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INTERAÇÃO ENTRE VACINAS VIVAS ATENUADAS PARA COCCIDIOSE E COMPOSTOS FITOGÊNICOS NO DESEMPENHO ZOOTÉCNICO DE FRANGOS DE CORTE

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CHAPECÓ, SC, BRASIL

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INTERAÇÃO ENTRE VACINAS VIVAS ATENUADAS PARA COCCIDIOSE E COMPOSTOS FITOGÊNICOS NO DESEMPENHO ZOOTÉCNICO DE FRANGOS DE CORTE

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Elaborada por
Ricardo Marques de Andrade

como requisito parcial para obtenção do grau de
Mestre em Zootecnia

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Chapecó, novembro de 2020.

DEDICATÓRIA

Dedico este trabalho aos meus pais, que sempre foram a base de tudo e a meu Filho Miguel Zeni Marques de Andrade, que torna meus dias mais leves e move tudo que há em mim, com sua doçura, inocência, amor e carinho. Com vocês ao meu lado todos os sonhos se tornam possíveis.

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RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Zootecnia
Universidade do Estado de Santa Catarina

INTERAÇÃO ENTRE VACINAS VIVAS ATENUADAS PARA COCCIDIOSE E COMPOSTOS FITOGÊNICOS NO DESEMPENHO ZOOTÉCNICO DE FRANGOS DE CORTE

AUTOR: Ricardo Marques de Andrade
ORIENTADOR: Fernando de Castro Tavernari
Chapecó, novembro de 2020

A coccidiose aviária é considerada a parasitose de maior impacto na avicultura mundial, com um custo global estimado em 3 bilhões de dólares anuais, a doença causada pelos protozoários intracelulares do gênero *Eimeria*, destrói as células do epitélio intestinal gerando mortalidade e redução do desempenho. Nas últimas décadas, o uso de drogas anticoccidianas tem se mostrado eficiente no controle da doença, entretanto, o crescente surgimento de microrganismos resistentes, a pressão dos consumidores por alimentos isentos de resíduos e a criação de leis que proíbem o uso destas drogas em diversos países tornam necessário o desenvolvimento de novas estratégias de controle. Portanto, este estudo teve como objetivo determinar se as vacinas vivas associadas a compostos fitogênicos fornecidos via ração poderiam melhorar o desempenho, a saúde intestinal, os níveis de globulina e a ação coccidiostática em frangos de até 42 dias de idade, desafiados com Eimeria spp, em comparação com um programa anticoccidiano tradicional. Foram utilizadas 800 aves divididas em cinco tratamentos de oito repetições cada (n = 20): CN - Controle negativo (ausência de aditivos na ração e sem desafio de coccídio); CP - Controle positivo (ausência de aditivos e com desafio de coccídio aos 21 dias); PAA - Programa de aditivos anticoccidianos, incluindo salinomicina e nicarbazina, com desafio de coccidiano aos 21 dias de vida; VAC - Vacinação no dia 1 de vida (Hipracox HIPRA®) contra coccidiose e subsequente desafio com coccidiano aos 21 dias; VAC + BCF - Vacinação (dia 1 de vida), adição de 200 ppm do blend composto fitogênico (BCF) à base de carvacrol e cinamaldeido à dieta, com desafio de coccídio aos 21 dias de vida. As aves do grupo PAA ganharam peso mais significativo e maior peso corporal aos 21 dias do que os grupos CN e 42 dias de idade do que os grupos CN e VAC+BCF. A excreção de oocistos de *Eimeria* nas excretas das aves foi maior no CP do que no CN, assim como o CP teve contagens mais

altas do que CN, PAA e VAC+BCF. Um maior escore médio de lesões intestinais para *Eimeria acervulina* foi observado em aves VAC e VAC+BCF, enquanto para *Eimeria maxima*, o maior escore foi encontrado em PC. Níveis séricos mais elevados de proteína total devido ao aumento das globulinas foram observados em aves de PAA e VAC do que CN. Nossos resultados sugerem que a vacinação para coccidiose logo após o nascimento dos pintinhos permite um desempenho semelhante ao programa convencional de controle da coccidiose via dieta.

Palavras-chave: Avicultura, óleos essenciais, extratos herbáceos, imunidade celular, anticoccidianos, *Eimeria spp.*

ABSTRACT
Master's Dissertation
Programa de Pós-Graduação em Zootecnia
Universidade do Estado de Santa Catarina

INTERAÇÃO ENTRE VACINAS VIVAS ATENUADAS PARA COCCIDIOSE E COMPOSTOS FITOGÊNICOS NO DESEMPENHO ZOOTÉCNICO DE FRANGOS DE CORTE

AUTHOR: Ricardo Marques de Andrade
ADVISER: Fernando de Castro Tavernari
Chapecó, Novembro de 2020

Avian coccidiosis is considered the parasitic disease with the greatest impact on poultry worldwide, with an estimated global cost of 3 billion dollars annually, the disease caused by intracellular protozoa of the genus *Eimeria*, destroys the cells of the intestinal epithelium, generating mortality and reduced performance. In the last decades, the use of anticoccidial drugs has been shown to be effective in controlling the disease, however, the growing emergence of resistant microorganisms, the pressure of consumers for residue-free food and the creation of laws that prohibit the use of these drugs in several countries make it necessary to develop new control strategies. Therefore, this study aimed to determine whether live vaccines associated with phytogenic compounds supplied via feed could improve performance, intestinal health, globulin levels, and coccidiostatic action in broilers up to 42 days of age, challenged with *Eimeria* spp, compared with a traditional anticoccidial program. We used 800 birds divided into five treatments of eight repetitions each (n = 20): NC – Negative control (absence of additives in the feed and without coccidian challenge); PC – Positive control (absence of additives and with coccidian challenge at 21 days); AAP – Anticoccidial additives program, including salinomycin and nicarbazine, with coccidian challenge at 21 days of life; VAC – Vaccination on day 1 of life (Hipracox HIPRA®) against coccidiosis and subsequent coccidian challenge at 21 days; VAC+BPC - Vaccination (day 1 of life), the addition of 200 ppm of blend phytogenic compound (BPC) based on carvacrol and cinnamaldehyde to the diet, with coccidian challenge at 21 days of life. The birds in the AAP group more significant weight gain and greater body weight at 21 days than the NC and 42 days of age than the NC and VAC+BPC groups. The excretion of *Eimeria* oocysts in the excreta of birds was higher in PC than in NC, and in the litter, PC had higher counts than NC, AAP, and VAC+BPC. A higher mean

score of intestinal lesions for *Eimeria acervulina* was observed in VAC and VAC+BPC birds, while for *Eimeria maxima*, the highest score was found in PC. Higher serum levels of total protein due to the increase in globulins were observed in birds of AAP and VAC than NC. Our findings suggest that vaccination for coccidiosis shortly after the birth of chicks allows performance similar to the conventional coccidiosis control program via diet.

Keywords: Poultry, essential oils, herbal extracts, cellular immunity, anticoccidialsls, *Eimeria spp*.

SUMÁRIO

1. CAPÍTULO I
REVISÃO DE LITERATURA

1.1 Introdução

A coccidiose aviária é a doença das aves mais relatada em todo o mundo (BIGGS, 1982), é uma parasitose causada pelos protozoários do gênero *Eimeria* (PEEL e LANDMAN, 2003), que segundo Entzeroth *et al*. (1998) pertencem ao filo Apicomplexa, Classe Sporozoea, Família Eimeriidae. Ao todo, são reconhecidas sete espécies de *Eimeria* com relevância para a avicultura, *Eimeria acervulina, E. maxima, E. tenella, E. brunetti, E. necatrix, E. mitis e E. praecox* e cada espécie possui características específicas de prevalência, patogenicidade, imunogenicidade e local de infecção (ROSE e LONG, 1980). Estes protozoários invadem e destroem as células do epitélio intestinal das aves causando danos às vilosidades e dilatação das criptas (WITLOCK, 1977; TAN, 2014), resultando em piora na digestão e absorção de nutrientes com consequente redução na taxa de crescimento (ADEDOKUN, 2012 e TAN, 2014).

Embora na maioria dos casos a imunidade se desenvolva após infecções repetidas, a infecção leva a perdas econômicas (VERMEULEN, 2001), que segundo Dalloul e Lillehoj (2006) e Shivaramaiah *et al*. (2014) associados aos gastos com o controle da doença geram custos globais estimados em mais de três bilhões de dólares anuais.

1.2 Ciclo biológico das eimerias

Os protozoários do gênero *Eimeria* apresentam um ciclo de vida complexo composto de duas fases, uma fase exógena realizada no ambiente e outra fase endógena, realizada dentro do hospedeiro (BLAKE e TOMLEY, 2014). A fase exógena ocorre a partir da liberação de oocistos não esporulados (não infectantes) nas fezes, que ao entrar em contato com oxigênio, umidade e calor, sofrem esporulação dando origem aos oocistos esporulados (infectantes), por estar na dependência de fatores ambientais, esta fase varia em sua duração podendo levar de quatro a seis dias, sendo os oocistos viáveis por vários meses no ambiente em condições favoráveis (MCDOUGALD, 2013). Os oocistos esporulados contêm quatro

esporocistos em seu interior e cada um destes carrega dois esporozoítos, após a ingestão dos oocistos esporulados por parte do hospedeiro, inicia então a fase endógena, onde sob a ação do trato digestório os esporozoítos são liberados na luz intestinal invadindo os enterócitos (LAL et al., 2009). A fase endógena compreende duas etapas, a primeira é a etapa de reprodução assexuada, chamada de esquizogonia. Os esporozoítos liberados na luz intestinal invadem as células do epitélio passando a ser chamados de trofozoítos, que se multiplicam intensamente formando vacúolos intracelulares denominados esquizontes, repletos de células infectantes que a partir deste estágio passam a se chamar merozoítos. Após uma intensa multiplicação dos merozoítos, o aumento de volume dos esquizontes causa o rompimento dos enterócitos, liberando uma nova geração de merozoítos infectantes que invadem as células adjacentes do epitélio, dando origem a um novo ciclo (NORTON E CHARD, 1983). Dependendo da espécie da Eimeria envolvida, o número de ciclos de reprodução assexuada (esquizogonia) é variável, para então dar início então ao ciclo de reprodução sexuada (gametogonia). Depois de pelo menos dois ciclos de esquizogonia, os merozoítos sofrem diferenciação sexual dando origem aos gametas masculino e feminino, que após a fecundação, originam um oocisto não esporulado que é liberado no ambiente através das fezes do hospedeiro, completando o ciclo de vida do parasita (MCDOUGALD, 2013).

1.3 Transmissão da coccidiose aviária

A transmissão da doença ocorre através das fezes das aves infectadas (FERNANDO et al., 1987) onde são eliminados oocistos não esporulados, que em condições climáticas favoráveis (WALDENSTED et al., 2001) sofrem esporulação, tornando-se infectantes e capazes de suportar condições adversas durante meses, até sua ingestão por um novo hospedeiro (KAWAZOE, 2009).

1.4 Estratégias de controle

Embora boas práticas de produção possam auxiliar a reduzir o risco de transmissão dos parasitas causadores da coccidiose, são essenciais medidas adicionais para um controle completo da doença (McDONALD e SHIRLEY, 2009). O uso de aditivos anticcocidianos na ração de frangos de corte tem desempenhado um papel importante no controle das doenças entéricas nas últimas décadas (JONES e RICKE, 2003) e de acordo com Chapman et al. (2010), as drogas anticoccidianas utilizadas pela indústria avícola são classificadas em duas categorias, os compostos ionóforos e as drogas sintéticas. Estes aditivos podem ser agentes coccidiostáticos como os anticoccidanos químicos, que impedem a replicação e o crescimento do parasita ou agentes coccidicidas como os anticoccidianos ionóforos, que destroem o agente. Seu uso isolado ou em combinações provou ser um mecanismo eficaz na luta contra a coccidiose aviária (MacDOUGALD e FITZ-COY, 2009) e a utilização comercial durante anos tem mostrado que drogas anticoccidianas são seguras se utilizadas nas concentrações aprovadas (CHAPMAN, 1992). No entanto, o surgimento de cepas de *Eimeria* resistentes a estas drogas e a ineficácia dos tratamentos, levou à prática comum do uso alternado de drogas de diferentes grupos químicos para evitar o desenvolvimento de resistência (THOMKE e ELWINGER, 1998).

Embora esta estratégia seja economicamente viável e eficaz (CHAPMAN et al., 2010), a crescente preocupação dos consumidores sobre a inclusão de drogas profiláticas na alimentação animal (GREATHEAD e KAMEL, 2006), o uso restrito de drogas anticoccidianas em países europeus desde 2006 e o total banimento proposto para 2021(COUNCIL DIRECTIVE OF 2011/50/EU OF THE EUROPEAN COUNCIL), fazem com que métodos de controle alternativos para a coccidiose sejam urgentemente considerados (NOIROT, 2010). Consequentemente, o desenvolvimento e o uso de vacinas e outros métodos alternativos têm demonstrado um crescimento considerável (QUIROZ-CASTAÑEDA e DATAN-GONZALES, 2015).

1.4.1 Vacinas vivas para coccidiose

A imunidade para *Eimeria* é espécie-específica (MATHIS et al., 2017) e estimulada pelos primeiros estágios do desenvolvimento do parasita, particularmente os esquizontes, e posteriormente é ampliada e mantida por múltiplas reexposições aos oocistos presentes na

cama. Assim, a reciclagem da infecção, após a administração de oocistos vivos, é fundamental para a desenvolvimento de imunidade protetora (CHAPMAN e CHERRY, 1997). Segundo Allen e Fetterer (2002), atualmente são utilizados dois tipos de vacinas vivas no controle da coccidiose, atenuadas e não atenuadas, em ambas a eficácia consiste na reciclagem de doses iniciais muito baixas de oocistos e no gradual desenvolvimento de uma imunidade sólida. Williams (2002) cita que o uso de vacinas vivas não atenuadas é limitado pelo risco induzido pelas *Eimeria* vacinais, então é acompanhado do uso de drogas para controlar a patogenicidade dos agentes. No entanto, o sucesso das vacinas vivas atenuadas se deve ao fato de haver menor risco de ocorrência da doença, pois há redução na proliferação da *Eimeria* e como resultado menor dano ao intestino do animal (SHARMAN et al., 2010). Um dos métodos de atenuação das espécies de *Eimeria* é a técnica de seleção por precocidade, que permite a obtenção de populações de parasitas que completam seu ciclo de vida até 30 horas mais rápido do que as cepas de campo, resultando em parasitas com uma virulência atenuada e capacidade reprodutiva reduzida (McDONALD e SHIRLEY, 2009; SHIRLEY e BEDRNÍK, 1997; INNES e VERMEULEN, 2006).

1.4.2 Compostos fitogênicos

Segundo Randrianarivelo et al. (2010), uma das alternativas ao uso de antimicrobianos na ração é a utilização de compostos fitogênicos, definidos por Puvuca et al. (2013) como compostos bioativos derivados de plantas com efeito positivo no crescimento e na saúde dos animais, frequentemente utilizados para definir óleos essenciais e extratos herbáceos. Windisch *et al.* (2006) divide estes compostos em quatro subgrupos: ervas, condimentos, óleos essenciais (compostos lipofílicos extraídos por vaporização ou destilação a álcool) e oleorresinas (compostos extraídos por solventes não aquosos). De acordo com Kroismay et al. (2008), dentro do grupo dos compostos fitogênicos são encontrados diversos princípios ativos e substâncias que variam substancialmente em sua composição química, sendo em sua maioria compostos fenólicos (carvacrol, timol, eugenol, curcumina e pimenta) (Lee et al., 2004), desta forma, os efeitos destes compostos fitogênicos na performance das aves também são muito variáveis, não apenas na dinâmica da microbiota intestinal (DORMAN e

DEANS, 2000), mas também no metabolismo animal (Lee et al., 2004). Reconhecidamente, alguns compostos possuem atividade antimicrobiana, antiviral, antifúngica e antioxidante (BRENES e ROURA, 2010).

Na última década vários estudos demonstraram a eficiência de produtos derivados de plantas como agentes anticoccidianos naturais (DUFFY et al., 2005). Giannenas et al. (2003), observou que óleos essenciais extraídos do orégano, principalmente timol e carvacrol, exerceram efeito anticoccidiano sobre a *Eimeria tenella* reduzindo os sinais clínicos da doença e as lesões intestinais e Jamroz et al. (2006), relatou que óleos essenciais à base de carvacrol e cinamaldeído, bem como oleoresina de capsaicina estimularam a produção e secreção de mucina no intestino, possivelmente dificultando a adesão dos patógenos no intestino. Já Remmal et al. (2013) avaliou o impacto *in vitro* de óleos comerciais a base de carvacrol, carvona, isopulego, timol e eugenol sobre oocistos de *Eimerias*, observando a lise dos oocistos de *E. tenella* (45%), *E. maxima* (32%), *E. acervulina* (10%), *E. necatrix* (6%) e *E. mitis* (7%) através de a liberação de substâncias internas em 273 nm.

Embora os mecanimsode ação dos óleos essenciais ainda não seja conhecido (LIU, 2013), com a identificação dos princípios ativos dos compostos fitogênicos e algum progresso nos estudos da mecânica destes compostos nos animais, aumentaram os esforços em pesquisa para a utilização destas substâncias como substituta dos antimicrobianos na alimentação animal (LI et al., 2012). No entanto, os resultados destes estudos foram em grande parte inconsistentes (SI et al., 2006; LIU et al., 2014) e o fator econômico para a obtenção desses produtos pode ser um entrave para seu uso em larga escala na cadeia produtiva. (REMMAL et al., 2013).

1.5 OBJETIVOS

1.5.1 Objetivo geral:

Avaliar se o uso de anticoccidiano e de vacinas vivas atenuadas para coccidiose, associada ou não à aditivos fitogênicos fornecidos via ração, exercem efeito no desempenho de frangos de corte criados até 42 dias de idade.

1.5.2 Objetivos específicos:

2. Avaliar o impacto do uso de vacinas vivas atenuadas para coccidiose associado ou não a aditivos via ração na mortalidade, ingestão de ração, ganho de peso diário, conversão alimentar e peso ao abate de frangos de corte criados até 42 dias.
3. Classificar em escores as lesões macroscópicas do epitélio intestinal das aves submetidas aos tratamentos e desafiadas para coccidiose.
4. Avaliar a dinâmica de excreção de oocistos nas fezes das aves submetidas aos tratamentos e desafiadas para coccidiose.

2. CAPÍTULO II
MANUSCRITO

Os resultados desta dissertação são apresentados na forma de um manuscrito, com sua formatação de acordo com as orientações da Revista “Research Veterinary Science”

Interação entre vacinas vivas para coccidiose e compostos fitogênicos na dieta de frangos de corte: impactos no desempenho zootécnico e nas concentrações de globulina

Submetido

MANUSCRITO

Interaction between live vaccines for coccidiosis and phytogenic compounds in the diet of broilers: impacts on zootechnical performance and globulin concentrations

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ABSTRACT

This study aimed to determine whether live vaccines associated with phytogenic compounds supplied via feed could improve performance, intestinal health, globulin levels, and coccidiostatic action in broilers up to 42 days of age, challenged with *Eimeria* spp, compared with a traditional anticoccidial program. We used 800 birds divided into five treatments of eight repetitions each (n = 20): NC – Negative control (absence of additives in the feed and without coccidian challenge); PC – Positive control (absence of additives and with coccidian challenge at 21 days); AAP – Anticoccidial additives program, including salinomycin and nicarbazine, with coccidian challenge at 21 days of life; VAC – Vaccination on day 1 of life (Hipracox HIPRA®) against coccidiosis and subsequent coccidian challenge at 21 days; VAC+BPC - Vaccination (day 1 of life), the addition of 200 ppm of blend phytogenic compound (BPC) based on carvacrol and cinnamaldBPCyde to the diet, with coccidian challenge at 21 days of life. The birds in the AAP group more significant weight gain and greater body weight at 21 days than the NC and 42 days of age than the NC and VAC+BPC groups. The excretion of *Eimeria* oocysts in the excreta of birds was higher in PC than in NC, and in the litter, PC had higher counts than NC, AAP, and VAC+BPC. A higher mean score of intestinal lesions for *Eimeria acervulina* was observed in VAC and VAC+BPC birds, while for *Eimeria maxima*, the highest score was found in PC. Higher serum levels of total protein due to the increase in globulins were observed in birds of AAP and VAC than NC. Our findings suggest that vaccination for coccidiosis shortly after the birth of chicks allows performance similar to the conventional coccidiosis control program via diet.

Keywords: Poultry, herbal components, immunization, anticoccidials.

1. INTRODUCTION

Avian coccidiosis is caused by protozoa of the genus *Eimeria* and is considered the Parasitic disease carrying the most significant impact in industrial poultry (Dalloul and Lillehoj 2006). These protozoa invade and destroy the intestinal epithelium cells of birds, causing damaging villi and dilating the crypts. This results in worsening digestion and absorption of nutrients with a consequent reduction in the growth rate (Abebe and Gugsa, 2018). Although good production practices can help to reduce the risk of transmission of parasites that cause coccidiosis, additional measures are essential for complete control of the disease.

Traditionally, the use of anticoccidial drugs in the diet of broiler chickens is efficient in controlling the disease. Although this strategy is economically viable and practical, the growing development of microorganisms resistant to these active principles, pressure from consumers for the production of waste-free food, and the restriction of using these drugs in animal production in several countries make it necessary to develop alternative control tools.

In the 21st century, several studies demonstrated the potential of live vaccines and phytogenic compounds to control coccidiosis (Eckert et al., 2021; Galli et al., 2020). Carvacrol is one of the main active components of oregano and has anti-inflammatory, antioxidant, anti-tumor, analgesic, antiparasitic, and anti-genotoxic effects (Suntres et al., 2015). Cinnamaldehyde is one of the active components found in cinnamon (*Cinnamomum* spp), used as an animal performance enhancer because it protects the intestinal mucosa, stimulating antioxidant enzymes, modulating inflammatory reactions, and antimicrobial action (Pirgozliev et al. 2019). In addition, cinnamaldehyde has a coccidiostatic effect against *Eimeria maxima* and *Eimeria acervulina* (Lee et al., 2011).

Live vaccines initiate immune responses to protect against the consequences caused by *Eimeria* spp. Thus, they can restore sensitivity to drugs; that is, vaccine strains are sensitive to drugs and can control coccidiosis (Vereecken et al., 2021). Nevertheless, little is known about the concomitant use of these two tools and the impact of their interaction on broiler performance. Therefore, the objective of this study was to compare the effects of live vaccines associated with phytogenic compounds supplied via feed to a traditional

anticoccidial program concerning performance, intestinal health, and serum biochemistry in broilers reared up to 42 days of age and challenged with coccidiosis.

2. MATERIALS AND METHODS

The experiment was carried out in the poultry sector of the Center for Applied Science and Research in Monogastrics of the University of the West of Santa Catarina - Xanxerê, a project approved by the Ethics Committee on the Use of Animals of the same institution (36/2019).

2.1 Animals, management, and experimental design

We used 800 male broiler chicks of the Ross 308 line from 1 to 42 days old, distributed on the first day of housing in a completely randomized design, composed of five treatments and eight replicates each, comprising 20 birds in each experimental unit.

The birds were housed in boxes with concrete floors, with an area of 1.5 m^2, and equipped with a tubular feeder and a nipple drinker. The litter was composed of reusable wood shavings from one cycle, adequately treated with a fermentation process, with a 5–6 cm thickness. To demonstrate the absence of a challenge for coccidiosis, an evaluation of oocysts per gram of excreta (OOPG) of the litter was carried out before the birds were housed, and the absence of *Eimeria* oocysts was verified. The light program and the environmental management (temperature, humidity, and ventilation) followed the commercial management and the lineage manual. The birds received water and feed ad libitum.

The rations were formulated according to the recommendations of the Brazilian Tables for Poultry and Swine (Rostagno et al., 2017), divided into three phases, initial (1–21 days), growth (22–35 days), and final (36–42 days). The main ingredients included in the diet were ground corn, soybean meal, and soybean oil, as detailed in Table 1.

To guarantee the presence of a coccidian challenge, the birds were individually challenged for coccidiosis at 21 days of age, in which they received orally the live vaccine (Bio-Coccivet, Biovet®), with a dose eight times greater (approximately 28 thousand oocysts/bird) than recommended by the manufacturer, which contains sporulated oocysts of

E. acervulina, *E. maxima*, and *Eimeria tenella*. The age of 21 days was defined for the challenge. The vaccine and the infective dose were based on previous studies by our research group (Galli et al., 2020). To verify the presence and effectiveness of the challenge, one of the treatments was defined as a negative control, in which the birds did not receive the coccidian challenge.

The treatments were defined as follows: NC – Negative control (absence of additives in the diet and without coccidian challenge); PC – Positive control (absence of additives and with coccidian challenge at 21 days of age); AAP – Anticoccidial additives program, comprising salinomycin 50 ppm + nicarbazine 50 ppm (Salinocarb, Ilender®) in the initial phase, salinomycin 72 ppm in the growth phase (Salinacox, Ilender®), and feed without the presence of anticoccidial in the final phase, with challenge coccidian at 21 days of age; VAC – Vaccination on day 1 of life against coccidiosis and coccidian challenge at 21 days of age; VAC+BPC – Vaccination on day 1 of life and addition of 200 ppm phytogenic compound in the diet containing carvacrol and cinnamaldehyde (Active, GRASP®), with coccidian challenge at 21 days of age. For the vaccination of birds, included in the groups VAC and VAC+BPC, the chicks were vaccinated by spraying on the first day of housing with the live attenuated vaccine for coccidiosis at 0.007 ml/bird (Hipracox HIPRA®). The formulation of this vaccine is shown in Table 2.

2.2 Performance evaluation

The birds were weighed on days 1, 7, 21, and 42 days, the same period that the amount of feed consumed (supplied – surplus) of each cycle was measured. Feed conversion (FC) was calculated by the total amount of feed ingested divided by the weight gain of the birds. Knowing this information, as well as mortality, the index of productive efficiency (IPE) was calculated during the entire production cycle (days 1 to 42), according to the following formula: IPE = (body weight x viability) / (age at slaughter x feed conversion).

2.3 Count of oocysts in excreta and litter

At 28 days of age (seven days after the coccidian challenge), samples of fresh excreta and poultry litter were collected at four points, at random, to represent the entire

area of each experimental unit. Approximately 20 g of fresh excreta and 300 g of litter were collected from each experimental unit for analysis. The samples were placed in properly identified plastic bags, then homogenized and diluted in a saturated NaCl solution in a 1:10 ratio, with the oocyst count performed in a MacMaster chamber, as described by Hodgson (1970).

2.4 Sample collection

Blood was collected from eight birds per treatment after 28 days of the experiment. The birds were manually contained, and using an insulin syringe, blood was collected from the ulnar vein. Subsequently, this material was placed in a tube without anticoagulant to obtain the serum. This material was then centrifuged at 3500 rpm for 10 minutes, and the separated serum was frozen (-20 °C) for biochemical analysis.

2.5 Assessment of intestinal injury score

At 28 days of age, one bird was slaughtered per experimental unit by cervical dislocation and disarticulation, according to animal welfare rules and euthanasia rules described by the CONCEA euthanasia practice guidelines (Brasil/MCTI, 2013), to determine the intestinal injury score. For this analysis, macroscopic lesions of the intestinal epithelium of the duodenum, jejunum, ileum, and cecum were classified according to the scale of Johnson and Reid (1970).

2.7 Histopathology

At 28 days, jejunum samples were collected and preserved in vials with 10% formaldehyde solution. Slides with histological sections were made and stained with hematoxylin and eosin. Under a light microscope, the morphological structure of the intestinal portions collected was evaluated. In this way, the villus length and crypt depth were evaluated according to the methodology described by Caruso and Demonte (2005). Histological images of the slides were captured using a digital microcamera (Electronic Eyepiece Camera Video) coupled to the trinocular biological microscope (model TNB-41T-PL, OPTON) and a specific program for capturing histological images (Images J). More

details of the methodology used to measure villus length and crypt depth were described by Galli et al. (2020).

2.8 Serum biochemistry

The total protein and albumin concentrations were determined using commercial analytical kits (Analisa®) and a semi-automatic biochemical analyzer (Bioplus 2000®). The serum globulin level was calculated as follows: globulin = total proteins – albumin.

2.9 Statistical analysis

The experimental results were subjected to the Shapiro–Wilk normality test and subsequently subjected to variance analysis. In case of significant difference, the experimental data were compared using the Tukey test at 0.05 probability, using the statistical software R.

3. RESULTS

3.1 Performance

Performance results are displayed in Table 3, which were observed no differences ($P > 0.05$) at seven days of age. However, from 1 to 21 days of age, birds in the AAP group had higher body weight ($P < 0.008$) and greater weight gain ($P < 0.009$) than birds in the PC. From 1 to 42 days of age, birds in the AAP group had higher body weight ($P < 0.010$) and greater weight gain ($P < 0.011$) than PC and VAC+BPC. There were no differences in feed intake or FC in all groups at all times ($P > 0.05$). The IPE did not vary across groups ($P > 0.05$; Tabl 3).

3.2 Oocyst count in excreta and litter

The oocyst counts in the excreta were lower in the birds in the NC and AAP groups than the PC ($P = 0.026$). However, there were no differences in the oocyst counts in the excreta from birds of the other treatments ($P > 0.05$). In addition, higher oocyst counts were

found in the litter of PC chickens than the NC, AAP, VAC, and VAC+BPC groups (P = 0.013; Fig. 1).

3.3 Assessment of intestinal injury score

Scores of intestinal lesions caused by *E. acervulina* differed between treatments, with birds in the VAC and VAC+BPC treatments having higher injury scores than birds in the PC group (P < 0.004). Scores of intestinal injury caused by *E. maxima* also differed between treatments; that is, birds in the PC group had higher injury scores than birds in the NC, AAP, and VAC+BPC groups (P < 0.003). There were no changes in the rates of injuries caused by *E. tenella* between groups (P > 0.05; Table 4).

3.4 Histopathology

Intestinal micrometry is shown in Table 5, which no diferences were found for villus height, crypt depth, and villus: crypt ratio between groups (P > 0.050).

3.5 Serum biochemistry

Protein levels are shown in Table 6. The birds in the AAP and Vac groups had higher total protein levels than the NC, PC, VAC+BPC groups (P = 0.011), similar to what we observed for globulins (P = 0.024).

4. DISCUSSION

Infections caused by *Eimeria* spp destroy the cells of the intestinal epithelium of birds, damaging the villi and dilating the crypts (Witlock, 1977; Tan, 2014), resulting in worsening digestion and absorption of nutrients with a consequent reduction in the growth rate (Adedokun, 2012; Tan, 2014). Similar observed in present results. Furthermore, in severe cases, coccidiosis can cause the host's death (Shirley et al., 2005). However, coccidiosis can occur clinically or subclinically (Haug 2008), and its manifestations depend on the infecting dose, the environment in which the animals are inserted, and host-related factors, including age, health, and physiological condition (Tafti and Mansourian, 2008). In the present study, the group of chickens used as a positive control, challenged with coccidia, had less weight gain, similar to what occurred in the negative control group;

suggesting that factors other than coccidiosis interfere with growth, because the birds of the NC had not been infected experimentally.

Birds belonging to the conventional anticoccidial program showed more significant weight gain at 21 and 42 days. This fact can be explained by the antimicrobial action of the ionophore anticoccidial salinomycin or its imAAPct on the intestinal microbiota of birds (Johansen, 2007). Lanckriet et al. (2010), when using this coccidiosis control program, showed a reduction in the count of *Clostridium perfringes* in bird intestines, which may have influenced growth. The challenge for coccidiosis using the live vaccine at a high dose is to avoid inducing disease, similar to what was observed in another study (Oviedo-Rondón 2005).

Broilers in the positive control group had a higher count of oocysts in feces and litter, which was expected because conventional or alternative coccidia are necessary to control coccidiosis, which is reflected in environmental contamination. Costa and Ávila (2003) reported that management techniques for the reuse of poultry litter, including canvas fermentation, cannot eliminate the sporulated oocysts of *Eimeria* spp., and therefore, the use of anticoccidials remains necessary to maintain high chicken meat productivity.

There was no difference between the other treatments, and these results may be related to the pre-symptomatic period of the disease or sample collection time (28 days of age), which corresponds to 7 days after the challenge. The reduction in the presence of oocysts in the poultry litter supplemented with anticoccidial agents such as salinomycin, an iontophoretic anticoccidial agent, can be explained by the alteration of the permeability of the cell membrane of *Eimeria* spp, leading to the death of the parasite. Chapman (2010) found that nicarbazine, an anticoccidial chemical, acts by inhibiting the development of the schizogony phase in the biological cycle of *Eimeria* (Aslian et al., 2014). In our study, the count of oocysts in fresh feces was statistically the same in the challenged groups, except for the AAP group. Unlike the excreta, in the litter of the AAP, VAC, and VAC+BPC groups, a lower oocyst count was observed. These results suggest a prophylactic (anticoccidial) vaccine and phytogenic, which may have induced the immunity that interfered in replicating the parasite, with consequent lower elimination of oocysts into the litter. Resinger et al. (2011) reported that phytogenic additives improved performance and immune system modulation, which can help birds face challenges for coccidiosis. In our

study, phytogenics did not increase weight gain, unlike the report of Giannenas et al. (2004), who found that the addition of phytogenic compounds in the diet improved the performance of broilers challenged with coccidiosis.

Lee et al. (2011) observed that cinnamaldehyde decreased the cell viability of *Eimeria* spp oocysts and increased the antibodies of the microneme protein. CinnamaldBPCyde increased the expression of interferon-gamma, which is related to the activation of macrophages to combat pathogens. Bozkurt et al. (2013) reported that carvacrol has an antiparasitic activity for *Eimeria* spp due to its ability to bind to the sporozoite membrane, which causes loss of the parasite's calcium ions, an essential mineral for invasion. In our study, the reduction of contamination in chicken litter suggests a direct effect of this phytogenic on the oocysts present in the excreta, inactivating or disrupting them, which deserves to be investigated in future studies.

The highest average score of intestinal lesions caused by *E. acervulina* was observed in birds challenged and subjected to vaccination and vaccination + phytogenic, which may be a consequence of these birds having at two different times of infection by *Eimeria*; that is, in the first days of life and later in the challenge held on the 21st, as both were made with live vaccines. Mathis et al. (2007) explain that immunity to *Eimeria* is stimulated by the first stages of development of the parasite, particularly schizonts, and is subsequently extended and maintained by multiple reexposures to oocysts present in the bed, thereby recycling the infection after administration of live oocysts is essential for the development of protective immunity (Chapman and Cherry, 1997).

Sharmam (2010) mentioned that *E. maxima* is one of the species with the highest pathogenicity, which may explain the higher average score of intestinal lesions caused by this species in the chickens of the positive control group when there was no conventional or alternative performance enhancer. Researchers criticize the individual assessment of the average score of intestinal injuries because, according to them, it has no considerable diagnostic value for *E. maxima*; however, it is useful when associated with histopathological evaluations of the injuries (Goodwin et al. 1998). In our study, although there was a difference in the weight gain of the birds, there was no change in the height of the villi or depths of the crypts. Consequently, the villus/crypt ratio was similar between treatments, which may be attributed to the low-level challenge of the birds in the

experimental station and having used not an inoculum of *Eimeria* spp but rather a live attenuated vaccine.

The increase in serum total protein levels may be related to better use of dietary protein and being related to improved health (OSO et al., 2014); however, in this case, we believe it is related to the immune response. Because the increase in total protein is directly related to the increase in globulins, an immune response is associated with greater production of antibodies (immunoglobulins) or acute phase proteins (Shamna et al., 2017). The higher levels of globulins in birds in the AAP group must have directly impacted the better zootechnical performance because healthy birds with strong immunity have better growth than those with coccidiosis (Perin et al., 2019). Likewise, the higher concentration of globulin also contributed to the growth of chickens in the Vac group, which may have been a consequence of an antibody production generated in an immune response to the vaccine used in the first route of life, boosted by the challenge performed on the 21st day of life. One explanation for not having observed the same effect in the VAC+BPC treatment is the anti-inflammatory effect of phytogenics, already reported in broilers that consumed bend of the herbal compounds used in this study (Galli et al., 2020b).

5. CONCLUSION

Vaccination for coccidiosis shortly after the birth of the chicks allows a performance similar to the conventional coccidiosis control program via diet; however, when this vaccine is associated with a phytogenic product, weight gain is negatively affected, being similar to birds in the control groups. The animals that have greatest weight gain are the ones that have the highest serum concentration of globulins, showing a positive correlation between performance and robust immune response. The main benefit of using phytogenics in bird diet is the reduction in the contamination of *Eimeria* spp oocysts in the poultry litter.

Declaration of competing interest

The authors declare no conflict of interest.

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Ethics committee

All procedures the Comitê de Ética approved this project *do Uso de Animais na Pesquisa* (CEUA) of the *Universidade do Oeste de Santa Catarina*, under protocol number 36/2019, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA).

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Table 1: Ingredients and basal feed used for all experimental groups.

	1 – 21 days	22–35 days	36–42 days
Corn (g/kg)	479.57	509.73	614.49
Soybean meal (g/kg)	433.08	391.84	304.46
Soybean oil (g/kg)	44.95	61.35	49.18
Bicalcium phosphate (g/kg)	18.94	14.53	10.92
Limestone (g/kg)	8.32	8.36	7.14
Iodized salt (g/kg)	4.60	4.25	4.07
DL-Methionine, purity 99% (g/kg)	3.34	3.16	2.54
L-Lysine HCL, purity 77% (g/kg)	2.21	1.81	2.22
L-Threonine, purity 98% (g/kg)	0.82	0.66	0.64
L-Valine, purity 96,5% (g/kg)	0.17	0.31	0.31
Premix of vitamins[1] (g/kg)	2.00	2.00	2.00
Premix of minerals[2] (g/kg)	2.00	2.00	2.00
Calculated Composition			
Metabolizable Energy (Kcal/kg)	3050	3200	3250
Crude protein (%)	24.3	22.62	19.54
Calcium (%)	0.94	0.83	0.66
Available phosphorus (%)	0.47	0.38	0.31
Digestible lysine (%)	1.36	1.23	1.06
Digestible methionine + cysteine (%)	0.96	0.91	0.79
Digestible threonine (%)	0.88	0.81	0.70
Digestible tryptophan (%)	0.28	0.26	0.21
Digestible Valine (%)	1.00	0.95	0.82
Sodium (%)	0.22	0.21	0.20

[1] Minimal vitamin levels per kg of food: vitamin A (5000000 IU); vitamin D3 (1.000.000 IU); vitamin E (15000 IU); vitamin K3 (1.500 mg); vitamin B1 (1.500 mg); vitamin B2 (3.000 mg); vitamin B6 (2.000 mg); vitamin B12 (7.000 mcg); folic acid (500 mg); nicotinic acid (15 g); AAPntothenic acid (7000 mcg); choline (80 g); biotin (100 mg); minimum humidity (40 g); maximum mineral matter (500 g).

[2] Minimal mineral levels per kg of food: copper (10 g); iron (50 g); iodine (1.000 mcg); manganese (80 g); selenium (300 mg); zinc (70 g); minimum humidity (20 g); maximum mineral matter (980 g).

Table 2: Composition of the commercial attenuated live vaccine for coccidiosis (Hipracox®).

Eimeria	Cepa	Oocysts (n) per dose
Eimeria acervulina	003	300–390
Eimeria maxima	013	200–260
Eimeria mitis	006	300–390
Eimeria praecox	007	300–390
Eimeria tenella	004	250–325

Table 3: Means and standard error of including different programs to mitigate the occurrence of coccidiosis on broiler performance.

		Treatments						
		PC	NC	AAP	VAC	VAC + BPC	P-value	CV (%)
1–7 days	BW (g)	173 ± 1.19	181 ± 1.60	176 ± 1.40	178 ± 0.91	178 ± 1.73	0.134	2.63
	WG (g)	128 ± 1.29	136 ± 1.65	130 ± 1.46	133 ± 0.73	133 ± 1.61	0.139	3.59
	FCo (g)	168 ± 2.09	171 ± 6.10	164 ± 4.60	168 ± 2.66	164 ± 2.64	0.213	6.42
	FC	1.32 ±0.01	1.26 ± 0.04	1.26 ±0.03	1.26 ±0.02	1.23 ± 0.02	0.324	6.70
1 –21 days	BW (g)	908±22.97^{b}	949 ±14.80^{ab}	996±15.61^{a}	943±11.57^{ab}	934±11.03^{ab}	**0.008**	5.41
	WG (g)	863±23.00^{b}	905±14.33^{ab}	949±15.56^{a}	897±11.45^{ab}	889±11.41^{ab}	**0.009**	5.64
	FCo (g)	1140±27.13	1204±17.58	1244±23.08	1204±15.90	1165±17.88	0.176	5.96
	FC	1.32±0.01	1.33±0.01	1.31±0.01	1.34±0.01	1.31±0.01	0.243	2.75
1–42 days	BW (g)	2538±28.67^{b}	2630±47.59^{ab}	2750±58.21^{a}	2638±45.25^{ab}	2525±45.15^{b}	**0.010**	5.66
	WG (g)	2489±28.58^{b}	2581±47.04^{ab}	2699±57.29^{a}	2588±44.22^{ab}	2476±44.59^{b}	**0.011**	5.68
	FCo (g)	4020±31.77	4093±80.88	4231±89.03	4177±80.15	4034±70.47	0.166	4.95
	FC	1.62±0.02	1.59±0.02	1.57±0.01	1.61±0.02	1.63±0.02	0.168	3.55
	IPE	383±11.40	395±8.49	406±3.83	382±11.83	375±11.19	0.217	7.34

* * Different letters on the same line indicate a significant difference by the Tukey test, at 0.05 significance.

BW – body weight; WG – weight gain; FCo – feed consumption; FC – feed conversion; IPE – index of productive efficiency; NC – control negative; PC – positive control; AAP – Anticoccidial program consisting of salinomycin + nicarbazine for 1–21 days and salinomycin for 22–35 days; Vac - Vaccination; Vac + BPC - Vaccination together with 200 ppm of phytogenic compounds.

Table 4: Score of intestinal injury caused by coccidiosis analyzed at 28 days of life, corresponds to seven days after challenge with a high dose of oocysts of *Eimeria* spp.

	Eimeria acervulina	*Eimeria maxima*	*Eimeria tenella*
Negative control	0.00 ± 0.00^{b}	0.13 ± 0.12^{b}	0.13 ± 0.12
Positive control	0.50 ± 0.19^{ab}	0.75 ± 0.16^{a}	0.25 ± 0.16
Anticoccidial Program	0.25 ± 0.16^{ab}	0.00 ± 0.00^{b}	0.25 ± 0.16
Vaccine	0.88 ± 0.22^{a}	0.25 ± 0.16^{ab}	0.13 ± 0.12
Vaccine + phytogenic compounds	0.75 ± 0.16^{a}	0.13 ± 0.12^{b}	0.00 ± 0.00
P-value	**0.004**	**0.003**	0.637
CV (%)	45.00	61.00	91.00

* Different letters in the same column indicate a significant difference by the Tukey test, at 0.05 significance

Table 5: Means and standard error of the effects of including different programs to mitigate the occurrence of coccidiosis on intestinal histomorphometric AAPrameters in broilers.

	Treatments						
	NC	PC	AAP	VAC	VAC+BPC	P-value	CV (%)
Villus height (µm)	1678.75 ± 72.60	1760.5 ± 45.50	1673.75 ± 54.60	1761.37 ± 49.50	1646.50 ± 62.30	0.526	9.52
Crypt depth (µm)	167.28 ± 9.33	159.94 ± 6.08	173.69 ± 10.27	186.44 ± 7.11	164.68 ± 7.35	0.224	10.79
Villus/crypt ratio	11.08 ± 0.75	11.41 ± 0.45	10.11 ± 0.44	9.94 ± 0.54	10.78 ± 0.75	0.532	11.95

* Different letters on the same line indicate a significant difference by the Tukey test, at 0.05 significance.
NC – negative control; PC – positive control; AAP – Anticoccidial program composed of salinomycin + nicarbazine from 1 to 21 days and salinomycin from 22 to 35 days; Vac - Vaccination; Vac + BPC - Vaccination together with 200 ppm of herbal extracts.

Table 6: Means and standard error of the effects of including different programs to mitigate the occurrence of coccidiosis on total protein, albumin, and globulin in broilers at 28 days of age.

	Treatments						
	NC	PC	AAP	VAC	VAC + BPC	P-value	CV (%)
Protein (g/dL)	5.41 ± 0.21^{bc}	5.74 ± 0.10^{b}	6.39 ± 0.36^{a}	6.84 ± 0.31^{a}	5.03 ± 0.30^{c}	**0.011**	6.02
Albumin (g/dL)	1.98 ± 0.03	2.4 ± 0,18	2.07 ± 0.19	1.87 ± 0.12	1.70 ± 0.51	0.089	4.12
Globulin (g/dL)	3.43 ± 0.15^{b}	3.34 ± 0.12^{b}	4.32 ± 0.20^{a}	4.97 ± 0.25^{a}	3.33 ± 0.26^{b}	**0.024**	5.58

* Different letters on the same line indicate a significant difference by the Tukey test, at 0.05 significance.
NC – negative control; PC – positive control; AAP – Anticoccidial program composed of salinomycin + nicarbazine from 1 to 21 days and salinomycin from 22 to 35 days; Vac - Vaccination; Vac + BPC - Vaccination together with 200 ppm of herbal extracts.

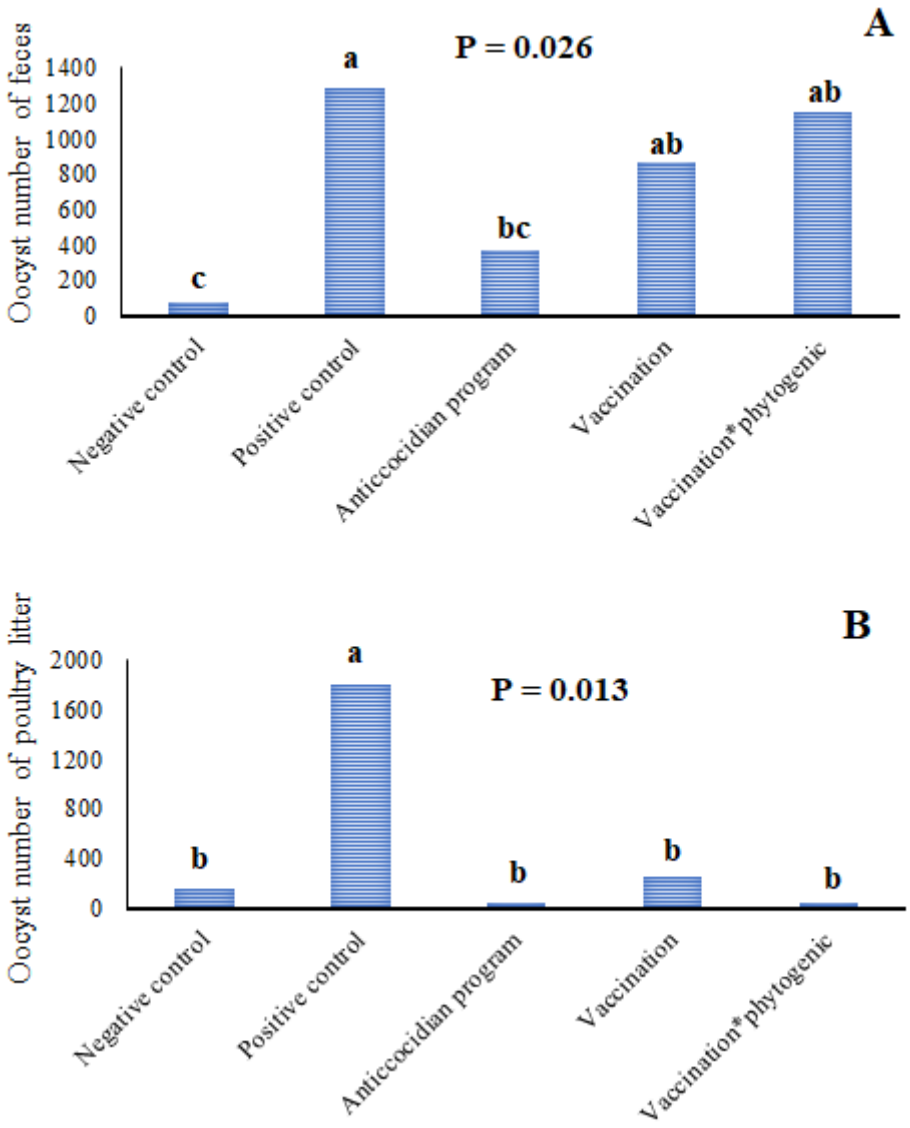

Figure 1. Count of Oocysts per gram (OOPG) of *Eimeria* in fresh feces and broiler litter subjected to different mitigation alternatives to avian coccidiosis.
NC - negative control; PC - positive control; AAP - Anticoccidial program composed of salinomycin + nicarbazine from 1 to 21 days and salinomycin from 22 to 35 days; Vac - Vaccination; VAC+BPC - Vaccination on day 1 of life associated with 200 ppm of phytogenic compounds.

3. CAPÍTULO III

CONSIDERAÇÕES FINAIS

Podemos concluir que o programa tradicional, baseando na utilização do anticoccidiano ionóforo salinomicina e do anticoccidiano químico nicarbazina exerce impacto positivo sobre os parâmetros de qualidade intestinal e desempenho zootécnicos das aves, reduzindo a contagem de oocistos na cama e demonstrando ser mais eficiente do que programas alternativos, baseados na utilização de vacinas vivas atenuadas para coccidiose e compostos fitogênicos, permanecendo, portanto, como uma ferramenta viável para o controle da parasitose.

A utilização de vacinas vivas atenuadas para coccidiose, mesmo quando associadas à compostos fitogênicos à base de carvacrol e cinamaldeído, pode causar lesões intestinais, entretanto, sem exercer impacto negativo no desempenho zootécnico das aves de modo geral. Programas alternativos baseados nestas ferramentas de controle demonstram potencial quando avaliada a contagem de oocistos nas fezes, sugerindo a necessidade de mais estudos e avaliações, especialmente em um cenário crescente de restrições parciais ou totais ao uso de anticoccidianos ionóforos e químicos na alimentação animal.

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ANEXO

O CAMPO ABAIXO É DE PREENCHIMENTO DA CEUA

PARECER CONSUBSTANCIADO DA CEUA/UNOESC

DE ACORDO COM O EXPOSTO ACIMA, A CEUA PROCEDE COM O SEGUINTE RELATO:

I – DADOS DE IDENTIFICAÇÃO		
Protocolo de Pesquisa CEUA nº 36/2019	Pesquisa (x)	
Título do Projeto: Interação entre vacinas vivas atenuadas para coccidiose e compostos fitogênicos no desempenho zootécnico de frango de corte		
Data de Início: 10/09/2019		
Data do Término: 31/05/2020		
Pesquisador Responsável: Tiago Goulart Petrolli		
Unoesc de: Xanxerê	Área: Ciências Agrárias	Curso: Mestrado em Sanidade e Produção Animal

II – ANÁLISE DO PROTOCOLO

Justificativa/Relevância (item 2.5): Verifique se o pesquisador apresenta uma justificativa coerente para a utilização dos animais, seja pela falta de dados na literatura e a importância clínica e/ou científica do projeto, considerando principalmente o que é descrito sobre os métodos alternativos.

O uso de drogas com efeito anticoccidiano na ração de frangos de corte tem demonstrado ser eficiente no controle da doença, entretanto, o crescente desenvolvimento de microrganismos resistes a estes princípios ativos, a pressão por parte dos consumidores pela produção de alimentos livres de resíduos e a restrição do uso destas drogas na produção animal em diversos países tornam necessário o desenvolvimento de ferramentas de controle alternativas. Nos últimos anos diversos estudos tem demonstrado o potencial de vacinas vivas e compostos fitogênicos no controle da coccidiose, no entanto, pouco se sabe sobre o uso concomitante destas duas ferramentas e o impacto de sua interação no desempenho de frangos de corte.

Metodologia: Descreva de forma sucinta a metodologia e análise da questão dos desconfortos, dos riscos, do número de animais, respectivas medidas preventivas e curativas quando necessário etc.

Serão 900 pintos machos adquiridos de incubatório e alojados no aviário da UNOESC Xanxerê. Eles serão divididos em 4 grupos com 10 repetições cada.

A ração inicial e de crescimento terão 3 versões cada (uma sem aditivos, outra com adição de anticoccideo e outra com compostos fitogênicos) e a ração final terá 2 versões (uma sem aditivos e outra com compostos fitogênicos).

Todos os grupos serão desafiados para coccidiose nos 22 dias de idade.

As aves serão pesadas nos dias 7,14, 21, 28m 35 e 42 para avaliação do ganho de peso. A ração também será pesada diariamente para avaliar o consumo em relação ao ganho de peso. E a mortalidade será registrada diariamente. Será coletado 300-500gramas de fezes frescas nos mesmos dias da pesagem e será realizada a contagem de oocitos. Será coletado sangue de forma quinzenal. E serão sacrificadas 2 aves após 7 dias do desafio para avaliação macro e microscópica do intestino.