
days 1–42																
BWG, g	2345	2362	2269	2266	2125	2246	2177	2320	20.2	NS	NS	NS	NS	NS	NS	NS
FI, g	4422	4407	4217	4300	4211	4304	4198	4440	38.3	NS	NS	NS	NS	NS	NS	NS
FCR, g feed/g BWG	1.89	1.87	1.86	1.9	1.98	1.92	1.93	1.91	0.008	*	NS	NS	NS	*	*	NS

NS – P > 0.05; *- P ≤ 0.05; VAC- anticoccidial vaccine; CP- crude protein level, N- normative; I- increased; HE- herbal extract blend; SEM – standard errors mean.

of Fabricius were excised and weighed. The breast muscle and abdominal fat contents were expressed as a percentage of the cold carcass. The weights of the liver, gizzard, spleen and bursa of Fabricius were expressed as a percentage of the live weight.

After the end of the feeding experiment (43 d of age), the pH of the intestinal (duodenum, ileum, ceca) and crop contents were measured. The sample was the content of individual sections pooled from 4 chickens. The analysis was performed in 5 replications.

2.4. Statistical analysis

The data were analysed as three-way ANOVA with anticoccidial vaccination, dietary crude protein level and herbal extract blend supplementation as the main factors. A two-way ANOVA with dietary crude protein level and herbal extract blend supplementation as the main factors was used to analyse their effect on the OPG results in vaccinated chickens. The Duncan's multiple range test was used to determine the interactions and differences of the treatment's main effects with a probability of P ≤ 0.05.

3. Results

One of the experimental factors in the current study was a varied level of crude protein in the diet. The results of analyses of nutrients in the experimental diets for the starter or grower-finisher feeding phase are presented in Table 1.

3.1. Performance results

Performance results obtained in the starter, grower-finisher and entire rearing period are included in Table 2. The average BWG in the starter feeding phase (1–21 d of age) was 569 g, feed intake – 903 g, and feed conversion calculated per 1 kg of BWG – 1.59 kg/kg. In the grower-finisher period (22–42 d of age), these values were −1 695 g, 3 434 g, 2.03 kg/kg, and for the entire experimental period 2 262 g, 4 313 g, 1.91 kg/kg, respectively.

Vaccination against coccidiosis had a significant negative effect on all performance parameters in the first experimental period. Significantly lower (by 10%) FI in all the groups of vaccinated birds worsened FCR, which resulted in a statistically confirmed decrease in BWG when compared to the non-vaccinated groups. Statistical analysis showed no effect of other experimental factors, nor any impact of their interactions on performance in this period.

In the second experimental period, a statistically confirmed tendency to worsened feed utilization in the vaccinated groups (2.05 vs 2 g/g BWG) was also found. FI and BWG were comparable for the vaccinated and non-vaccinated groups. A statistically significant interaction between VAC and CP was found. A higher crude protein level in the diet of the vaccinated birds significantly increased BWG to a level comparable to the BWG of the non-vaccinated birds.

Analysis of the performance results for the entire experimental period showed, as a consequence, a significant negative effect of VAC on the BWG and FCR. Moreover, a positive significant interaction between CP and VAC on BWG was recorded, as in the second experimental period. A significant effect of interaction VACxHE on 1–42 FCR was also observed, where supplementation of the herbal extract blend significantly decreased the FCR in vaccinated birds. When analysing the effect of CPxHE, the administration of both factors slightly decreased the positive effect of individual factors on the 1–42 FCR.

Table 3
Effect of experimental factors on results of slaughter analysis (n = 8).

Factors									SEM	Significance (P-value)						
VAC	−				+											
CP	N		I		N		I									
HE	−	+	−	+	−	+	−	+		VAC	CP	HE	VACxCP	VACxHE	CPxHE	VACxCPxHE
Carcass yield, %	75.9	78.1	75.8	75.6	75.5	76.6	77.2	76.2	0.321	NS	NS	NS	NS	NS	NS	NS
Breast meat yield, % carcass	29.1	29.1	29.1	28.9	29.3	29	30.2	30.4	0.243	NS	NS	NS	NS	NS	NS	NS
Abdominal fat, % carcass	0.601	0.591	0.262	0.356	0.499	0.596	0.407	0.379	0.032	NS	*	NS	NS	NS	NS	NS
Liver, % LBW	1.84	1.93	1.87	1.82	1.84	1.83	1.86	1.85	0.02	NS	NS	NS	NS	NS	NS	NS
Gizzard, % LBW	1.2	1.22	1.18	1.17	1.33	1.21	1.17	1.21	0.019	NS	NS	NS	NS	NS	NS	NS
Spleen, % LBW	0.092	0.098	0.088	0.074	0.085	0.101	0.082	0.091	0.003	NS	NS	NS	NS	NS	NS	NS
Bursa of Fabricius, % LBW	0.215	0.256	0.266	0.199	0.243	0.242	0.256	0.26	0.008	NS	NS	NS	NS	NS	NS	NS

NS – P > 0.05; *- P ≤ 0.05; VAC- anticoccidial vaccine; CP- crude protein level, N- normative; I- increased; HE- herbal extract blend; LBW- live body weight; SEM – standard errors mean.

3.2. Slaughter analysis

The results of the slaughter analysis (Table 3) showed no effect of any of the experimental factors on the studied parameters, other than the lowering effect of increased CP level on abdominal fat content ($P > 0.05$).

3.3. Oocyst count

The logarithmically transformed OPG values [$\log_{10}$ (OPG + 1)] in the samples collected throughout the experimental period are presented in Table 4. The largest increase in oocyst excretion was observed on the 14th d post vaccination, and gradually decreased in the following sampling periods in all treatments. The analysis of OPG in samples collected on the 41st d post vaccination indicated the significant effect of supplementation with HE and the significant interaction of CPxHE, where HE administration resulted in an increase of OPG. It is likely that this increase, indicated in just 2 of 6 replicates, could be the result of contamination from the unvaccinated groups.

3.4. pH of the gastrointestinal tract contents

The experimental factors did not affect the pH of the contents of the examined intestinal sections (Table 5). Vaccination against coccidiosis significantly lowered the pH of the crop content, however it did not affect the acidity of the contents of further sections of the gastrointestinal tract.

Table 4
Oocyst shedding [$\log_{10}$ (OPG* + 1]; (n = 6).

Factors					SEM	Significance (P-value)		
CP	N		I					
HE	−	+	−	+		CP	HE	CPxHE
5 d pv	3.078	3.296	3.268	3.368	0.051	NS	NS	NS
6 d pv	3.627	3.588	3.597	3.867	0.059	NS	NS	NS
7 d pv	4.334	4.479	4.475	4.566	0.053	NS	NS	NS
14 d pv	3.699	3.905	3.945	3.819	0.12	NS	NS	NS
21 d pv	3.394	3.385	3.3	3.361	0.08	NS	NS	NS
28 d pv	2.908	2.929	3.092	3.326	0.074	NS	NS	NS
35 d pv	2.29	2.875	2.35	2.109	0.149	NS	NS	NS
41 d pv	2.179 a	2.205 a	1.262 b	2.71 a	0.171	NS	*	*

NS – P > 0.05; *- P ≤ 0.05; a, b- means in raws with different letters differ significantly at P ≤ 0.05; CP- crude protein level, N- normative; I- increased; HE- herbal extract blend; d pv- days post vaccination; SEM – standard errors mean.

Table 5
pH of chickens crop and intestinal content at 43 d of age (n = 5)

Factors									SEM	Significance (P-value)						
VAC	–				+											
CP	N		I		N		I									
HE	–	+	–	+	–	+	–	+		VAC	CP	HE	VACxCP	VACxHE	CPxHE	VACxCPxHE
pH of crop content	4.76	4.63	4.77	4.75	4.63	4.6	4.61	4.61	0.022	*	NS	NS	NS	NS	NS	NS
pH of duodenum content	6.37	6.35	6.37	6.41	6.38	6.34	6.38	6.37	0.012	NS	NS	NS	NS	NS	NS	NS
pH of ileum content	6.72	6.75	6.38	6.16	6.88	6.62	6.86	6.57	0.076	NS	NS	NS	NS	NS	NS	NS
pH of ceca content	7.03	7.05	7.02	6.92	6.96	7.01	7.04	6.94	0.02	*	NS	NS	NS	NS	NS	NS

NS – $P > 0.05$; *- $P \leq 0.05$; VAC- anticoccidial vaccine; CP- crude protein level, N- normative; I- increased; HE- herbal extract blend; SEM – standard errors mean.

4. Discussion

Growing public concerns over food safety, along with consumer pressure for the development of natural-origin approaches as alternatives to chemoprophylaxis, make vaccination against coccidiosis a more desirable method to control this widespread parasitism. On the EU market, only attenuated live anticoccidial vaccines are registered.

In the current study the applied nutritional treatments were expected not to exert coccidiostatic properties that could interfere with the circulation of the vaccine strains, and thus the acquiring of immunity. The analyses of OPG values in the excreta samples confirm the lack of this mode of action of CP or HE. According to the declaration of the vaccine's producer, immunity should be built up to 14 d post vaccination, and this was reflected in the profile of oocyst shedding which decreased continuously from 14 d post vaccination in all of the vaccinated groups. The exception was the increased OPG value recorded on 41 d post vaccination, negatively influenced by herbal extracts supplementation, or by the interaction of combined administration of increased CP level and HE. Although these effects were statistically significant, it should be emphasized that the recorded OPG values seemed to remain at very low levels and did not affect BWG in the HE-treated groups at the end of the grower-finisher period.

The data obtained in the current study confirm the possibility of performance deterioration, i.e. decreased FI and BWG as well as increased FCR, due to anticoccidial vaccination within the starter feeding phase. None of the nutritional factors or their interactions had a significant effect on the performance in this period. However, administration of HE, CP or both of them numerically increased BWG by 16, 9 or 10%, respectively, in comparison to the vaccinated untreated group. The adverse effect of vaccination on 1–21 d BWG was also indicated in the study by da Silva et al. (2009), but without significantly worsened FI or FCR. A decreased BWG and increased FCR were noticed within 3 wks. following vaccination in the study of Lehman et al. (2009), where the variable protein level in the diet (21 vs. 23%) did not influence performance. In a study with a series of experiments evaluating various protein levels in starter diets (from 20 to 24%) on the performance of broilers vaccinated with a live anticoccidial vaccine, the performance parameters were also affected during the pre-challenge period (Lee et al., 2011), but an increasing protein level (up to 22%) was found to significantly ameliorate this negative effect. According to the authors, a proper protein level in the starter diet is crucial to avoid performance deterioration due to vaccination.

When analysing experimental data for the entire rearing period (1–42 d of age), a significant, negative effect of VAC on the BWG and FCR was also found despite the growth rate in the second feeding period not being significantly affected by any experimental factors. This information indicates that the transient performance deterioration in the starter phase could remain persistent and harmful. The problem with compensating decreased body weight gain as an effect of vaccination was also seen in the studies of Lehman et al. (2009) and Williams (1998), although in a study by da Silva et al. (2009) the vaccinated birds were able to compensate for the initial decreased BWG by 36 d of age. The application of increased CP and HE to the diet of the vaccinated birds was believed to ameliorate this undesirable result of the vaccine. In the current study, the positive effect of increased crude protein level on BWG was statistically confirmed during the second and entire experimental periods, where the interactions of VAC and CP were found. Moreover, the values of FCR in the entire experimental period indicate the favourable, significant effect of the interaction VACxHE, where HE-supplementation improved the feed utilization rate in the vaccinated birds. The beneficial effect of a similar herbal extract blend on the lowering of the feed conversion ratio was also found in a previous study of clinical coccidiosis (Arczewska-Włosek and Świątkiewicz, 2012), as well as under conditions of natural, low exposure to coccidia (Arczewska-Włosek and Świątkiewicz, 2015).

Phytogenic products have been involved in many studies to investigate their antioxidative, antibacterial, immunostimulating and anticoccidial effects as a method in a coccidiosis prevention program (Abbas et al., 2012; Arczewska-Włosek and Świątkiewicz, 2014; Bozkurt et al., 2016). The positive effect of *Origanum vulgare* compound (Orego-Stim®), a herb that was also a component of the extract blend in the current study, was found in a study conducted on cocci-vaccinated broilers under an organic rearing system (Waldenstedt, 2003). This herbal compound, rich in thymol and carvacrol, positively influenced performance by increasing BWG and FI, without affecting FCR. Another herb included in the supplemented extract blend with a positive effect confirmed by earlier research is *Echinacea purpurea*, tested as a first herb in vaccinated birds, mainly due to its well-known immunomodulatory, antioxidant and anti-inflammatory properties (Allen, 2003). The herb (1 or 5 g/kg) was administered for the first 2 wks. only, and significantly ameliorated growth reduction due to vaccination by improved BWG of the vaccinated birds at 14 d of age. This positive effect persisted for the next 2 wks. Interestingly, supplementation with *E. purpurea* preparations did not influence the BWG of unvaccinated challenged chickens, but in vaccinated birds the addition of herbs significantly increased this parameter. The author recommended Echinacea as a potential adjuvant for live vaccines against coccidiosis (Allen, 2003).

Contrary to the above favourable effect of phytogenic supplementation in the diet of vaccinated birds are the results obtained by Oviedo-Rondón et al. (2005, 2006a,b) in subsequently performed studies with 2 essential oil blends, Crina® POULTRY or Crina® ALTERNATE, containing thymol, eugenol, curcumin and piperin. The investigated essential oil blends did not consistently promote better performance in cocci-vaccinated broilers (Oviedo-Rondón et al., 2005), or even lead to lower performance, higher lesion scores and oocyst counts in cocci-vaccinated birds (Oviedo-Rondón et al., 2006a). According to the authors, the reason for such effects could be the result of the cytotoxic properties of phenolic compounds included in essential oils on the villus tips of the intestinal mucosa, which disrupted the development of immune response in vaccinated birds.

Also, a moderate effect of the combined use of vaccination and another essential oil blend with carvacrol, 1.8-cineole, camphor, thymol, α- fenchone, α-terpinyl acetate and sabinene as the main active components was found in a study of Küçükyılmaz et al. (2012). Supplementation with this specific essential oil blend was not able to ameliorate the deleterious effect of vaccination on performance up to 18 d of age.

Analysing the slaughter parameters, another positive effect of increased CP in the diet was found to be a decrease in carcass fat deposition. A similar effect of higher levels of protein in the diet was noted by Lehman et al. (2009). Moreover, the researchers noted a higher carcass yield due to the increased dietary CP level, the negative effect of vaccination in respect to this parameter as well as the significant interaction of these factors. In the current study, there was no significant effect of vaccination or nutritional treatment on the relative weights of immunological organs such as the spleen or bursa of Fabricius. Contrary to these findings, da Silva et al. (2009) recorded lower absolute and relative bursa weights and bursa diameters, but also lower lymphocyte bursa depletion or less damage in bursal lymphocytes due to vaccination against coccidiosis.

5. Conclusion

In conclusion, the results of the current study confirm the transient detrimental effect of live anticoccidial vaccination on performance that could not be fully compensated for by the birds before attaining slaughter age. Due to the positive, significant interactions of vaccination and increased CP level or HE- supplementation on performance in the grower-finisher and entire experimental period, these nutritional methods may be considered as support tools in terms of vaccination, although they were not able to significantly improve the performance of the vaccinated birds in the starter period.

Conflict of interest

There is no conflict of interest.

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