UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL

ADITIVOS ALTERNATIVOS AOS ANTIBIÓTICOS PROMOTORES DE CRESCIMENTO NO DESEMPENHO DE FRANGOS DE CORTE SOB DESAFIO SANITÁRIO

Letícia Soares
Zootecnista

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Orientador: Profa. Dra. Nilva Kazue Sakomura

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TÍTULO: ADITIVOS ALTERNATIVOS AOS ANTIBIÓTICOS PROMOTORES DE CRESCIMENTO NO DESEMPENHO DE FRANGOS DE CORTE SOB DESAFIO SANITÁRIO

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DADOS CURRICULARES DO AUTOR

LETÍCIA SOARES – filha de Roosevelt José Soares e Marisa Soares, nasceu no dia 24 de outubro de 1989, na cidade de Jundiaí, São Paulo. Em março de 2008 ingressou no curso de Zootecnia na Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista – câmpus de Jaboticabal, São Paulo, graduando-se em março de 2013. No período de agosto de 2011 a julho de 2012 foi bolsista de iniciação científica pelo CNPq, sob orientação da Profa. Dra. Sandra Aidar de Queiroz. Em março de 2013 ingressou no curso de mestrado em Zootecnia pela mesma instituição, onde obteve bolsa pelo CNPq, sob orientação da Profa. Dra. Nilva Kazue Sakomura e defendendo esta dissertação em fevereiro de 2015.

“[...] Não vou me deixar embrutecer, eu acredito nos meus ideais. Podem até maltratar meu coração, que meu espírito ninguém vai conseguir quebrar [...]"

(Um dia perfeito - Renato Russo)

“[...] O caminho não está pronto, mas é preciso sempre caminhar muito mais. O caminho se mostra enquanto persistente, caminhar sempre pra frente [...]”

(O horizonte é logo ali – O Rappa)

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SUMÁRIO

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CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o Protocolo nº 12536/14 do trabalho de pesquisa intitulado **"Ácidos orgânicos em substituição à antibióticos para frangos de corte"**, sob a responsabilidade da Prof.ª Dr.ª Nilva Kazue Sakomura está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 07 de julho de 2014.

Jaboticabal, 07 de julho de 2014.

Prof.ª Dr.ª Paola Castro Moraes
Coordenadora – CEUA

ADITIVOS ALTERNATIVOS AOS ANTIBIÓTICOS PROMOTORES DE CRESCIMENTO NO DESEMPENHO DE FRANGOS DE CORTE SOB DESAFIO SANITÁRIO

RESUMO – O objetivo deste trabalho foi avaliar o uso de aditivos alternativos aos antibióticos promotores de crescimento sobre o desempenho, morfometria intestinal e controle de enterite necrótica em frangos de corte, para isso foram realizados dois ensaios. No primeiro, foram utilizados 1920 pintinhos machos Cobb 500®, distribuídos em 8 tratamentos e 8 repetições com 30 aves em cada parcela. Os tratamentos foram: Controle positivo, sem aditivos na dieta e sem desafio sanitário; Controle negativo (CN), sem aditivos e com desafio sanitário; CN + antibiótico, CN + prebiótico A; CN + antibiótico + prebiótico A; CN + probiótico; CN + ácido orgânico; CN + prebiótico B. Foram avaliados peso corporal, ganho de peso, consumo de ração, e conversão alimentar, bem como os parâmetros intestinais altura de vilo, profundidade de cripta, relação vilo:cripta e número de células caliciformes. Não houve diferença significativa no desempenho animal e qualidade do trato intestinal entre os tratamentos testados. Entretanto, houve aumento significativo no número de células caliciformes no jejuno de aves tratadas com prebiótico A e com antibiótico + prebiótico A. No segundo ensaio, foram utilizados 1360 pintinhos machos Cobb 500®, distribuídos em 5 tratamentos e 8 repetições com 34 aves em cada parcelas. Os tratamentos foram: Não desafiado, sem aditivo na dieta e sem desafio sanitário; Desafiado, sem aditivo e com desafio sanitário; Controle positivo, Desafiado + enramicina 10 ppm; Desafiado + Premium Lac AP; Desafiado + Premium Lac BP. Foram avaliados peso corporal, ganho de peso, consumo de ração, e conversão alimentar, bem como escore de lesão intestinal causada por enterite necrótica. No período de 1 a 42 dias, houve diferença estatística apenas para consumo de ração. Entretanto, no período de 22 a 35 dias quando houve a inoculação com *C. perfringens*, o tratamento não desafiado apresentou melhores resultados, já o tratamento desafiado apresentou resultados similares ao antibiótico, que foi similar ao Premium Lac AP para todas as variáveis avaliadas, indicando que o Premium Lac AP é tão efetivo quanto o antibiótico no controle de enterite necrótica. Premium Lac AP e

Premium Lac BP se destacaram com a menor incidência de lesões intestinais, sugerindo maior eficiência no controle da doença.

Palavras – chave: Ácidos orgânicos, *Clostridium perfringens*, morfometria intestinal, prebiótico, probiótico, *Saccharomyces cerevisae*

ALTERNATIVE ADDITIVES TO ANTIBIOTIC GROWTH PROMOTER ON THE PERFORMANCE OF BROILERS SUBMITTED TO IMMUNE CHALLENGE

ABSTRACT - The aim of this study was evaluate the use of alternative additives to antibiotic growth promoter on performance, intestinal morphometric and necrotic enteritis control of broilers, for this were realized two trials. In the first trial, were used 1,920 male Cobb 500® broilers, distributed into 8 treatments and 8 replicates in a completely randomized design, with 30 birds per pens. The treatments were: Positive Control (PC), with no additives in the diet and no sanitary challenge; Negative control (NC), with no additives in the diet but with sanitary challenge; NC + antibiotic; NC + prebiotic A; NC + antibiotic + prebiotic A; NC + probiotic; NC + organic acid; NC + prebiotic B. We evaluated body weight, body weight gain, feed intake, and feed conversion ratio, as well as the intestinal parameters villus height, crypt depth, villus:crypt ratio, and goblet cell count. No significant differences were found in animal performance between the tested treatments tested. No significant differences in the quality of the intestinal tract were seen. However, the number of goblet cells in the jejunum significantly increased in birds treated with prebiotic A and with antibiotic + prebiotic A. In the second trial, were used 1,360 male Cobb 500® broilers, distributed into 5 treatments and 8 replicates in a completely randomized design, with 34 birds per pens. The treatments were: Unchallenged, diet with no additives inclusion and no sanitary challenge; Challenged, diet with no additives inclusion but with sanitary challenge; Positive Control, diet with antibiotic (enramycin 10 ppm) and sanitary challenge; Challenged + Premium Lac AP; Challenged + Premium Lac BP. We evaluated body weight, body weight gain, feed intake, and feed conversion ratio, as well as intestinal lesion score caused by necrotic enteritis were evaluated. There was statistic difference in the period from 1 to 42 days only for feed intake. However, in the period from 22 to 35 days when there were *C. perfringens* inoculation, unchallenged treatment had best results. Challenged treatment showed similar results to antibiotic, which in turn was similar to Premium Lac AP for all variables evaluated, indicating that the Premium Lac AP was as effective as antibiotic for necrotic enteritis control.

Premium Lac BP and Premium Lac AP stood out for lower incidence of intestinal lesions, suggesting greater efficiency in controlling the disease.

Keywords: Organic acid, *Clostridium perfringens*, intestinal morphometric, prebiotic, probiotic, *Saccharomyces cerevisae*

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

Introdução

A atividade avícola brasileira tem apresentado nas últimas décadas grandes avanços na produção, destacando-se pelos altos índices de produtividade, técnicas de manejo, matéria prima de qualidade e custos relativamente baixos, conferindo ao Brasil maior inserção no mercado internacional, apresentando-se como o maior exportador e terceiro maior produtor mundial de carne de frango (MAPA, 2014).

Um importante fator que contribuiu para esse desenvolvimento foi o uso de antibióticos e quimioterápico na prevenção, controle e tratamento de doenças infecciosas desde 1940, promovendo melhoria na eficiência alimentar e crescimento animal (Niewold, 2007). Segundo Ito et al. (2005), os antibióticos promotores de crescimento (APCs) são prescritos para controlar ou equilibrar a proliferação de bactérias gram positivas, como *Bifidobacterium sp., Clostridium perfingens, Lactobacillus sp. e Bacteroides fragilis* que liberam metabólitos tóxicos, causam competição por nutrientes com o hospedeiro e estímulo excessivo do sistema imune local, comprometendo o ganho de peso do animal.

Por outo lado, seu uso crônico em baixas concentrações é conhecido por induzir a resistência bacteriana contra os antibióticos utilizados (Gyles, 2008), e passou a ser visto como um fator de risco tanto para a saúde humana como animal, devido à possível indução de resistência cruzada.

Não é de hoje que a União Europeia (UE) tem se manifestado contra o uso de APCs, desde 1973 deu início a retirada de alguns antibióticos e então, em 2006 proibiu o uso de todos os antibióticos em baixa dosagem (5~40 ppm) na alimentação animal (Marshall e Levy, 2011). Porém, a remoção dos APCs tem provocado redução no desempenho animal e aumentado a incidência de algumas doenças como a coccidiose e a enterite necrótica subclínica (Dibner and Richards, 2005).

Buscando atender as exigências mercadológicas e minimizar os problemas sanitários, as empresas avícolas passaram a investir em aditivos que possam substituir os antibióticos promotores de crescimento nas rações, mantendo os

mesmos índices zootécnicos alcançados por eles. Entre os chamados “aditivos alternativos”, estão os probióticos, prebióticos e ácidos orgânicos.

Aditivos alternativos

Probióticos

Segundo o Compêndio Brasileiro de Alimentação Animal (2004), probióticos são cepas específicas de várias espécies de microrganismos que agem como auxiliares na recomposição da microbiota intestinal dos animais, diminuindo a prevalência dos microrganismos patogênicos ou indesejáveis.

Os probióticos geram benefícios quando introduzidos no trato gastrintestinal, uma vez que competem com a microbiota patogênica por nutrientes e locais de adesão no epitélio intestinal (Junqueira & Duarte, 2005), além disso, são responsáveis pela produção de vitaminas do complexo B e enzimas digestíveis, sintetizam metabólitos como as bacteriocinas, ácidos orgânicos e peróxidos de hidrogênio que têm ação antibacteriana, especialmente em relação às bactérias patogênicas, e estimulam o sistema imunológico do hospedeiro devido a carga antigênica da microbiota intestinal, aumento da produção de anticorpos, ativação de macrófagos, proliferação de células T e produção de intérferon (Ohimain and Ofongo, 2012), melhorando o ganho de peso e a eficiência alimentar das aves, justamente por competirem com os patógenos no intestino e evitarem lesões no vilo, permitindo a regeneração da mucosa intestinal (Sato et al., 2002).

Apresentam uma abordagem funcional na nutrição animal, saúde intestinal e uma potencial alternativa para a substituição de APCs (Applegate et al., 2010), no entanto, resultados contraditórios ao uso de probióticos têm sido relatados quanto a melhoria dos índices zootécnicos e redução da colonização intestinal por microrganismos patogênicos. Essas diferenças nos resultados são atribuídas às diferenças na dosagem do produto, estirpe da bactéria administrada e número de microrganismos viáveis, estabilidade do produto durante a fabricação da ração, variações no estado fisiológico do animal e o equilíbrio da microbiota no intestino do animal (Huyghebaert et al., 2011).

O probiótico ideal, deve ser composto por estirpes de bactérias nativas e resistentes aos processos alimentares, assim como a acidez estomacal, efeitos dos sais biliares e enzimas digestivas, além de serem de rápida proliferação. Para alcançar melhores resultados, devem ser utilizados já nos primeiros dias de vida da ave, a fim de que as bactérias presentes no produto colonizem e se multipliquem no trato intestinal das aves, iniciando suas atividades benéficas ao hospedeiro antes de ser contaminado por algum patógeno, favorecendo o equilíbrio entre os microrganismos benéficos por exclusão competitiva (Lorençon et al., 2007).

Prebióticos

O conceito de prebiótico engloba ingredientes alimentares que estimulam seletivamente o crescimento e/ou atividade de bactérias benéficas no intestino e que não sofrem ação das enzimas digestivas. Diversas substâncias como carboidratos, peptídeos, lipídeos, fibras e álcoois podem ser classificados como prebióticos, sendo os oligossacarídeos de cadeia curta, como os mananoligossacarídeos (MOS), os frutoliogossacarídeos (FOS) e os glucoligossacarídeos (GOS) os mais estudados por apresentarem melhores resultados (Benites et al., 2008).

A principal forma de ação dos prebióticos é sobre a modulação benéfica da microbiota nativa presente no hospedeiro. Especula-se também, que alguns prebióticos específicos poderiam agir diretamente sobre a translocação intestinal de patógenos, impedindo a sua aderência às células epiteliais e ativando a resposta imune adquirida (Colett, 2000), uma vez que as bactérias podem reconhecer sítios de ligação nos oligossacarídeos como sendo da mucosa intestinal, assim, reduzem a colonização intestinal por bactérias patogênicas, a incidência de infecções e proporcionam condições para que a mucosa intestinal se mantenha inteiramente apta às suas funções de secreção, digestão e absorção (Steen and Nielsen 2000, Qureshi et al., 2000).

Os prebióticos derivados de MOS são os mais utilizados na produção avícola, produzidos a partir da parede celular de *Saccharomyces cerevisiae*, apresentam a capacidade de modular o sistema imunológico e a microbiota intestinal, podem se ligar a uma ampla variedade de micotoxinas e assim preservar a integridade da superfície

de absorção intestinal, bloquear a aderência de das bactérias patogênicas ao ocupar os sítios das células epiteliais e reduzir a taxa de renovação da mucosa (turnover). Também são capazes de induzir a ativação dos macrófagos, saturando os receptores de manose das glicoproteínas da superfície celular (Saad, 2010), promovendo melhoria no desempenho zootécnico na presença de desafios sanitários.

Historicamente, o uso de prebióticos na produção avícola é recente quando comparado ao uso em humanos e animais pets. Os resultados são variáveis em função do ambiente em que as aves são criadas e do desafio sanitário a que estão expostas (Borges, 2003). As respostas obtidas com a utilização de compostos de potencial ação prebiótica podem estar relacionadas com as condições do lúmen e das paredes intestinais do hospedeiro, ou ainda com a presença de bactérias degradadoras dos compostos testados nos diferentes compartimentos do trato gastrintestinal (Mosenthin & Bauer, 2000).

Ácidos orgânicos

Os ácidos orgânicos são substâncias que contém uma ou mais carboxilas em sua molécula, em geral quando o termo é empregado na produção animal, refere-se aos ácidos fracos de cadeia curta (C1-C7), principalmente aos ácidos propiônico, fórmico, acético, cítrico, lático, benzóico e fumárico (Dibner & Buttin, 2002). São comumente encontrados na natureza como componentes normais de tecidos vegetais e animais, além disso, são formados através da fermentação microbiana no trato intestinal ou nas rotas metabólicas intermediárias, constituindo parte importante do suprimento energético dos animais hospedeiros (Bellaver & Scheuermann, 2004).

Esses ácidos orgânicos são adicionados à dieta, visando a redução do pH do trato digestivo com objetivo de facilitar a digestão e controlar a microbiota. O modo de ação dos ácidos orgânicos parece ser através da acidificação da dieta com redução do pH estomacal e aumento na ação da pepsina na digestão dos peptídeos, secreção de suco pancreático, tripsina e quimiotripsina promovendo o aumento da digestibilidade das dietas, assim como a digestibilidade ileal verdadeira dos aminoácidos (Partanen, 2001), essa redução de pH pode reduzir a proliferação de microrganismos indesejáveis (Davidson, 2001).

Além disso, a forma não dissociada dos ácidos orgânicos tem a capacidade de atravessar a membrana celular dos microrganismos, alterando o pH citoplasmático, afetando metabolismo celular, transporte de aminoácidos e fosfatos, inativação de enzimas, e aumento da pressão osmótica celular resultante do acúmulo de ânions polares dentro da célula (Russel, 1992). Na tentativa de reestabelecer a homeostase celular, o microrganismo inicia um processo de retirada de prótons (K^+) acumulados em seu interior pela bomba de ATPase, que por ser um processo ativo promove o esgotamento e morte da bactéria até o rompimento da parede celular (Gauthier, 2005).

A ação antimicrobiana dos ácidos orgânicos também acaba interferindo na saúde das células e integridade intestinal, pois a presença de microrganismos no trato digestivo eleva potencialmente a competição por nutrientes, acelera a passagem do alimento, aumenta a descamação de células intestinais e estimula a secreção de mucina pelas células caliciformes (Apajalahti, 2005).

O uso de ácidos orgânicos como controlador da carga microbiana no trato digestório e promotor de melhoria da morfologia intestinal tem demonstrado resultados interessantes.

Enterite necrótica

A enterite necrótica é uma das doenças entéricas de maior importância econômica, prejudica o desempenho produtivo, aumenta a conversão alimentar e reduz o ganho de peso, foi descrita pela primeira vez em frangos de corte por Parish em 1961 na Inglaterra e desde então, já foi reportada em diferentes países como Austrália, Estados Unidos, Perú, Canadá, Dinamarca, Alemanha, China, Índia e Brasil.

Causada pelo *Clostridium perfringens*, bactéria anaeróbia gram-positiva e oportunista, que está entre as primeiras bactérias a colonizar o ceco dos frangos, porém, encontrada em pequena quantidade no intestino delgado devido ao pH ácido e à elevada concentração de CO_2 (Adams, 2006).

A enterite necrótica afeta frangos entre duas a seis semanas de idade, apresenta-se nas formas aguda e subclínica, sendo a forma aguda caracterizada por surtos com elevada mortalidade e aves deprimidas, apresentando diarreia, anorexia, desidratação e penas eriçadas (Opengart et al., 2008). Já a forma subclínica, de maior

ocorrência nos plantéis, apresenta apenas uma redução geral no desempenho das aves (Hofshagen, 1992) devido aos danos causados na mucosa intestinal que diminuem a digestão e absorção de nutrientes. As lesões causadas pelo *Clostridium perfringens* resultam em necrose coagulativa da mucosa intestinal, seguida de hemorragia.

Um fator predisponente para a enterite necrótica são as lesões causadas pela coccidiose, enfermidade causada pela ingestão de oocistos esporulados de Eimeria presentes no ambiente, alimento e água (Van Immerseel et al., 2004), uma vez que as lesões causadas no intestino servem de porta de entrada para o *Clostridium perfringens*. Além disso, fatores ambientais, tais como qualidade da cama, densidade populacional, local de criação e qualidade dos ingredientes da ração (Dekich, 1998) também têm grande importância na multiplicação da bactéria e, consequentemente, são considerados fatores de risco para o desenvolvimento da doença.

Os antibióticos ainda são muito utilizados para o controle da enterite necrótica, porém, devido as pressões em prol da eliminação dos APCs na produção avícola vem aumentando a necessidade do uso de aditivos alternativos com caráter profilático (Timbermont, 2011).

Morfometria e integridade intestinal

O bom desempenho das aves depende da obtenção adequada de energia, água, sais minerais, lipídeos, carboidratos, vitaminas e aminoácidos. Entretanto, para que isso ocorra, o sistema digestório deve apresentar características estruturais que possibilitem a ingestão e a passagem do alimento pelo trato (Boleli et al, 2002).

O intestino delgado é responsável pelo processo de digestão e absorção dos nutrientes contidos nos alimentos (Maiorka & Macari, 2002), seu elemento funcional é a mucosa intestinal, constituída por um complexo sistema de células em multiplicação que estão se renovando constantemente com padrões bem definidos geneticamente.

Em aves, a mucosa do intestino delgado possui dobras microscópicas denominadas vilosidades ou vilos, que proporcionam aumento na superfície interna do órgão, e consequentemente na área de digestão e absorção intestinal (Boaro, 2009), o tamanho dos vilos depende do número de células que o compõem.

Nos vilos e criptas estão presentes as células caliciformes secretoras de gliciproteínas, importantes na manutenção e desenvolvimento do epitélio intestinal, essas células protegem o epitélio durante os processos digestivos, possuem poder lubrificante sobre os alimentos sólidos e funcionam como uma barreira protetora impedindo o contato de microrganismos com as células (Murarolli, 2008).

A competição entre bactérias e hospedeiro por nutrientes e a formação de metabólitos depressores do crescimento no intestino podem ter efeitos negativos sobre a mucosa do intestinal (Van Leeuwen, 2002).

Segundo Baurhoo et al. (2007), as vilosidades longas estão correlacionadas com a melhora na saúde intestinal, proporcionando melhor uniformidade e integridade da mucosa, além de proporcionar maior absorção de nutrientes. As características do intestino variam de acordo com diversos fatores, tais como, o tipo de dieta, a presença de aditivo alimentar ou promotor de crescimento adicionado à ração, presença de microbiota bacteriana, incidência de doenças entéricas e intensidade de desenvolvimento corporal (ITO et al., 2004).

Na produção avícola, um dos maiores desafios é a manutenção de integridade da mucosa intestinal e do equilíbrio da microbiota em padrões benéficos ao hospedeiro.

Objetivo

Avaliar o uso de aditivos alternativos aos antibióticos promotores de crescimento sobre o desempenho, morfometria intestinal e controle de enterite necrótica em frangos de corte.

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CAPÍTULO 2 - ALTERNATIVE ADDITIVES TO ANTIBIOTIC GROWTH PROMOTERS ON THE PERFORMANCE OF BROILERS

Abstract

The use of antibiotics as a prophylactic treatment is seen as a risk factor for human and animal health. Several organizations are against the use of this feed additive, and have demanded restrictions on its use in production. This study aimed to evaluate the effects of alternative additives in comparison to antibiotic growth promoters on the performance and quality of the intestinal tract of broilers submitted to sanitary challenge. We used 1,920 male Cobb 500® broilers, distributed into eight treatments and eight replicates in a completely randomized design, totaling 64 pens with 30 birds each. The treatments were: Positive Control (PC), with no additives in the diet and no sanitary challenge; Negative control (NC), with no additives in the diet but with sanitary challenge; NC + antibiotic; NC + prebiotic A; NC + antibiotic + prebiotic A; NC + probiotic; NC + organic acid; NC + prebiotic B. We evaluated body weight, body weight gain, feed intake, and feed conversion ratio, as well as the intestinal parameters villus height, crypt depth, villus:crypt ratio, and goblet cell count. No significant differences were found in animal performance between the tested treatments tested. No significant differences in the quality of the intestinal tract were seen. However, the number of goblet cells in the jejunum significantly increased in birds treated with prebiotic A and with antibiotic + prebiotic A.

Keywords: probiotic, prebiotic, organic acid, *Saccharomyces cerevisae,* intestinal morphology, sanitary challenge

Abbreviations: AGP, antibiotic growth promoters; PC, positive control; NC, negative control; MOS, mannan oligosaccharides; CV, coefficient of variation

1. Introduction

Poultry represents one of the most dynamic agriculture sectors worldwide, due to advances in various segments that make up the production chain of the meat complex.

To achieve high levels of productivity, antibiotics and chemotherapeutics are commonly used for prophylactic purposes and also as poultry growth promoters (Cromwell, 1999). Growth promoters are routinely used in diets to control pathogenic agents during the digestive process, improving performance parameters and maximizing production.

Nowadays, the use of antibiotics as a prophylactic treatment is seen as a risk factor for human and animal health due to concerns about potential increases of resistant pathogenic microorganisms. Several organizations are against the use of this feed additive, and have demanded restrictions on its use in production animals (Albino et al., 2006). For instance, the European Commission decided to prohibit the inclusion of antibiotic growth promoters (AGPs) in animal feed (Regulation EC nº. 1831/2003) (Huyghebaert et al., 2011), whereas in the Brazilian market, the use of growth promoters is still allowed as long the AGP is properly registered with the Ministry of Agriculture, Livestock and Food Supply, and the correct use, dosage, and withdrawal period are respected.

Due to increasing concerns about the use of AGPs on human health, mainly due to alleged induction of cross-resistance of pathogenic bacteria to humans (Santos et al., 2005), the search for an alternative additive has intensified. This alternative additive has to maintain the high productivity of animals without affecting the quality of the final product. Some alternatives already in use are probiotics, prebiotics, and organic acids. While some studies have not detected any difference in animal performance, others have indicated that these alternative additives improve animal intestinal performance. Thus, results have been controversial.

This study aimed to evaluate the effects of alternative additives in comparison to antibiotic growth promoters on the performance and quality of the intestinal tract of broilers submitted to sanitary challenge.

2. Material and methods

The experiment was conducted at the Poultry Science Laboratory of the Department of Animal Science, University of Agrarian and Veterinary Sciences – UNESP, Jaboticabal – SP, in the period from February to March of 2013.

2.1. Animals, experimental design and diets

For this experiment, we used a total of 1,920 one-day-old male broiler chicks of the Cobb 500® lineage. The experimental design used was a completely randomized design with eight treatments and eight replicates, totaling 64 pens with 30 birds each. The tested treatments using different additives are described in Table 1.

Table 1. Treatment evaluated

Positive Control (PC) diet with no additives inclusion and no sanitary challenge
Negative control (NC) diet with no additives inclusion and with sanitary challenge
Antibiotic (avilamycin) 0.0035 kg/100kg
Prebiotic A (concentrated mixture of *Saccharomyces cerevisae*) 0.01 kg/100kg
Antibiotic (avilamycin) 0.0035 kg/100kg + prebiotic A 0.01 kg/100kg
Probiotic (*Bacillus subtillis*) 0.005 kg/100kg
Organic acid (fumaric acid) 0.06 kg/100 kg
Prebiotic B (*Saccharomyces cerevisae*) 0.1 kg/100kg

Diets were formulated to meet all the nutritional requirements of the birds according to the phase, based on the recommendations of the Brazilian Tables for Poultry and Swine (Rostagno et al., 2011). Table 2 describes the control diet and its nutritional composition for initial (1–21d) and growth (22–42d) phases. The diets of the other treatments are based on the same

formulation, varying only in the amount of inert ingredient, which was replaced by additives according to their inclusion.

Table 2. Percentage and nutritional composition of the control diets of broilers in the initial (1–21 days) and growth phase (22–42 days)

	Initial	**Growth**
Ingredients	**g/kg**	**g/kg**
Corn	618.43	633.20
Soybean meal	331.04	297.10
Dicalcium phosphate	16.85	12.32
Soybean oil	10.18	37.07
Limestone	8.46	7.75
Salt	4.33	4.00
DL-Methionine	3.26	2.60
L-Lysine HCl (78%)	3.13	2.28
L-Threonine	1.32	0.78
Inert[1]	2.00	2.00
Vitamin supplement[2]	0.50	0.45
Mineral supplement[3]	0.50	0.45
Total	1000.00	1000.00
Nutritional composition calculated		
Crude protein	21.60	20.00
Metabolizable energy (Kcal/kg)	3000	3200
Lysine (Dig.)	1.253	1.101
Met+Cys (Dig.)	0.902	0.804
Methionine (Dig.)	0.606	0.525
Threonine (Dig.)	0.814	0.715
Tryptophan (Dig.)	0.219	0.200
Calcium	0.867	0.717
Phosphorus (av.)	0.424	0.335
Sodium	0.213	0.198
Potassium	0.795	0.737

[1] Washed sand. [2] Initial (kg of product): vit. A, 30,000,000 UI; vit. D3, 6,000,000 UI; vit. E, 60,000mg; vit. K, 8,000mg; vit. B1, 6,000mg; vit. B2, 12,000mg; vit. B6, 12,000mg; vit. B12, 60,000mcg; niacin, 80,000mg; pantothenic acid, 30,000mg; biotin, 240mg; folic acid, 3,000mg; vit. C, 100,000mg; BHT, 125mg. Growth (kg of product): vit. A, 10,000,000 UI; vit. D3, 2,000,000 UI; vit. E, 20,000mg; vit. K, 2,000mg; vit. B1, 2,000mg; vit. B2, 4,000mg; vit. B6, 4,000mg; vit. B12, 20,000mcg; niacin, 30,000mg; pantothenic acid, 10,000mg; biotin, 60mg; folic acid, 1,000mg; vit. C, 50,000mg; BHT, 125mg. [3]Initial and Growth (kg of product): selenium, 360mg; iodine, 1,400mg; iron, 96,000mg; copper, 20,000mg; manganese, 156,000mg; zinc, 110,000mg.

2.2. Facilities and management

The control of temperature, humidity, and air exchange were performed automatically by exhausters and climate control system, according to the age of the birds. Incandescent lamps were used as heating source according to the need of animals until 14 days of age, as well as infant tubular feeders, thereafter being replaced by adult tubular feeders. Water and feed were provided *ad libitum* during the experimental period. The lighting program adopted was 23 hours of light and 1 hour dark until the 7th day of life, and from 8th day the lighting program was 20 hours of light and 4 hours dark.

All birds were vaccinated against coccidiosis on first day of life (via ocular) and at 11 days old they were vaccinated against Newcastle disease (via drinking water).

2.3. Immune challenge

The immune challenge consisted of using reused litter and supply contaminated water with reused poultry litter. The litter was added in proportion of 1 kg of litter for each 4 liters of water (1:4 m/v) and its extract was supplied once a week, from the 5th day of life. During the procedure, the birds had water withdrawn for 2 hours and afterwards had access to water contaminated with reused poultry litter extract for 6 hours.

2.4. Performance evaluation

At 14, 21, and 42 days old, the birds and the leftover diet were weighed to determine average body weight, body weight gain, feed intake, and feed conversion ratio.

2.5. Histological analysis

At 21 days of age, two birds from each pen were slaughtered and samples of approximately 3 cm were taken from duodenum, jejunum, and ileum sections of the intestine for histological analysis of villus height, crypt depth, and goblet cell count. Samples were carefully collected and washed in distilled water, identified, and fixed in Bouin solution. They were then transferred to a solution of 70% alcohol, where they remained until preparation of the slides.

For each slide 30 measures of villus height and crypt depth were made. The measurements of villus height were taken from the basal region, which coincides with the upper portion of the crypts, until the apex. The measurements of crypt depth were made from the base to the crypt–villus transition region. To evaluate the number of goblet cells, 30 counts per segment of intestine were performed, one count per villus, in a field of 250 μm using 10X objective.

2.6. Statistical analysis

Data were analyzed by general linear model procedures of SAS 9.0 (SAS Institute, 2001). The means were tested by Duncan multiple-range test with 5% significance.

3. Results

To determine the efficiency of the tested additives, parameters associated with performance and intestinal morphology were evaluated, among which increased villus height, crypt depth, villus:crypt ratio, and goblet cells were associated with a better performance.

There was no statistically significant difference ($p > 0.05$) between treatments for the performance parameters here studied during both tested periods (1 to 21 and 1 to 42 days). The use of antibiotics, prebiotic A, antibiotic + prebiotic A, probiotic, organic acid, and prebiotic B

did not affect animal performance when compared to performance observed in controls, as presented in Table 3.

Table 3. Performance in tested initial and growth periods in broiler chicks fed diets with different additives (mean ± standard deviation)

Treatments	Feed Intake (g)	Body Weight (g)	Body Weight Gain (g)	Feed Conversion Ratio (g/g)
1 to 21 days				
Positive Control[a]	1,108±50.51	812±34.69	764±34.46	1.450±0.02
Negative Control[b]	1,117±35.70	815±26.01	767±25.85	1.455±0.03
Antibiotic[c]	1,118±37.73	824±19.83	777±19.86	1.440±0.02
Prebiotic A[d]	1,111±43.31	823±35.02	775±35.10	1.434±0.02
Antibiotic + prebiotic A[e]	1,118±34.60	832±22.88	784±22.93	1.436±0.03
Probiotic[f]	1,099±32.37	818±16.90	771±16.91	1.426±0.02
Organic acid[g]	1,122±26.27	835±20.98	787±21.11	1.425±0.03
Prebiotic B[h]	1,100±42.17	822±35.08	774±34.96	1.420±0.03
CV (%)	3.50	3.34	3.54	1.76
Probability	0.8478	0.6850	0.6871	0.2773
1 to 42 days				
Positive Control	4,566±205.56	2,856±59.92	2,808±60.03	1.604±0.01
Negative Control	4,725±117.96	2,976±75.50	2,929±75.33	1.613±0.02
Antibiotic	4,636±104.97	2,952±74.48	2,905±74.45	1.596±0.02
Prebiotic A	4,664±180.83	2,928±87.96	2,880±87.94	1.619±0.03
Antibiotic + prebiotic A	4,704±129.29	2,983±80.09	2,935±80.02	1.603±0.02
Probiotic	4,650±150.68	2,955±67.78	2,907±67.83	1.599±0.02
Organic acid	4,682±128.49	2,956±75.51	2,909±75.55	1.610±0.01
Prebiotic B	4,573±170.70	2,911±80.47	2,863±80.31	1.597±0.02
CV (%)	3.37	2.59	2.63	1.17
Probability	0.4977	0.0606	0.0610	0.1901

[a]Positive Control (PC), diet whit no additives inclusion and no sanitary challenge;
[b]Negative Control (NC), diet whit no additives inclusion, with sanitary challenge;
The additives were supplemented in the NC
[c]Antibiotic (Avilamycin) 0.0035 kg/100kg;

[d]Prebiotic A (concentrated mixture of *Saccharomyces cerevisae*) 0.01 kg/100kg;
[e]Antibiotic (Avilamycin) 0.0035 kg/100kg + prebiotic A 0.01 kg/100kg;
[f]Probiotic (*Bacillus subtillis*) 0.005 kg/100kg;
[g]Organic acid (Fumaric acid) 0.06 kg/100 kg;
[h]Prebiotic B (*Saccharomyces cerevisae*) 0.1 kg/100kg;

There was no statistically significant difference ($p > 0.05$) for the morphometric parameters analyzed, except for the number of goblet cells in the jejunum ($p < 0.05$). Prebiotic A and antibiotic + prebiotic A resulted in higher numbers of goblet cells in the jejunum, similar to those observed in broilers treated with antibiotics and negative controls. Prebiotic B presented the lowest values, being similar to positive control, probiotic, and organic acid as presented in Table 4.

Even though the number of goblet cells in the jejunum differed between treatments, no difference was detected in the performance of the birds.

Table 4. Morphology of duodenum, jejunum, and ileum of broilers at 21 days fed diets with different additives (mean ± standard deviation)

Treatments	Villus Height (µm)	Crypt Depth (µm)	Villus:Crypt	Goblet Cells
Duodenum				
Positive Control[a]	1,483±156.01	169±14.17	9.04±1.23	18.18±1.16
Negative Control[b]	1,379±235.01	162±20.72	8.66±1.24	18.35±0.98
Antibiotic[c]	1,502±174.26	169±20.76	9.12±1.12	18.74±2.74
Prebiotic A[d]	1,488±155.63	160±13.03	9.51±1.02	18.36±2.00
Antibiotic + prebiotic A[e]	1,434±184.55	154±10.27	9.46±1.42	18.80±1.98
Probiotic[f]	1,303±233.29	155±15.59	8.50±1.16	18.03±2.63
Organic acid[g]	1,389±159.91	168±18.87	8.55±1.15	17.71±1.46
Prebiotic B[h]	1,532±132.02	156±11.72	9.94±0.83	17.36±1.61
CV (%)	12.66	9.96	12.74	11.31
Probability	0.2152	0.3124	0.1585	0.8656
Jejunum				
Positive Control	816±75.55	118±14.33	7.21±0.78	19.36±1.79^{BC}
Negative Control	804±137.44	121±16.58	7.10±1.00	21.41±2.99^{AB}
Antibiotic	765±79.25	111±11.48	7.03±0.64	20.58±1.23^{ABC}
Prebiotic A	799±72.59	112±14.90	7.30±1.04	22.40±3.13^{A}
Antibiotic + prebiotic A	759±65.10	109±8.60	7.18±0.69	22.18±1.42^{A}
Probiotic	852±112.25	115±11.16	7.32±1.17	19.07±1.60^{BC}
Organic acid	827±130.19	129±14.63	6.57±0.80	19.39±2.55^{BC}
Prebiotic B	784±141.37	118±11.58	6.81±1.09	18.42±2.51^{C}
CV (%)	13.24	11.29	12.98	11.10
Probability	0.6674	0.0894	0.7247	0.0029
Ileum				
Positive control	664±83.72	109±7.34	6.23±0.64	24.18±3.98
Negative control	672±42.79	115±9.85	6.37±0.50	27.64±4.05
Antibiotic (avilamycin)	608±93.89	109±5.41	5.95±0.84	25.93±3.66
Prebiotic A	624±65.87	103±4.10	6.18±0.67	28.69±3.39
Antibiotic + prebiotic A	590±60.59	105±7.34	5.76±0.49	26.31±3.62
Probiotic	597±55.65	108±9.09	5.85±0.51	26.32±3.51
Organic acid	585±58.07	103±8.54	5.79±0.43	28.59±3.34
Prebiotic B	625±61.44	109±10.40	5.85±0.54	26.13±1.75
CV (%)	10.97	7.83	9.74	13.10
Probability	0.1347	0.1024	0.2971	0.1900

[a]Positive Control (PC), diet whit no additives inclusion and no sanitary challenge;
bNegative Control (NC), diet whit no additives inclusion, with sanitary challenge;
The additives were supplemented in the NC
[c]Antibiotic (Avilamycin) 0.0035 kg/100kg;
[d]Prebiotic A (concentrated mixture of *Saccharomyces cerevisae*) 0.01 kg/100kg;
[e]Antibiotic (Avilamycin) 0.0035 kg/100kg + prebiotic A 0.01 kg/100kg;

[f]Probiotic (*Bacillus subtillis*) 0.005 kg/100kg;
[g]Organic acid (Fumaric acid) 0.06 kg/100 kg;
[h]Prebiotic B (*Saccharomyces cerevisae*) 0.1 kg/100kg;
Means with no common letters in the row differ significantly ($P < 0.05$) by Duncan test

4. Discussion

Antibiotic growth promoters have been used on a large scale to mask deficiencies in feed formulations, in the quality control of ingredients, and sanitary control of facilities. However, the European Union imposes barriers to the use of AGPs and there is therefore an increasing search for alternatives that maintain the health and performance of poultry. Many additives have been tested to replace these antibiotics, but their effectiveness is not always clear and their modes of action require further investigation.

No significant differences were found in animal performance among the treatments tested. No significant differences in the quality of the intestinal tract were seen. However, the number of goblet cells in the jejunum significantly increased with treatment with prebiotic A and with antibiotic + prebiotic A.

The fact of no statistic difference has been observed between positive and negative controls in the periods from 1 to 21 days and 1 to 42 days for animal performance and quality of the intestinal tract indicates that sanitary challenge was not effective. It is possible that the contamination produced by adding poultry litter to drinking water of birds is not enough to cause an imbalance in the health of poultry.

The inefficiency of the sanitary challenge can explain the lack of response of tested products. According to Baurhoo et al. (2007), the beneficial effects of most additives are clearer in suboptimal and stressful condition, such as a disease condition, thermic stress, high stocking density and bad management practices.

In this study, the chickens were raised under suitable conditions of environment, management and stocking density, which provided an environment of a low level of stress and pathogenic challenge. In addition, factors such as additive type, dosage, and duration of use can affect animal responses to different tested additives (Yang et al., 2009).

Bozkurt et al. (2012), Ghasemi and Taherpour (2013) and Fernandes et al. (2014) showed that supplementation with organic acid, probiotic, prebiotic or antibiotic growth promoter had no effect on weight gain and feed conversion ratio. These findings support the results of the present study (Table 3).

Probiotics have been used in an attempt to improve the microbial balance in the gastrointestinal tract by bacterial antagonism and competitive exclusion in order to minimize the stimulation of the immune system and energy expenditure. Furthermore, they improve the digestion of nutrients, directly improving performance. However, the probiotic evaluated in this study showed no statistically significant results for feed intake, body weight, body weight gain, and feed conversion ratio, when compared to the other treatments and the control. These findings agree with those described by Willis and Reid (2008) reported that the inclusion of probiotics in the diet (with a minimum concentration of 1.04×10^8 CFU / g) had no significant effect on the performance of broilers, as well as Li et al. (2008) and Aliakbarpour et al. (2013) who found no response in body weight gain and feed conversion ratio in birds fed diets containing probiotics.

On the other hand, Toghyani et al. (2011), Aliakbarpour et al. (2012) and Nunes et al. (2012) describe positive responses to weight gain and feed efficiency using probiotics to replace antibiotics in the broiler diet. Salim et al. (2013) attributed the improvement in performance

and feed efficiency in broilers fed probiotics to the effects of the additive in the maintenance of a beneficial microbial population, which improves digestion and increase performance.

As presented in Table 3, there was no effect of the use of prebiotic on feed intake, body weight, body weight gain, and feed conversion ratio. These results are in agreement with those of Haldar et al. (2011), Kim et al. (2011) and Shanmugasundaram and Selvaraj (2012) who reported that supplementation with prebiotics in the diet of broilers had no significant effect on body weight gain and feed conversion ratio.

Different from what was observed in this study, Reisinger et al. (2012), Sohail et al. (2012), Nikpiran et al. (2013) and Onwurah et al. (2014) found significant improvements in the performance of broilers fed diets containing *Saccharomyces cerevisiae*. These results can be explained as a function of prebiotics in controlling the microbial activity and composition. Thereby, the prebiotics help to maintain a beneficial microbiota, by suppressing the growth of pathogens through different mechanisms, and as a consequence, they improve utilization of nutrients and animal development and increase cell proliferation.

When working with the addition of organic acids in broiler diets to facilitate digestion, control the microbiota, and reduce the proliferation of undesirable microorganisms, Houshmand et al. (2011) and Venkatasubramani et al. (2014) found no effect of organic acids on the body weight gain and feed conversion ratio. These results are in agreement with those found in the present study (Table 3).

According to Dibner et al. (2007), the variables such as buffering capacity of dietary ingredients, cleanliness of production environment and heterogeneity of gut microflora affect the efficiency of organic acids and many times fail to produce measurable improvement in broiler performance. However, Negara et al. (2009), Adil et al. (2011) and Kamal et al. (2014)

reported positive effects of organic acids on body weight gain and feed conversion ratio in poultry.

The intestinal morphometry is widely used as a criterion for evaluating the effects of additives in nutrition and intestinal physiology, but in many cases there is no correlation between the performance and quality of the intestinal tract (Vieira et al., 2008).

The results of the analysis of intestinal morphology of this study (Table 4) corroborate the results obtained by Santos et al. (2004), who found no differences in villus height of the duodenum between the control group and birds fed diets containing probiotics based on *Lactobacillus acidophilus* and *Lactobacillus casei*, as well as Abudabos et al. (2013) working with *Bacillus subtilis*. Duarte et al. (2014) found no differences in crypt depth of the duodenum when worked with *Bacillus cereus*.

Different from what was observed by Gutierrez-Fuentes et al. (2013), Al-Fataftah and Abdelqader (2014) and Duarte et al. (2014), who found increase in villus height, crypt depth and villus:crypt ratio in the duodenum of chickens fed diets containing probiotic.

Yang et al. (2007) and Lima (2010) did not observe differences in the duodenum variables when using dry yeast and antimicrobial. However, Panda et al. (2009) and Oliveira et al. (2009) affirmed that adding of MOS to diet of broilers provides improve intestinal quality.

Working with isolated organic acids, Cengiz et al. (2012), found no significant effect on villus height nor on villus:crypt ratio in the duodenum, as observed in this study. However, this author found higher values for crypt depth in the antibiotic group, a finding in disagreement with those here described.

The results obtained for treatment with probiotic are consistent with the findings of Aliakbarpour et al. (2012) and Hayakawa et al. (2014), who reported no effect of adding probiotics on jejunum histomorphometry. On the other hand, Abudabos et al. (2013) and Al-

Fataftah and Abdelqader (2014) found changes in the villus height and crypt depth of jeujum and ileum.

When Ao et al. (2012) worked with prebiotic and organic acids found no changes in the quality of the intestinal mucosa in the jejunum and ileum of broilers, agreement with the results presented. However, Ghosh et al. (2011) and Sayrafi et al. (2011) observed beneficial effects on intestinal histomorphometry.

Differences in the number of goblet cells in the jejunum, observed in this study, corroborate the results of Bedford (2000) and Gilmore and Ferretti (2003). These mucus-secreting cells are important in the maintenance and development of the intestinal epithelium, protecting the epithelium during the digestive process, have lubricity role with solid food (Murarolli, 2008) and also act as a protective barrier against pathogens due contain antimicrobial substances and IgA immunoglobulins (Sklan, 2005).

The mucus part of the nonspecific immune response, and when there is a great expression of goblet cells indicates that there may be some kind of sanitary challenge that requires increased production of mucus. In contrast, when the mucus in large quantities can bring harm to the health of the bird as it increases intestinal transit, reducing the absorption of nutrients.

The exact mechanism related to the increased number of goblet cells is still not clearly established and this divergence in results can be explained by the dosage of the products tested and also variations between different intestinal segments.

The fact that no significant differences were found between the control and the other treatments might be explained by a failure in the methodology used associated to the sanitary challenge. Therefore, it is not possible to investigate the potential differences among the treatments here tested. This hypothesis may also explain why the results produced by different studies are so controversial. Further studies aimed at comparing these treatments should be done

in order to standardize the methodology for an effective sanitary challenge that is capable of reproducing the challenges faced in the field.

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CAPÍTULO 3 - ORGANIC ACIDS BLENDS ON THE BROILER PERFORMANCE AND NECROTIC ENTERITIS CONTROL

Abstract

Necrotic enteritis is a common infectious disease in broiler production caused by *Clostridium perfringens*, a gram-positive anaerobic bacteria found worldwide which causes high economic losses every year due to intestinal lesions, decreased water and feed intake and growth depression. This study aimed to evaluate the effects of organic acids blends Premium Lac AP (lactic, acetic and butyric acid) and Premium Lac BP (lactic, acetic, butyric acid and tributyrin) in comparison to antibiotic (enramycin) on broiler performance and necrotic enteritis control. Were used 1,360 male Cobb 500® broilers, distributed into five treatments and eight replicates in a completely randomized design, totaling 40 pens with 34 birds each. The treatments were: Unchallenged, diet with no additives inclusion and no sanitary challenge; Challenged, diet with no additives inclusion but with sanitary challenge; Positive Control, diet with antibiotic (enramycin 10 ppm) and sanitary challenge; Challenged + Premium Lac AP; Challenged + Premium Lac BP. The diets were formulated to meet all the requirements of the birds. Body weight, body weight gain, feed intake, and feed conversion ratio, as well as intestinal lesion score caused by necrotic enteritis were evaluated. There was statistic difference ($P > 0.05$) in the period from 1 to 42 days only for feed intake, where antibiotic, Premium Lac AP and Premium Lac BP did not differ, but had lower results compared to unchallenged and challenged treatments. However, in the period from 22 to 35 days when there were *C. perfringens* inoculation, unchallenged treatment had best results. Challenged treatment showed similar results to antibiotic, which in turn was similar to Premium Lac AP for feed intake, body weight, body weight gain and feed conversion ratio, indicating that the Premium Lac AP was as effective as antibiotic for necrotic enteritis control. Regarding the intestinal lesions caused by necrotic enteritis, Premium Lac BP and Premium Lac AP stood out for lower incidence of lesions, suggesting greater efficiency in controlling the disease.

Keywords: Organic acid, sanitary challenge, *Clostridium perfringens, Eimeria maxima*, intestinal lesion.

1. Introduction

Necrotic enteritis is a common infectious disease in broiler production caused by *Clostridium perfringens*, a gram-positive anaerobic bacteria found worldwide which causes high economic losses every year due to intestinal lesions, decreased water and feed intake, growth depression and, consequently, increased feed conversion rate.

In the last decades, the control and prevention of necrotic enteritis were based on the administration of antibiotics such as virginiamycin, zinc bacitracin and enramycin supplied in poultry feed. However, since 2006 the European Commission decided to prohibit the inclusion of antibiotic growth promoters (AGPs) in animal feed (Regulation EC nº. 1831/2003) (Huyghebaert et al., 2011), intensifying the search for alternative feed additives.

A common strategy to eliminate the use of antibiotics in the production chain and to maintain the health and productivity index is the use of alternative additives such as organic acids. In the same way as the antibiotics, they also act in the control of intestinal microbiota due to their physical-chemical characteristics that influence the pH of the gastrointestinal tract and give them bactericidal and bacteriostatic activity (Ricke, 2003).

Although there are many studies with supplementation of organic acids in poultry production and necrotic enteritis control, the results are contradictory. This fact could be due to differences in mode of action, environmental condition, dose and response criterion assessed (Gunal et al., 2006; Hernández et al., 2006; Isabel and Santos, 2009; Houshmand et al., 2011). Therefore, organic acids still need to be further studied in order to achieve a common census on the efficiency of use of such additives as an alternative to AGPs. Thus, the objective of this study was to evaluate the effects of organic acids blends Premium Lac AP (lactic, acetic and butyric acid) and Premium Lac BP (lactic, acetic, butyric acid and tributyrin) when compared to antibiotic (enramycin) on the poultry performance and necrotic enteritis control.

2. Material and methods

The experiment was conducted at the Poultry Science Laboratory of the Department of Animal Science, University of Agrarian and Veterinary Sciences – UNESP, Jaboticabal – SP, in the period from August to September of 2014.

2.1. Animals, experimental design and diets

In this study 1,360 one-day-old male broiler chicks of Cobb 500® strain were used. The experimental design was a completely randomized design with five treatments and eight replicates each, totaling 40 pens with 34 birds each in density of 11.4 birds/m². The tested treatments are described in Table 1.

Table 1. Treatments evaluated

Unchallenged - diet meeting all nutritional requirements, no additives and no sanitary challenge
Challenged - diet meeting all nutritional requirements, with sanitary challenge but no additives
Positive control (PC) - diet meeting all nutritional requirements, with antibiotic (enramycin 10 ppm) and sanitary challenge
Challenged + Premium Lac AP (8, 8, 6 and 4 kg/t for pre-initial, initial, grower and finisher phases, respectively)
Challenged + Premium Lac BP (8, 8, 6 and 4 kg/t for pre-initial, initial, grower and finisher phases, respectively)

Diets were formulated to meet all the nutritional requirements of the birds according to the phase, based on the recommendations of the Brazilian Tables for Poultry and Swine (Rostagno et al., 2011). Meat and bone meal was used to meet phosphorus requirement, further to be an ingredient with favorable characteristics to proliferation of *Clostridium perfringens*, acting as important factor to necrotic enteritis development. Salinomycin 12% was not used in the initial phase because it is an anticoccidial agent and act against *Eimeria maxima*, making the immune challenge impossible.

Table 2 describes the control diets and their nutritional composition for pre-initial (1–10 days), initial (11-21 days), growth (22–35 days) and finisher (36-42 days) phases. The diets of the other treatments were based on the same formulation, varying only in the amount of corn ingredient to PC, which was replaced by antibiotic according to its inclusion. The organic acid blend was included “on top”.

Table 2. Nutritional composition of the control diets of broilers in the pre-initial (1-10 days), initial (11-21 days), growth (22-35 days) and finisher (36-42 days) phases

Ingredients	**Pre initial** g/kg	**Initial** g/kg	**Growth** g/kg	**Finisher** g/kg
Corn	611.74	629.81	639.90	661.10
Soybean meal	300.27	284.29	269.19	252.37
Meat and bone meal	55.06	50.10	41.60	35.40
Soy oil	10.80	17.14	31.76	34.37
Limestone	3.28	4.28	4.40	4.16
Salt	3.47	3.44	3.34	3.49
DL-Methionine	3.48	3.17	2.79	2.45
L-Lysine (54.6%)	5.93	5.52	4.60	4.37
L-Threonine	1.47	1.25	0.92	0.79
Salinomycin 12%	0.50	-	0.50	0.50
Vitamin Px.[1]	0.50	0.50	0.50	0.50
Mineral Px.[2]	0.50	0.50	0.50	0.50
TOTAL	1000.00	1000.00	1000.00	1000.00
Nutritional composition calculated				
Crude Protein	22.43	21.42	20.30	19.38
Metabolizable energy (Kcal/kg)	2987	3050	3157	3200
Digestible lysine	1.29	1.22	1.12	1.06
Digestible Met+Cys	0.93	0.88	0.82	0.77
Digestible methionine	0.64	0.60	0.55	0.51
Digestible threonine	0.84	0.79	0.73	0.69
Digestible tryptophan	0.22	0.21	0.20	0.19
Calcium	0.90	0.84	0.74	0.66
Available Phosphorus	0.45	0.41	0.35	0.31
Sodium	0.22	0.21	0.20	0.20
Potassium	0.78	0.75	0.72	0.69

[1] Pre initial and Initial (kg of product) - vit. A, 30.000.000 UI; vit. D3, 6.000.000 UI; vit. E, 60.000mg; vit. K, 8.000mg; vit. B1, 6.000mg; vit. B2, 12.000mg; vit. B6, 12.000mg; vit. B12, 60.000mcg; niacin, 80.000mg; pantothenic acid, 30.000mg; biotin, 240mg; folic acid, 3.000mg; vit. C, 100.000mg; BHT, 125mg. Grower and Finisher (kg of product) - vit. A, 10.000.000 UI; vit. D3, 2.000.000 UI; vit. E, 20.000mg; vit. K, 2.000mg; vit. B1, 2.000mg; vit. B2, 4.000mg; vit. B6, 4.000mg; vit. B12, 20.000mcg; niacin, 30.000mg; pantothenic acid, 10.000mg; biotin, 60mg; folic acid, 1.000mg; vit. C, 50.000mg; BHT, 125mg. [2]Pre initial, Initial, Grower and Finisher (kg of product) - selenium, 360mg; iodine, 1.400mg; iron, 96.000mg; copper, 20.000mg; manganese, 156.000mg; zinc, 110.000mg.

2.2. Facilities and management

The birds were raised in a positive-pressure house with control of temperature done by curtains and fans management. During the initial phase, incandescent lamps were used as heating source according to the poultry needs. It was used wood shaves as litter. Each pen was equipped with nipple drinkers and infant tubular feeders, until 14 days of age, thereafter replaced by adult tubular feeders. Water and feed were provided *ad libitum* during the experimental period. The lighting program adopted was 24 hours of light. All birds were vaccinated against Newcastle and IBD at seven days of age (via drinking water) and at 14 days of age were vaccinated against IBD (via drinking water).

2.3. Immune challenge

The immune challenge consisted of a dose 1.0 mL/bird inoculated orally with *Eimeria maxima* with a concentration of 5 x10^4 CFU/mL on the 17th day of age, and two administrations of 1.0 mL/bird inoculated orally with *Clostridium perfringens* with a concentration of 2.5 x 10^6 CFU/mL on the 21st and another on the 24th days of age.

2.4. Performance evaluation

At 10, 21, 35 and 42 days old, the birds and the feed leftovers were weighed to determine average body weight, body weight gain, feed intake, and feed conversion ratio.

2.5. Intestinal analysis

At 28 days old, two birds per pen were slaughtered for intestinal analysis. The birds were randomized selected and slaughtered by asphyxiation with CO_2. Afterward, the birds were weighed and their intestines were removed and opened longitudinally to evaluate the intestinal

necrotic enteritis by lesion score, which ranged from 0 to 5, according to the criteria described in Table 3.

Table 3. Criteria used to evaluation of necrotic enteritis lesion

Score	Characterization
0	Normal: no necrotic enteritis lesions and the small intestine has normal elasticity
1	Mild: small intestinal wall is thin and flaccid, covered with thickened mucus
2	Minor: 1-6 necrotic enteritis pocks, minor ulceration and necrosis of the intestinal wall
3	Moderate: more than 6 necrotic enteritis pocks or coalescing of pocks
4	Severe: extensive area(s) of necrosis and ulceration of the small intestinal membrane
5	Dead or moribund: bird that probably died within 24 hours

2.6. Statistical analysis

Data were analyzed by general linear model procedures of the statistical software SAS 9.0 (SAS Institute, 2001). The means were tested by Duncan multiple-range test with 5% significance. To perform the statistical analysis outliers were identified by SAS and then removed.

3. Results

To determine the efficiency of Premium Lac AP and Premium Lac BP in comparison to antibiotic for necrotic enteritis control, parameters associated with performance and intestinal lesion were evaluated. The decrease on incidence of lesion scores were associated with a better performance.

There was no statistically significant difference ($P > 0.05$) between treatments for the studied variables in the period from 1 to 10 days (Table 4), as expected, since there was no immune challenge in this period.

In the period from 1 to 21 days was observed significant effect ($P < 0.05$) in body weight, body weight gain and feed conversion ratio. The unchallenged treatment showed better results,

which was similar to the challenged, however the challenged birds presented a trend of reduction on performance, indicating a clear effect of *Eimeria maxima* inoculation at 17 days of age. Birds receiving diets containing antibiotic, Premium Lac AP and Premium Lac BP presented similar results to challenged treatment (Table 4).

In the period from 1 to 35 days, there was statistically significant difference ($P < 0.05$) for the studied variables, when the best results was obtained by the unchallenged treatment, showing the effectiveness of the immune challenge. The challenged treatment showed similar results to antibiotic, which in turn was similar to Premium Lac AP for all studied variables. These results indicated that Premium Lac AP was as effective as antibiotic for necrotic enteritis control (Table 4). Premium Lac BP presented the worst results when compared with other treatments, and these differences are more evident in growth phase (Table 5).

There was statistic difference ($P > 0.05$) only for feed intake, antibiotic, Premium Lac AP and Premium Lac BP did not differ, but had lower results compared to unchallenged and challenged treatments. The lack of difference for other variables can be explained by the dilution of immune challenge effect caused during grower phase throughout the production period.

Table 4. Performance parameters evaluated for challenged and unchallenged broiler fed diets with the inclusion or not of additives (mean ± standard deviation)

Treatments	Feed Intake (g)	Body Weight (g)	Body Weight Gain (g)	Feed Conversion Ratio (g/g)
		1 to 10 days		
Unchallenged[1]	279.15 ± 5.09	297.43 ± 6.01	249.75 ± 6.24	1.12 ± 0.04
Challenged[2]	284.59 ± 12.19	297.92 ± 8.39	250.06 ± 8.19	1.16 ± 0.10
Antibiotic[3]	282.79 ± 15.34	298.74 ± 2.31	248.34 ± 7.46	1.14 ± 0.07
PLAP [4]	293.88 ± 18.23	293.22 ± 8.13	245.27 ± 8.13	1.18 ± 0.07
PLBP [5]	286.59 ± 16.56	297.48 ± 3.25	249.85 ± 3.18	1.22 ± 0.14
CV(%) [6]	4.91	2.06	2.77	7.75
Probability	0.33	0.49	0.66	0.23
		1 to 21 days		
Unchallenged	1214.12 ± 26.63	1016.47 ± 18.14 A	968.75 ± 18.06 A	1.25 ± 0.01 AB
Challenged	1190.14 ± 46.25	990.62 ± 26.58 AB	942.71 ± 26.49 AB	1.26 ± 0.03 ABC
Antibiotic	1173.75 ± 47.47	987.11 ± 37.26 AB	939.37 ± 37.37 AB	1.25 ± 0.02 A
PLAP	1166.87 ± 59.53	949.80 ± 44.56 B	901.87 ± 46.57 B	1.29 ± 0.03 C
PLBP	1193.62 ± 83.33	972.15 ± 54.84 B	924.50 ± 54.82 B	1.29 ± 0.06 BC
CV (%)	4.72	4.01	4.20	2.80
Probability	0.49	0.03	0.03	0.04
		1 to 35 days		
Unchallenged	3335.12± 54.68 A	2417.75 ± 43.13 A	2370.25 ± 43.20 A	1.41 ± 0.03 A
Challenged	3160.57 ± 83.71 B	2229.57 ± 50.73 B	2181.57 ± 50.73 B	1.45 ± 0.03 B
Antibiotic	3084.50 ± 54.38 B	2186.37 ± 53.40 BC	2138.50 ± 53.58 BC	1.44 ± 0.02 B
PLAP	3059.50 ± 119.51 B	2157.87 ± 86.53 CD	2110.12 ± 86.36 CD	1.45 ± 0.03 B
PLBP	3055.50 ± 127.38 B	2101.87 ± 69.50 D	2054.12 ± 69.78 D	1.49 ± 0.05 C
CV (%)	3.07	2.84	2.90	2.24
Probability	< 0.05	< 0.05	< 0.05	< 0.05
		1 to 42 days		
Unchallenged	4401.75 ± 80.37 A	2895.75 ± 123.37	2847.87 ± 122.87	1.55 ± 0.06
Challenged	4301.00 ± 128.79 A	2889.12 ± 334.59	2841.12 ± 334.74	1.53 ± 0.12
Antibiotic	4171.87 ± 71.67 B	2733.25 ± 144.67	2685.37 ± 144.82	1.56 ± 0.07
PLAP	4159.12 ± 151.52 B	2733.00 ± 275.26	2684.87 ± 274.91	1.56 ± 0.13
PLBP	4162.12 ± 158.81 B	2664.86 ± 181.09	2617. 14 ± 181.18	1.61 ± 0.08
CV (%)	2.91	8.17	8.31	5.99
Probability	< 0.05	0.20	0.20	0.66

[1]Unchallenged - meeting all nutritional requirements, with no additives and no sanitary challenge
[2] Challenged - meeting all nutritional requirements and with no additives, but with sanitary challenge
[3]Antibiotic - meeting all nutritional requirements, with additive (enramycin 10 ppm) and sanitary challenge
[4] PLAP - Premium Lac AP
[5] PLBP – Premium Lac BP
[6]Coefficient of variation
Means with no common letter in row differ significantly ($P < 0.05$) by Duncan test.

Table 5 shows the performance results in the growth phase, highlighting the effect of *Clostridium perfringens* inoculation at 21 and 24 days for feed intake, body weight, body weight gain and feed conversion ratio.

There was statistically significant difference ($P < 0.05$) for the studied variables in the period from 22 to 35 days. The best results were obtained by unchallenged treatment, which confirms that the immune challenge was effective. Challenged treatment showed similar results to antibiotic, which in turn was similar to Premium Lac AP for all variables, indicating that the Premium Lac AP was as effective as antibiotic for necrotic enteritis control. Premium Lac BP presented the worst results, suggesting that the addition of tributyrin on organic acids blend damaged the product activity.

Table 5. Performance parameters evaluated for challenged and unchallenged broiler from 22 to 35 days fed diets with the inclusion or not of additives (mean ± standard deviation)

	22 to 35 days			
Treatments	**Feed Intake (g)**	**Body Weight (g)**	**Body Weight Gain (g)**	**Feed Conversion Ratio (g/g)**
Unchallenged[1]	2234.87 ± 46.83 A	2417.75 ± 43.13 A	1401.25 ± 36.03 A	1.595 ± 0.023 A
Challenged[2]	2040.00 ± 90.92 B	2229.57 ± 50.73 B	1260.00 ± 79.46 B	1.669 ± 0.044 B
Antibiotic[3]	1992.25 ± 67.03 BC	2186.37 ± 53.40 BC	1200.00 ± 54.77 B	1.662 ± 0.038 B
PLAP [4]	1986.00 ± 91.11 BC	2157.87 ± 86.53 CD	1206.25 ± 78.73 B	1.647 ± 0.069 AB
PLBP [5]	1955.12 ± 77.85 C	2101.87 ± 69.50 D	1131.35 ± 60.34 C	1.733 ± 0.065 C
CV(%) [6]	3.75	2.84	5.16	3.07
Probability	< 0.05	< 0.05	< 0.05	< 0.05

[1]Unchallenged - meeting all nutritional requirements, with no additives and no sanitary challenge
[2] Challenged - meeting all nutritional requirements and with no additives, but with sanitary challenge
[3]Antibiotic - meeting all nutritional requirements, with additive (enramycin 10 ppm) and sanitary challenge
[4] PLAP - Premium Lac AP
[5] PLBP – Premium Lac BP
[6]Coefficient of variation
Means with no common letter in row differ significantly (P<0.05) by Duncan test.

When evaluating the presence of lesions caused by necrotic enteritis at 28 days of age, it was observed greater presence of score 2 lesion for challenged treatment, indicating the effectiveness of the immune challenge. Between the challenged treatments, Premium Lac AP

and Premium Lac BP showed higher incidence of score 0 lesion, suggesting greater efficiency on necrotic enteritis lesion control. Only the treatment with antibiotic presented incidence of score 3 lesion, and also it was the one with lower incidence of score 0 lesion (Table 6), but the performance of this group showed a good recovery, being similar to Premium Lac AP as presented in Table 5.

Table 6. Intestinal analysis at 28 days old of broiler chickens fed diets tested (% of slaughtered animals with their scores)

Treatment	Score 0	Score 1	Score 2	Score 3
Unchallenged[1]	37.50	50.00	12.50	0.00
Challenged[2]	12.50	50.00	37.50	0.00
Antibiotic[3]	6.25	62.50	18.75	12.50
PLAP [4]	31.25	50.00	18.75	0.00
PLBP [5]	25.00	50.00	25.00	0.00

[1]Unchallenged - meeting all nutritional requirements, with no additives and no sanitary challenge
[2] Challenged - meeting all nutritional requirements and with no additives, but with sanitary challenge
[3]Antibiotic - meeting all nutritional requirements, with additive (enramycin 10 ppm) and sanitary challenge
[4] PLAP - Premium Lac AP
[5] PLBP – Premium Lac BP

4. Discussion

The objective of this study was to evaluate the effects of organic acid blend Premium Lac AP (lactic, acetic and butyric acid) and Premium Lac BP (lactic, acetic, butyric acid and tributyrin) when compared to antibiotic (enramycin) on the poultry performance and necrotic enteritis control. The results indicated that birds subjected to treatment with the blend of organic acids Premium Lac AP, showed similar performance to those treated with antibiotic, regarding the control of intestinal lesions, Premium Lac AP and Premium Lac BP also stood out for the lower incidence of lesions in relation to antibiotic.

The climatic conditions and the high housing density in has favored the development of pathogenic microorganisms such as *Escherichia coli*, *Salmonella spp*. and *Clostridium*

perfringens and therefore, the development of enteric diseases (Smith, 2011; Fascina et al, 2012).

The unchallenged treatment showed better results trend from 1 to 21 days when compared to challenged treatment, suggesting the effect of *Eimeria maxima* inoculation at 17 days of age. The short interval between inoculation and the end of the phase can be the limiting factor in that there were no greater differences between the two treatments. In the period from 22 to 35 days, it is evident the difference on bird performance between unchallenged and challenged treatments due to inoculations with *Clostridium perfringens* which have been concentrated at this period.

The fact of no statistical difference has been observed between unchallenged and challenged treatments in the period from 1 to 42 days can be explained by the lack of an increase in re-contamination of birds in the finisher phase and, consequently, the effect caused by the immune challenge during the growth phase was diluted throughout the rearing period.

The coccidiosis caused by *Eimeria maxima* inoculation acts as an important factor for the development of necrotic enteritis, since *Clostridium perfringens* is commonly found in small amount in the gastrointestinal tract of poultry (Al-Sheikhly and Truscott, 1977), but require a gateway to proliferate and damage the health of the animal. Allied to this, high energy diets, rich in protein ingredients (Gholamiandehkordi et al., 2007) and with high non-starch polysaccharide content such as wheat, rye and oats (Jia et al., 2009), changes in the feeding program, heat stress, litter moisture and high-density housing are also risk factors to the development of necrotic enteritis. The lack of effect on performance parameters observed of 1 to 10 days can be justified by controlled environmental conditions, new litter and absence of immune challenge.

These lack of effect was also observed by Houshmand et al. (2011) working with inclusion 1.5 g/kg of feed blend of organic acids (formic acid, citric acid, malic acid, lactic acid and tartaric acid) and Isabel and Santos (2009) found no difference in body weight of broilers from 1 to 15 days, treated with salts of organic acids (formic acid and propionic acid), confirming the results of this study (Table 4). However, Viola et al. (2008) showed that supplementation with blend of lactic, formic, acetic, and phosphoric acids has a positive effect on performance parameters of broilers from 1 to 7 days.

When *Eimeria maxima* and *Clostridium perfringens* colonize the intestinal flora, they compete with the birds by diet nutrients, prevent the action of bile acids, thereby reducing the digestion of fats and fat soluble vitamins (Engberg et al., 2000), causing, in addition, changes in the intestinal mucosa, impairing nutrient absorption and thus reducing the performance.

In the period from 1 to 21 days, as shown in Table 4, there was statistic difference for performance parameters. However, the challenged treatments, independently of additive used showed no effect, which can be explained by the lack of activity of these additives on protozoa control. It is known that enramycin acts against gram positive bacterial, and the studied organic acids blends act against gram negative and gram positive bacterial, according to the chain size.

Giannenas et al. (2014) reported improvement in feed conversion of turkeys in initial phase fed diets supplemented with 300 mg of benzoic acid per kg of feed, which contradicts the results presented in Table 4. Better feed conversion ratio in this phase might be associated with the bacteriostatic effect of benzoic acid, due inhibitory effect on microbial enzymes a-ketoglutaric acid dehydrogenase and succinic acid dehydrogenase (Bosund, 1962) and less competition for nutrients between host and native microbiota. Other than as observed in this study, Vale et al. (2004) and Gunal et al. (2006) reported that the use of propionic acid and formic acid had no effect on body weight gain and feed conversion ratio.

In the period from 1 to 35 days was found significant difference for all performance variables studied, these differences become even more evident when we observe the growth phase (22 to 35 days) separately (Table 5). The effect of organic acids may be associated with decreased gastric pH by organic acids and their salts and intestinal colonization with pathogenic microorganisms. Furthermore, the effect may be related to increase activity of proteolytic enzymes, which improve nutrient absorption and intestinal morphology and, consequently, influence the body weight and feed conversion ratio of the birds (Giannenas, 2006).

Vieira et al. (2008) and Viola et al. (2008) reported a reduction in feed intake when the birds were treated with organic acids, confirming the results observed in the treatments with Premium Lac AP and Premium Lac BP. Pinchasov and Jensen (1989) have found that the propionic acid had a significant action on the reduction of food intake, and Cave (1978) had reported the importance of organic acid in the regulation of satiety, since pH values between 3 and 4 potentiate the activation of pepsinogen in pepsin and reduce the rate of gastric emptying, increasing the food retention time in the stomach.

However, Gunal et al. (2006) and Izat et al. (1990) did not observe any effect of the organic acids in performance parameters, since well-nourished healthy chicks do not positively respond to growth promoters when they are housed under clean conditions and at a moderate stoking density.

There was no significant effect between treatments in the period from 1 to 42 days, due the lack of re-contamination of birds in the finisher phase and, consequently, the effect caused by the immune challenge during the growth phase was diluted throughout the rearing period. However, studies by Alçiçek et al. (2004) and Giannenas et al. (2014) reported positive effects of organic acids on body weight gain and feed conversion ratio in poultry from 1 to 42 days, due to better digestibility of the nutrients leads to a more balanced gut flora with the potential

to reduce the proportion of pathogenic bacteria, improving the efficiency of feed utilization and growth.

As shown in Table 6, the treatments containing organic acids had a lower incidence of intestinal lesion caused by necrotic enteritis, confirming the findings of Geier et al. (2010) and Stringfellow et al. (2009) showed a reduction in intestinal lesion score of birds subjected to treatment blend of acetic, formic, propionic, sorbic, caprylic and capric. The mechanism of action of organic acids involves reduction in intracellular pH via the entry of undissociated acids into the bacterial cell and subsequent dissociation in the cytoplasm (Skrivanova et al. 2005), reducing the amount of *Clostridium perfringens* and therefore lesions caused by them.

The performance study demonstrated that the Premium Lac AP can be used in broiler feed as an alternative to antibiotic on the control necrotic enteritis. Regarding the intestinal lesions caused by necrotic enteritis, Premium Lac BP and Premium Lac AP stood out for lower incidence of lesions, suggesting greater efficiency in controlling the disease.

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