UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA
CAMPUS BOTUCATU

ADITIVOS À BASE DE LEVEDURA NA ALIMENTAÇÃO DE FRANGOS DE CORTE: BEM-ESTAR, SAÚDE INTESTINAL E SISTEMA IMUNE

MARCOS ANTONIO NASCIMENTO FILHO

Tese apresentada ao Programa de Pós-Graduação em Zootecnia como parte dos requisitos para obtenção do título de Doutor em Zootecnia.

Botucatu, SP

Abril – 2023

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Abril – 2023

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP

BIBLIOTECÁRIA RESPONSÁVEL: ROSEMEIRE APARECIDA VICENTE-CRB 8/5651

Nascimento Filho, Marcos Antonio.
Aditivos à base de levedura na alimentação de frangos de corte : bem-estar, saúde intestinal e sistema imune / Marcos Antonio Nascimento Filho. - Botucatu, 2023

Tese (doutorado) - Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Medicina Veterinária e Zootecnia
Orientador: Ibiara Correia de Lima Almeida Paz
Capes: 50400002

1. Frango de corte - Alimentação. 2. Leveduras. 3. Microbiota. 4. Sistema imunológico. 5. *Saccharomyces cerevisiae.*

Palavras-chave: Desafio sanitário; Desempenho; Frangos de corte; Imunidade intestinal; Microbiota.

DEDICATÓRIA

À Deus, por tudo que tenho e sou.

Aos meus pais, pelo amor, fé e sabedoria.

À minha noiva, pelo companheirismo e confiança.

Toda a minha vida por vocês, sempre.

EPÍGRAFE

“It is better to be vaguely right than exactly wrong”

Carveth Read

AGRADECIMENTOS

À Deus, por me guiar e ser o alicerce da minha vida.

À minha mãe Silvana de Melo Moscoso, e ao meu pai Marcos Antonio Nascimento (in memoriam), obrigado por todo o amor incondicional, apoio e sacrifícios que fizeram por mim ao longo da vida. Sou imensamente grato por serem meus pilares e me guiarem pelos caminhos certos, pois tudo que alcancei até aqui foi adquirido pelos seus ensinamentos em família.

À minha noiva, Julia Gonçalves Almeida, por todo amor, paciência, companheirismo e confiança. Com seu sorriso meus dias ficam sempre mais alegres. Agradeço por você ser a metade que me completa ontem, hoje e sempre.

À Professora Associada Ibiara Correia de Lima Almeida Paz, por todo o apoio, motivação, amizade e conhecimento compartilhado como orientadora para o meu aperfeiçoamento profissional.

Aos integrantes do Grupo de Pesquisa em Ciência Avícolas que estiveram presentes nesta jornada de experimentos, sou grato pelos momentos descontraídos que passamos juntos, pelo apoio e também as experiências de trabalho em equipe.

Aos estagiários e funcionários do setor de avicultura da Faculdade de Medicina Veterinária e Zootecnia que participaram das atividades nos experimentos para a obtenção do meu título de doutor, agradeço pela amizade e suporte nas horas de trabalho.

Aos amigos da UNESP, Ana Beatriz, Laís Cordeiro, João Fiuza, Armando Contin, Izabela Bataglioli, Daniel Martins, Victoria Girnos, Gustavo Coelho, Fernanda Contin e Amanna Gonzaga que sempre me ajudaram e apoiaram durante as disciplinas e experimentos, e que, como grandes amigos formamos uma família ao qual serei sempre grato pelos conselhos e os momentos alegres que passamos juntos.

Aos meus grandes amigos, Jonathan Santos e Vinicius Cambito, que desde que nos conhecemos sempre estiveram ao meu lado me apoiando. Muitas pessoas nós conhecemos, mas existem apenas algumas que deixam uma marca especial em nossas vidas. Obrigado pelo carinho e amizade, e saibam que a nossa amizade é sem fim.

À empresa Aleris, pela parceria e doação dos aditivos utilizados para a condução dos experimentos, muito obrigado.

À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), pela concessão do auxílio a pesquisa (processo nº 2020/12641-4), muito obrigado.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

Aos professores do Programa de Pós-Graduação em Zootecnia da UNESP de Botucatu, agradeço todo o conhecimento compartilhado durante toda a jornada acadêmica.

À Faculdade de Medicina Veterinária e Zootecnia da Universidade Estadual Paulista "Júlio de Mesquita Filho, obrigado.

E a todos que de alguma forma contribuíram para realização desta tese, muito obrigado!

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CAPÍTULO I
CONSIDERAÇÕES INICIAIS

RESUMO

A indústria avícola é um setor produtivo rápido e dinâmico devido ao intenso desenvolvimento e aplicação de tecnologias na genética, nutrição e manejo dos frangos de corte. Por este motivo, os desafios encontrados atualmente na avicultura estimulam a constante adaptação dos sistemas contemporâneos aos novos modelos de produção, de forma a garantir maior saúde, bem-estar das aves e segurança alimentar. No que diz respeito a nutrição, o uso de aditivos à base de levedura tem sido considerado uma alternativa promissora para substituir os antimicrobianos como melhoradores de desempenho na alimentação animal. Compostos principalmente por mananoligossacarídeos, β-glucanos e nucleotídeos, esses prebióticos demonstram capacidade para modular a microbiota intestinal e respostas do sistema imune das aves, influenciando positivamente as características de produtividade. No entanto, diversos mecanismos de ação desses prebióticos e suas associações ainda foram pouco elucidados e demandam mais estudos. Sendo assim, o projeto de pesquisa propôs investigar os efeitos da suplementação de produtos à base de levedura *Saccharomyces cerevisiae* em rações para frangos de corte. Para isso foram realizados dois ensaios de desempenho, sendo: a) a avaliação dos efeitos da suplementação dos aditivos prebióticos sobre o bem-estar e a integridade intestinal de frangos de corte desafiados com *Eimeria*; e b) o uso dos aditivos no controle da infecção de *Salmonella* Heidelberg em frangos de corte e na modulação da microbiota intestinal e respostas do sistema imune. Foram verificados resultados positivos para a morfologia intestinal dos frangos de corte suplementados com os aditivos à base de levedura frente ao desafio de *Eimeria*, assim como alta produção de serotonina que está relacionada há melhores condições de bem-estar das aves. Além disso, frente ao desafio de *Salmonella* foi verificada a produção de imunoglobulinas e aumento da concentração de alguns ácidos graxos de cadeia curta para os frangos suplementados com aditivos à base de leveduras, indicando o aumento das respostas do sistema imune. A microbiota intestinal também foi alterada e a bactéria *Turicibacter* (biomarcador de serotonina) foi identificada em aves suplementadas com os aditivos. Em geral, os resultados demonstraram que a suplementação de aditivos à base de levedura é uma alternativa na nutrição de frangos de corte para estimular efeitos benéficos na saúde, desempenho e bem-estar das aves.

Palavras-chave: Desafio sanitário, Desempenho, Frangos de corte, Imunidade intestinal, Microbiota, *Saccharomyces cerevisiae*.

ABSTRACT

The poultry industry is a fast and dynamic productive sector due to its intense development and application of technologies in genetics, nutrition, and management of broiler commercial systems. As a result, the challenges currently encountered in aviculture stimulate the constant adaptation of the contemporary systems to new production models, in order to ensure greater welfare and health of chickens and food safety. Regarding to nutrition, the use of yeast-based additives has been considered a promising alternative to replace antibiotics as growth promoters in animal feed. Composed mainly of mannooligosaccharides, β-glucans and nucleotides, these prebiotics demonstrate capacity to modulate the intestinal microbiota and immune system responses of birds, positively influencing in productivity parameters. However, several modes of action of these prebiotics and their associations with other functional substances have not been entirely elucidated and require further studies. Thus, the research project proposed to investigate the effects of yeast-based products *Saccharomyces cerevisiae* in broiler chicken diets. The study was divided in two performance trials: a) evaluation of the effects of yeast-based additives on the well-being and intestinal integrity of broiler chickens challenged with *Eimeria*; and b) the use of yeast-based products in the control of *Salmonella* Heidelberg infection in broiler chickens and in the modulation of intestinal microbiota, and immune system responses. Positive results were verified for the intestinal morphology of broilers supplemented with yeast-based additives against the *Eimeria* challenge, as well as high production of serotonin which is related to better welfare conditions of the birds. In addition, in the face of the *Salmonella* challenge, the production of immunoglobulins and increased concentration of some short-chain fatty acids were verified for the broiler chickens supplemented with yeast-based additives, indicating an increase in the immune system response. The gut microbiota was also altered and the *Turicibacter* bacterium (serotonin biomarker) was identified in birds supplemented with the additives. Overall, the results demonstrated that supplementation of yeast-based additives is an alternative in broiler nutrition to stimulate beneficial effects on poultry health, performance and well-being.

Keywords: Broiler chickens, Challenge, Intestinal immunity, Microbiota, Performance, *Saccharomyces cerevisiae.*

1.1. INTRODUÇÃO

A produção avícola é uma atividade econômica que vem crescendo continuamente devido a aplicação de tecnologias dentro dos sistemas de criação. Enquanto o amplo avanço da nutrição, genética e manejo tem aumentado a eficiência dos plantéis de frangos de corte, a demanda pelo bem-estar, saúde das aves e qualidade do produto final tem se tornado cada vez mais uma preocupação pública.

Com o movimento da Europa em 2006, que baniu o uso de antimicrobianos melhoradores de desempenho (AMD) na alimentação animal (Castanon, 2007), uma crescente pressão social e comercial tem demandado regulamentações mais robustas, especialmente nos principais países produtores de carne de frango como o Brasil, maior exportador e terceiro maior produtor mundial (ABPA, 2023). Garantir a máxima produtividade das aves é essencial para a competitividade da indústria avícola, permitindo que o sistema opere tanto do ponto de vista econômico quanto sustentável. Portanto, a busca por novas abordagens nutricionais tem sido cada vez mais encorajada a fim de substituir os antimicrobianos nas rações (Ricke, 2018), uma vez que as aves continuam expostas naturalmente à patógenos do ambiente que podem ser nocivos ao organismo animal, prejudicando o seu desempenho.

Os aditivos zootécnicos equilibradores da microbiota intestinal, como por exemplo os prebióticos tem se revelado uma alternativa natural promissora e funcional nos sistemas de produção animal (Yadav et al., 2016), assim como potenciais substitutos aos AMD pois possuem propriedades benéficas similares, que atuam na melhora do desempenho e saúde de frangos de corte para máxima eficiência produtiva (Mehdi et al., 2018).

Prebióticos são substâncias alimentares não digeríveis ou não hidrolisáveis que podem estimular efetivamente o crescimento de microrganismos locais presentes na microbiota e gerar benefícios a saúde (Gibson et al., 2017). Neste contexto, a levedura inativa *Saccharomyces cerevisiae* e seus derivados, oriundos do processo de fermentação alcoólica, destacam-se como um agente biológico com capacidade prebiótica. O resultado do processo de obtenção do etanol, a partir da fermentação dos carboidratos do caldo de cana-de-açúcar, geram como coproduto a biomassa celular da levedura composta por compostos ativos, sendo a fração da parede celular (mananoligossacarídeos - MOS, β-glucanos, principalmente) e os componentes intracelulares das células autolisadas

(nucleotídeos, ácidos orgânicos, polifenóis, aminoácidos, vitaminas e minerais) (Świątkiewicz; et al., 2014).

A composição dos produtos à base de leveduras irá depender do tipo de processamento que a levedura será submetida, contudo sabe-se que a levedura *Saccharomyces cerevisiae* contém cerca de 29 - 64% β- 1,3/1,6 glucanos, 31% de MOS, 13% de proteína, 9% de lipídeos e 1 - 2% de quitina (Jaehrig et al., 2008). Algumas dessas substâncias, como os β-glucanos desempenham um papel interessante na imunidade de frangos de corte, uma vez que atuam nas respostas tipo 1 e 2 de defesa do organismo contra patógenos, desencadeando respostas na microbiota intestinal e reações imunomoduladoras de macrófagos, citocinas e linfócitos (Teng e Kim, 2018). Os MOS, por sua vez, servem como sítio de ligação para certas bactérias patogênicas no lúmen intestinal, aglutinando-se a fímbria específicas (Tipo I) e evitando que essas bactérias se liguem ao epitélio (Mirza, 2018). Além disso, os MOS são capazes de modular a resposta do sistema imune e desencadear uma série de estímulos durante o combate ao agente estressor. Da mesma forma, os nucleotídeos são considerados agentes imunomoduladores envolvidos em diversos processos biológicos, assim como substâncias ligadas a rápida proliferação de células do sistema imune e manutenção da integridade intestinal. (Świątkiewicz et al., 2014). Estas condições também favorecem o desempenho e o bem-estar das aves, gerando assim uma melhor resposta em produção.

Estudos recentes têm mostrado resultados positivos com a utilização de produtos à base de leveduras na alimentação de frangos de corte. Avaliando os efeitos da suplementação de levedura autolisada de *Saccharomyces cerevisiae* na saúde das aves, Bortoluzzi et al. (2018) verificaram que o aditivo modulou as respostas do sistema imune e da microbiota intestinal de frangos desafiados com vacina contra coccidiose por meio da redução da expressão gênica do receptor (TLR4) e interleucina do tipo 1 β (IL-1β). Pourabedin et al. (2016), avaliando os efeitos dos MOS sobre a microbiota cecal e a expressão de citocinas em frangos desafiados com *Salmonella* Enteritidis, verificaram atividade imunomoduladora sobre a microbiota e produção de polipeptídios de resposta inflamatória para o combate dos agentes patogênicos, reduzindo a colonização da *Salmonella*. Avaliando os efeitos de produtos à base de leveduras para frangos de corte, Alizadeh et al. (2016) verificaram que nucleotídeos são capazes de regular a expressão do gene TL4, associado com a proteção da barreira gastroepitelial e resposta imune contra patógenos, o que pode explicar o seu efeito sobre a proliferação de linfócitos e células do

epitélio intestinal. Em outro estudo, a suplementação de β-glucanos na dieta de frangos desafiados com *Clostridium perfringens* melhorou a saúde intestinal das aves por meio da inibição do crescimento de bactérias patogênicas e da produção de respostas e expressão de genes do sistema imune (Tian et al., 2016).

Sobre a ação dos aditivos prebióticos à base de leveduras na modulação da comunidade microbiana e integridade intestinal das aves, Bonato et al. (2020) verificaram que frangos de corte desafiados com *Salmonella* Enteritidis e suplementados com parede celular de levedura apresentaram melhor integridade intestinal e efeitos positivos sobre a microbiota do ceco e parâmetros imunológicos comparado as aves sem suplementação. Em outro estudo, a parede celular de levedura suprimiu a resposta inflamatória, promovendo a liberação de imunoglobulinas e aumento da produção de ácidos graxos de cadeia curta, o que sugere um potencial benéfico para a saúde dos frangos de corte no controle de infecções bacterianas (Xue et al., 2017). Com o objetivo de identificar os efeitos do MOS no perfil de microrganismos no ceco das aves, Corrigan et al. (2015) observaram que o prebiótico altera positivamente a diversidade das bactérias do ceco, aumentando a presença de microrganismos desejáveis que estão relacionados com a atividade hidrolítica e melhora na digestão dos alimentos. Shao et al. (2013), investigando o efeito protetor de β-1,3/1,6-glucanos sobre a morfologia e integridade intestinal, verificaram que a sua suplementação pode favorecer a integridade intestinal de frangos desafiados com *Salmonella* Typhimurium, devido ao aumento da expressão de proteínas da zona de oclusão intercelular. Os autores também observaram aumento significativo nos parâmetros histomorfométricos do intestino das aves como altura de vilo, relação vilo/cripta e número de células caliciformes, o que auxilia na manutenção de uma barreira da mucosa intestinal efetiva.

Efeitos diretos dos prebióticos sobre o desempenho e qualidade da carne de frangos de corte também têm sido relatados na literatura. Segundo Fomentini et al. (2016), a utilização de MOS melhora o ganho de peso, a conversão alimentar e a viabilidade das aves de 1 a 42 dias de idade. Da mesma forma, a suplementação de β-glucanos pode ser considerada uma alternativa aos aditivos melhoradores de desempenho, pois aumenta a eficiência alimentar e viabilidade dos frangos de corte, entretanto, sem apresentar efeitos na qualidade da carne (Moon et al., 2016). Por outro lado, Cho et al. (2013), observaram que a qualidade da carne foi beneficiada pelo uso de β-glucanos na ração de frangos, além de resultados significativos na melhora do desempenho zootécnico das aves. Em outro

estudo, a suplementação de nucleotídeos na ração de frangos sob estresse térmico também melhorou os parâmetros de desempenho e resposta imune sem afetar a qualidade da carne das aves (Salah et al., 2019).

A interdependência existente entre o sistema imune, a microbiota e o epitélio intestinal das aves para a manutenção da homeostase é complexa. Os estudos têm demonstrado que os prebióticos potencialmente modificam a interação entre o hospedeiro e a microbiota e melhoram o estado de saúde e desempenho dos frangos de corte (Teng e Kim, 2018). Neste contexto, os efeitos dos prebióticos tem desencadeado respostas sobre a manutenção funcional do organismo e a modulação dos microrganismos intestinais, aumentando a colonização de bactérias benéficas por meio de processos fermentativos. Da mesma forma, a ativação direta de metabólitos e células do sistema imune estimula a liberação de citocinas que agem no combate de microrganismos patogênicos, regulando as respostas do sistema imune inato e adaptativo (Mirza, 2018). Entretanto, estes efeitos sobre a saúde e desempenho dos frangos podem ser variáveis dependendo de fatores diretos e indiretos que influenciam as respostas das aves, como por exemplo a fase de criação, a dose utilizada do aditivo, a composição da ração, o ambiente e tipo de desafio existente.

Os benefícios dos prebióticos, em especial os produtos à base de leveduras são notórios, por isso, estes aditivos vêm sendo utilizados pela indústria avícola substituindo o uso de AMD a fim de atender as demandas mundiais em segurança e qualidade do produto final. Todavia, estes aditivos são responsáveis por diversos mecanismos de ação, muitos deles ainda pouco elucidados que demandam mais estudos, como as interações da microbiota intestinal e os produtos da fermentação bacteriana, os mecanismos de ação do organismo para as respostas anti-inflamatórias e a expressão de genes de resistência no combate de bactérias patogênicas de relevância a saúde pública, tal como a *Salmonella*, a nível molecular. Além disso, a investigação do uso de aditivos a base de levedura associados a microrganismos probióticos e outras substâncias funcionais demandam mais estudos para investigar os efeitos benéficos destas combinações em parâmetros de produção. Novas abordagens científicas que usufruam da biotecnologia e ferramentas de inteligência artificial sobre a interação microbiota-hospedeiro, e também, que avaliem os benefícios de aditivos no bem-estar das aves associado a qualidade da carne, devem ser estimuladas a fim de disponibilizar informações indispensáveis de viés econômico-sustentável. Nos últimos anos, a quantidade de estudos avaliando probióticos aumentou,

entretanto, não há na literatura estudos nacionais que relacionaram benefícios da suplementação de produtos à base de levedura (prebióticos) em dietas para frangos de corte correlacionando o bem-estar animal.

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CAPÍTULO II

INFLUENCE OF YEAST-BASED ADDITIVES ON INTESTINAL HEALTH AND WELL-BEING OF BROILER CHICKENS CHALLENGED WITH *EIMERIA*

Manuscript prepared according to the guidelines of Journal of Applied Poultry Research - submitted manuscript

SUMMARY

A study was conducted to investigate the effect of *Saccharomyces cerevisiae* yeast-based additives supplementation on the welfare and intestinal health of chickens challenged with *Eimeria*. A total of 1890 d-old male chicks were placed into 42 pens (45 birds/pen, 7 replicates), in a completely randomized design. Dietary treatments were as follows: negative control (no additives); positive control (salinomycin); NC + 500 mg/kg of yeast cell wall; NC + 455 mg/kg of yeast cell wall and 45 mg/kg of free nucleotides; NC + 500 mg/kg of autolyzed yeast cell wall associated with postbiotic; and NC + 250 mg/kg of autolyzed yeast cell wall associated with postbiotic. At 4 days of age, the birds were challenged with 20x *Eimeria* vaccine dosage. The performance was evaluated and analyses for intestinal health, serotonin and welfare were performed. All parameters of performance were affected by the challenge. Positive control showed the best growth performance compared to all treatments. Chickens fed yeast-based additives had overall good feed conversion ratio, viability and production efficiency factor, showing better economic efficiency compared to the antibiotic supplementation. Regarding intestinal morphometry, yeast-based prebiotic associated with postbiotic was able to partially respond against the infection, protecting distinctive intestinal segments, especially jejunum and ileum. Serotonin plasma level was increased by the prebiotic supplementation, in which also influenced the locomotion condition of the chickens by gait score evaluation. Birds supplemented with yeast-based additives associated with postbiotic were able to provide similar results to the antibiotic group. Therefore, the use of yeast-based additives can improve profitability, well-being of the birds and help immune response against pathogens.

2.1. DESCRIPTION OF PROBLEM

Commonly known and used in broiler feed, prebiotics have gained increasing notoriety through scientific research that shows their promising effects as a substitute for performance-enhancing antimicrobials. Prebiotics are non-digestible feed ingredients that are metabolized by beneficial bacteria and lead to immune responses to protect the intestinal ecosystem. Due to their cell biomass compounds, prebiotics can assist the body in homeostatic balance by modifying the interaction between the host and microbiota, resulting in improved health and development in broiler chickens (Teng and Kim, 2018).

Among the most common substances with prebiotic capacity, the yeast *Saccharomyces cerevisiae*, used in the process of alcohol fermentation, stands out as one of the most used and with more investments in the market (Ahiwe et al., 2019; Malairuang et al., 2020). The yeast cell biomass is composed of mannan-oligosaccharides and beta-glucans (active cell wall compounds) and other intracellular components of the autolyzed cells, such as nucleotides (Świątkiewicz et al., 2014). In addition, these metabolites have immunomodulatory action against pathogens (Brummer et al., 2010; Pourabedin et al., 2017) and estimulate rapid proliferation of immune system cells for the maintenance of intestinal integrity (Bonato et al., 2020), which makes them interesting for use in farm animal diets.

Besides to the reduction of pathogenic microorganisms, prebiotics act on the modulation of the intestinal microbiota that influences the behavior and physiology of the host (van der Eijk et. al., 2020). Prebiotic supplementation is associated with the production of short-chain fatty acids that can stimulate the expression of enzymes associated with serotonin synthesis, produced mainly in the intestine (Silva et. al., 2020). Therefore, the increase of beneficial bacteria in the microbiota is associated with better intestinal integrity, reduced stress and fear of birds, improving animal welfare (Baurhoo et. al., 2007; Teng and Kim, 2018).

Although the studies published to date on prebiotics mainly present a positive influence on the immune system and intestinal microbiota (Huff et. al., 2010; Pourabedin and Zhao, 2015), these additives are also responsible for several effects, usually positive, and still little explored. Some findings are often taken as secondary responses and do not receive special attention, since they are not part of the hypothesis initially proposed for conducting the studies. Examples of secondary responses observed in studies with

prebiotics involve improving the welfare indices of the birds (Kraimi et al., 2019), or even how the interaction between other natural substances and prebiotics added to the diets occurs (Al-Khalaifah, 2018). Most of the responses have practical indicators (e.g., well-being and blood assessments), which are efficient to the commercial scope due to the ease of measurement and low cost, in addition to being low invasive to the birds (Sohail et al., 2010; Almeida Paz et al., 2019). Therefore, research involving investigations to understand these benefits, already observed empirically, should be conducted. The objective of this study was to investigate the effect of supplementation of *Saccharomyces cerevisiae* yeast-based additives on the welfare and intestinal health of broiler chickens challenged with *Eimeria*.

2.2. MATERIAL AND METHODS

The experimental procedures were approved by the Institutional Animal Care and Use Committee, of the School of Veterinary Medicine and Animal Sciences of the São Paulo State University (FMVZ/UNESP), Botucatu, SP, Brazil (protocol number: 0133/2020).

2.2.1. Animals, Diets, and Housing

The study was conducted at the facilities of the FMVZ/UNESP. One-day-old male broiler chicks (n=1890) Ross AP95 were used in the experiment. Chicks were weighed (average live weight of 40.3 g) and placed into 42 pens (45 birds/pen and 7 replicates/treatment) in a completely randomized design. The birds were housed in floor pens (3 m^2) with new wood shaving litter in an environmentally controlled facility of exhaust fans and cooling system (negative pressure). Each pen was 1.36 m wide × 2.20 m long and was equipped with a tube feeder and a nipple drinker line.

The nutritional program consisted of four diets: pre-starter (1–7 d), starter (7–21 d), grower (21–35 d), and finisher (35–41 d) fed from 1 to 41 days of age. Chickens were fed an isonutritive and isoenergetic diet, prepared in mash form and formulated with corn, soybean meal, and 5% wheat bran (Table 1) to meet nutritional requirements for standard performance, according to Rostagno et al. (2017). Feed and water were available ad libitum. The experimental treatments were: basal diet, unsupplemented negative control (NC); basal diet supplemented with 600 mg/kg of salinomycin, positive control (PC); NC + 500 mg/kg of yeast cell wall (YCW); NC + 455 mg/kg of yeast cell wall and 45 mg/kg

of free nucleotides (YCW+N); NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic (ACW+P500); and NC + 250 mg/kg of autolyzed yeast cell associated with postbiotic (ACW+P250). Prebiotic additives (*Saccharomyces cerevisiae*; non-digestible dead yeast and derivatives) were added in powder form, with the guarantee levels: Yeast cell wall: Mannanoligosaccharides: 17%; β-glucans: 28%; Crude protein (CP): max. 35%. Free nucleotides: min. 15% nucleotides; min. 50% CP. Autolyzed yeast cell associated with functional substances: Postbiotics and *Bacillus subtilis* min. 1.0 x 10^7 CFU/g; CP: min. 25% (the association of strong and active components such as mannans and beta-glucans with any substance released by or produced through the metabolic activity of inactivated microbial cells (Kouhounde et al., 2022). For the concentration of free nucleotides, the process consists of extracting and concentrating RNA up to 80-90% and then hydrolyze it with enzymes. Additives were added by replacing an inert substance in the basal diet.

2.2.2. Challenge and Experimental Procedures

In order to cause an imbalance in the intestinal microbiota and generate immunological stress on birds without the occurrence of high mortality, at 4 days of age, the chicks were challenged with an attenuated multivalent vaccine of E. acervuline, E. maxima, E. praecox, E. tenella and E. mitis at a dosage 20 times higher than that recommended by the manufacturer (Bio-coccivet, Biovet Brazilian Laboratory S/A, São Paulo, Brazil). The infectious dose was defined based on a previous study (adapted from Belote et al., 2018), and all chickens were inoculated by oral gavage of 0,6 mL of the oocysts' suspension. In addition to the vaccine challenge, it was proposed to use a high density of birds per pen (15 chickens/m²) and the use of wheat bran in the feed ration in order to increase the environmental stress and the viscosity of the intestinal content for the experimental measurements. Biosecurity procedures were maintained among groups.

At 15 and 29 days of age, six birds per treatment were selected based on the average body weight of the pen, euthanized by cervical dislocation without feed withdrawal and segments of the gastrointestinal tract were collected by flushing with distilled water to analyze the histological condition of the duodenum, jejunum and ileum. All samples were dehydrated, infiltrated, and embedded in paraffin following common histological procedures. At 20 days of age, 14 birds per treatment were submitted to blood collection to evaluate gut leakage. Samples were obtained carefully, homogenized and immediately frozen at a −20°C for subsequent analysis. For well-being evaluation, from day 20, birds

were weekly monitored to measure their locomotion condition and at 39 and 40 days of age, the behavior and response of the birds to management were evaluated. Serotonin levels was assessed during day 20 and 40 of the experiment.

The chickens' growth performance was weekly measured by body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and viability. The production efficiency factor (PEF) and economic feed efficiency (EFE) were calculated using the following formulas:

Formula 1: PEF = ((BW (kg) × L) / (FCR × age at slaughter)) × 100

where L = livability.

Formula 2: EFE = (BWG × live chicken price per kg) / (FI × total feed cost per kg)

where price was measured in dollar (Houndonougbo et al., 2009).

2.2.3. Histological Analysis

In order to analyze the intestinal morphology using the I See Inside (ISI) methodology (Kraieski et al., 2017), one slide and 20 intestinal villi per bird were observed in 10X magnification under optical microscope (Nikon Eclipse E200, São Paulo, Brazil). If any type of change was detected, the parts were also observed using 20X and 40X magnification, with the same microscope. According to Kraieski et al., the ISI methodology (INPI BR 1020150036019) is an evaluation protocol based on a numeric score of alteration, in which is expressed as a guide to associate the intestinal lesions to some disturbed performance (Table 2). In this methodology, an impact factor (IF) is defined for each alteration in macroscopic and microscopic analysis, according to the reduction of organ functional capacity, based on previous knowledge from the literature and background research. The IF ranges from 1 to 3, with 3 being the most impacting to organ function. In addition, the extent of each lesion (intensity) or the observed frequency compared to non-affected organ is evaluated in each organ/tissue with score (S) ranging from 0 to 3: score 0 (absence of lesion or frequency), score 1 (alteration up to 25% of the area or observed frequency), score 2 (alteration ranges from 25 to 50% of the area or observed frequency), and score 3 (alteration extends to more than 50% of the area or observed frequency). To obtain the final value of the ISI index, the IF of each alteration is multiplied by the respective score number, and the results of all alterations are summed according to the formula ISI = Σ(IF$*$S), where IF = impact factor and S = Score. The sum

of the average of all parameters presented in Table 2 will give the total ISI value for this specific bird (each bird was considered a replicate for statistical analysis).

2.2.4. Intestinal Permeability

To evaluate the permeability of the intestinal mucosa, a dose of 8.0 mg/bird of Fluorescein Dextran Isothiocyanate (FITC-d; MW 3,000-5,000; Sigma Aldrich Co., St. Louis, MO) was administrated by gavage to quantify its passage into the bloodstream. After 2 hours and 30 minutes of oral administration, blood was collected, coagulated and centrifuged, and serum diluted 1:1 in PBS. FITC-d levels in the serum were measured at an excitation wavelength of 485 nm and an emission wavelength of 528 nm (Synergy HT, multi-mode microplate reader, BioTek Instruments, Inc., Winooski, VT). The measured fluorescence were compared to a standard curve with known FITC-d concentrations (Vicuña et al., 2015).

2.2.5. Serotonin, Gait Score and Welfare Indicators

The neurotransmitter serotonin was evaluated at 20 and 40 days of age. In each period, a total of 1.5 ml of blood was collected from seven birds per treatment. Samples were conditioned in anticoagulant tubes, centrifuged, and transferred to micro tubes. Serotonin (5-hydroxytryptain) plasma levels were measured using a commercial enzyme immunoassay kit (adapted from Chapman et al., 2008).

All behavior tests were carried out by the same observers and before known any experimental results. In order to assess the birds' walkability, all birds were observed for Gait Score, according to the methodology described by Stamp Dawkins et al. (2004). Each bird was individually encouraged to walk in the pen by an observer, which classified the bird in scores ranging from 0-2 based on its walkability (0 = birds walking normally; 1 = birds walking up to 10 steps with difficulty and imbalance between the legs; and 2 = birds barely walking from 1 to 4 steps and sitting down). Additionally, for the Latency to lie test, which assess the time that the bird takes to sit when exposed to an uncomfortable and aversive situation to its rearing, three chickens per replicate were placed in a box (75 × 50 × 20 cm), with feet submerged in 3 cm of water at room temperature and observed for a maximum of 360 seconds, when the test is interrupted (Almeida et al., 2017). As long as the bird remained standing, the better was the general condition and welfare of the chicken.

To assess the level of reactivity of the broiler chickens, the touch test was applied. The method used in this experiment was initially described by Chiozzini and Soster (2017) and adapted by Almeida Paz et al. (2019). To evaluate how calm the broiler chickens were, all experimental pens were evaluated by the same observer. After 2 min of immobile waiting inside the pen, the observer extended his arm, trying to touch the birds, and then counted the number of birds that could be touched. This method is based on the escape distance of birds, where the smaller it is, the calmer the animals will be.

2.2.6. Statistical Analysis

The data on performance, intestinal parameters, and serotonin production were submitted to ANOVA by PROC GLM (General Linear Models) of SAS 9.4 (2013). The homogeneity of variances was assessed by Levene's test and data normality was verified by Shapiro-Wilk test. When significant effect was verified, the variables were submitted to mean comparison by Tukey's test. For gait score, latency to lie, and touch test, data were submitted to a non-parametric analysis using chi-squared test in the R platform version 4.2.0 (2022). Statistical significance was considered at the level of 5%.

2.3. RESULTS AND DISCUSSION

2.3.1. Performance

All parameters of productive performance were affected by the *Eimeria* challenge ($P < 0.05$) during the experimental period (Table 3). Broiler chickens supplemented with salinomycin (PC) showed the best recovered results for growth ($P < 0.05$). Although dietary treatments containing yeast-based additives did not provide the best live weight and weight gain, the different prebiotic supplementation used in the present study showed good performance results compared to the negative control (NC) group (approximately +40grams body weight). From 1 to 21 days, *Eimeria* challenge showed a trend to decrease approximately 25% body weight of the chickens (based on commercial guideline table: Ross 308 AP - Broiler Performance Objective, Rev. 2022) for all treatments, indicating the efficacy of the inoculated vaccine without severe mortality. Throughout the experiment, the birds were able to recover from the induced dysbiosis, compensating the loss in performance, however, their maximum potential growth was notably impaired. (Yun et al., 2000).

In the present study, the supplementation of salinomycin was more efficient than yeast-based additives to support the immune system of the birds, leading to better responses for production. However, some authors reported positive benefits for growth (Sozcu and Ipek, 2017; Biswas et al., 2018; Leung et al., 2019) and nutrient digestibility (Nisar et al., 2021) using prebiotic, probiotic and/or postbiotic supplementation.

We can infer that the results obtained in this study were greatly influenced by the high challenge level imposed, since there was an association among high density, nutritional challenge, and *Eimeria* inoculation, leaving the chickens in a situation of great sanitary stress. It is likely that such imposition of a challenge has diminished the ability of these additives to modulate other responses, since, in situations of moderate stress, prebiotics have other direct and indirect functionalities more prominent than performance such as microbiota modulation, immune response against pathogens, and intestinal homeostasis (Liu et al., 2021). The association between these dynamic mechanisms can lead to pathways of response that, in some cases, positively stimulate the growth performance of the birds.

It is worth noting that, although the chickens' growth was significantly diminished, the PEF and EFE variables demonstrated that the yeast-based diets were less costly compared to NC (P=0.0007 and 0.0001, respectively). Such feed efficiency associated with direct and indirect functional benefits of the prebiotics found in this study stands out as a very positive result, especially considering its impact on the intestinal health and well-being of the chickens that will be discussed further.

Salinomycin is a coccidiostat ionophore used commercially to prevent coccidiosis and it can also inhibit the growth of other gram-positive bacteria (Rutkowski and Brzezinski, 2013). The use of salinomycin as antimicrobial substance improving growth is still allowed in Brazil and United States, however Europe has already banned its use in animal nutrition because of microbial resistance. Considering the vaccine challenge proposed in this experiment, it can explain the better performance response of the birds for positive control compared to the yeast-based supplemented groups. Iseri and Klasing (2013) suggested that epithelial alterations in the intestine can be one of the major causes of coccidiosis and it is strongly related to decrease in body weight gain, feed intake, and feed efficiency. When the host identifies pathogens in the organism, metabolic and immune responses are set as a mechanism of defense. It demands energy for higher maintenance of the organism, which cause a decrease in some regular functions such as

digestion and absorption (Belote et al., 2018). Due to *Eimeria* invasion, commensal bacteria in the intestinal microbiota decrease while pathogenic bacteria increase. In addition, the proliferation of schizonts and release of merozoites in the enterocytes cause inflammation and damage in the intestinal mucosa (Lu et al., 2021), decreasing its absorptive function. It suggests that gut morphology may be impaired and that the severity of the infection can reflect to intestinal permeability (Teng et al., 2020).

2.3.2. Histological Parameters and Intestinal Integrity

The total ISI score proved to be a good method for assessing intestinal integrity, indicating the effects of treatments used in different intestinal segments (Figures 1 and 2). At 12 dpi, duodenum was the most infected segment due to considerable presence of oocysts, congestion, and lamina propria inflammation. Although jejunum and ileum followed the same pattern, it was possible to note higher incidence of oocysts in the jejunum and thicker lamina propria in the ileum, both of high impact to the organ. The PC group presented the lowest ISI score in the duodenum, which was statistically different from most of the treatments ($P < 0.05$). Although birds fed ACW+P250 and ACW+P500 did not perform well in the duodenum, they had a good response to protect the jejunum and ileum, equaling to PC or showing statistically better results ($P < 0.05$). On the other hand, chickens consuming YCW+N did not present the same trend, showing higher and similar ISI score in all segments to NC. Lastly, YCW had intermedial scores in the duodenum and ileum but higher score for jejunum compared to the other treatments.

In the second evaluation (26 dpi), it was observed that the broiler chickens were able to partially reduce the alterations suffered by the intestinal epithelium of the three segments, reducing the ISI scores and improving organ functionality. The YCW+N and ACW+P250 showed lower ISI scores for duodenum, jejunum and ileum compared to all treatments. By the time from the challenge, the immune system of the birds was capable to defend the organism against the infection, even for those birds without supplementation, in which showed similar results to the supplemented groups. Unexpectedly, birds fed YCW showed the highest ISI scores for all segments, differing from YCW+N and ACW+P250 supplemented birds ($P < 0.05$).

The most important histopathological alterations occurred in the lamina propria, with greater inflammation in addition to the presence of oocysts. These parameters indicating disruptive intestinal mucosa have already been described by Belote et al. (2018) as easy

parameters to compare intestinal health between different treatments, once higher scores indicate directly worse animal condition.

An inflammatory reaction is characterized by congestion, expansion of blood vessels and the presence of inflammatory cells into the small intestine that can be caused by the diet, microorganisms, environment, management and other features. In fact, a combination of factors may play a role in inflammation. The results obtained in the present study indicated that the *Eimeria* challenge disturbs the intestinal morphology of chickens supplemented with yeast-based additives as well as antibiotic, however, these additives were able to partially respond against the infection, protecting distinctive intestinal segments. While the antibiotic substance was able to reduce duodenum damage, yeast-based additives were able to reduce damage in jejunum and ileum. Therefore, when we observed a decrease in inflammatory parameters, an improvement in the functionality of intestinal cells can be suggested.

Niewold (2007) reported that antibiotic growth promoters can reduce inflammation by protecting epithelial cell wall against exudation, avoiding the invasion and accumulation of inflammatory cells. Likewise, mannanoligosaccharides serve as a binding site for certain pathogenic lectins, binding them through type I fimbriae and preventing these hosts from binding to the surface of the epithelium (Mirza, 2018). Since these mannans are not hydrolysable, they easily reach the intestine and are excreted from there, carrying the invading microorganisms (Ferket et al., 2002; Sadeghi et al., 2013). Our study corroborates with previous research showing that prebiotics and probiotics can be promising additives to prevent intestinal disorders in broiler chickens challenged by pathogenic microorganisms such as C. perfringens (Tian et al., 2016; Li et al., 2017), and *Eimeria* (Leung et al., 2019).

Further histological alterations observed in these experimental birds were the epithelial thickness, inflammatory cell infiltration, and enterocyte proliferation in the intestine. According to Belote et al. (2018) the increase of these cells in the first week of life of the chickens can negatively affect performance parameters up to 28 days of age. However, evaluating immunity, increased mucin production as a barrier defense against pathogens indicates positive recovery responses (data not shown).

Goblet cells are glycoprotein producing cells localized in the epithelium and have the functions to protect intestinal mucosa against abrasive agents of the diet and enteric

pathogens as well as to help the final digestion. The production of mucin by these cells plays an important role in nonspecific immune response, serving as an important sanitary barrier. According to Duangnumsawang et al. (2021), mucin is the first defensive mechanism of the intestine as a response of any factor disturbing metabolic activity. Although the mechanism of action is not fully understood, studies confirmed that the structural components of mucins produced by goblet cells have antibacterial properties stimulated by β-defensin, lysozyme, avidin and IgA, which regulate the microbial immune response (Bar Shira and Friedman, 2018). Considering the supplementation of yeast-based additives, beta-glucans play an interesting role in broiler chicken immunity, as they act on the body's type 1 and 2 defense responses by triggering responses in the gut microbiota and immunomodulatory reactions of macrophages, cytokines, and lymphocytes (Guo et al., 2003; Xue et al., 2017; Teng and Kim, 2018), that might be associated with these epithelial cell responses. However, this rapid increase in mucin is itself a barrier to nutrient absorption. In general, the results obtained in this study allow us to affirm that chickens receiving yeast-based prebiotic supplementation showed good results for gut morphometry, especially in the jejunum and ileum. Therefore, the maintenance of intestinal cell integrity leads to reduced ISI score and intestinal cell alterations.

Regarding intestinal permeability, we noticed that the supplemented groups reflected in different degrees of intestinal integrity (Table 4). Low serum FITC-d was observed for YCW, indicating that this prebiotic supplementation allowed better intestinal integrity. No differences were found between the NC, PC and YCW+N groups, while in ACW+P500 and ACW+P250 this concentration was higher than in the other treatments ($P < 0.05$). Allied to the histomorphometry findings in this experiment, the intestinal integrity results for the ACW+P500 and ACW+P250 groups lead us to infer that the high infection occurred at the beginning of the challenge, leading to the rupture of the epithelial lining of the intestine, in which they can be recovered considerably by the time for these groups.

After studying the pertinent literature on the intestinal morphometry, the authors did not expect differences comparing both parameters, however, the recovery of FITC-d indicated that yeast-based additives associated with postbiotic (ACW+P500 and ACW+P250) showed greater dysfunction of the mucosal barrier compared to positive and negative controls. In addition, the use of yeast cell wall (YCW) resulted in better intestinal

integrity, which leads to a discussion about the best option for supplementation of broiler chickens in a great environmental challenge.

It is well known that enteric mucosa is the main channel and barrier to control absorption in the gastrointestinal tract (Ma et al., 2013). In addition, *Eimeria* ssp. infection causes general tissue damage and disruption of the lamina propria, indicating a high permeability in which can lead to undesirable molecules translocation from the intestinal lumen to the blood (McDougald and Fitz-Coy, 2013). This condition can be associated to numerous factors such as nutrition, density, age, management, environmental stress, sanitary condition, etc. (Gilani et al., 2021). The development of leaky gut will challenge the immune system which can result in infection, affecting the health and reducing the growth of the birds. In our study, the performance of yeast-based prebiotic associated with postbiotic in the intestinal integrity may be explained by the high ISI score for duodenum, where ACW+P500 and ACW+P250 presented high incidence of oocytes in this intestinal segment, leading to mucosa inflammation. As duodenum is the first intestinal segment, integrity of the lamina propria was compromised and higher FITC-d uptake was observed in the blood. The opposite occurred to YCW and PC, however, it is not a fact for NC, where a high ISI score for all intestinal segments and lower intestinal permeability were found.

Some authors are in agreement that intestinal integrity of broiler chickens can be improved by the supplementation of yeast-based additive (Yang et al., 2009; Bonato et al, 2020; Perricone et al., 2022). Based on its components, especially mannans, β-glucans, and nucleotides, yeasts are beneficial to intestinal morphology and resistance, modulating the cellular barrier and microbiota to support integrity (Wang et al., 2022). Evaluating the supplementation of yeast cell wall in broiler chicken diets, McCaffrey et al. (2021) found functional properties improving gut health and epithelial layer condition. These properties were also noticed by Hernández-Ramírez et al. (2021), who reported a modulation in mycotoxin infection, reducing negative impacts on production. Additionally, despite the fact that prebiotics can change the intestinal microbiota, Wang et al. (2016), suggest that these additives can improve the tight junction protein expression, which is a potential direct on-site modulation in the metabolism. Evaluating the effects of *Saccharomyces* supplementation for broiler chickens to control the *Campylobacter* jejunum infection, Massacci et al. (2019) found that prebiotics can modulate the intestinal ecosystem and cell structure, leading to a stronger epithelium and a higher number of beneficial

microorganisms in the gut. Such reports may partially explain the results obtained in this experiment. Although we have corroborative studies showing the benefits of yeast based-additives, for Behnamifar et al. (2019), probiotics and prebiotics are not effective to control coccidiosis, especially if compared with coccidiostat ionophores.

2.3.3. Welfare Measurements

The dietary treatments containing yeast-based prebiotics significantly improved the serotonin levels of broiler chickens (Figure 3). All birds showed better results compared to NC and PC groups, indicating that the use of these additives can improve the behavior and well-being of the chickens. Overall, ACW+P250 group presented the best serotonin plasma level, followed by ACW+P500, YCW+N, and YCW ($P < 0.0001$). In addition, the walkability of the birds was influenced by the different prebiotic supplementation programs tested (Table 5). The data obtained regarding gait score indicated that ACW+P250 promoted the best ability for chickens to walk ($P < 0.05$), contrasting to the results found for ACW+P500. On the other, the LTL and touch tests of the chickens were not influenced by the treatments ($P > 0.05$) (Table 6).

It is notable that yeast-based additives improved the serotonin blood levels of the chickens. The gastrointestinal tract and the brain are directly connected by the vagus nerve and immune signals with uncountable neurons and neurotransmitters, which are pathways to send information to one another in the body (Cerdó et al., 2017). This relationship plays a key role controlling health and well-being, especially thinking that the intestinal mucosa is responsible for about 90% of the serotonin production in the body by enterochromaffin cells, which synthesize, store and release serotonin (Gill et al., 2008; Gershon and Tack, 2007). It is known that serotonin plays an important role on behavior, emotion, and cognition for animals (Bacque-Cazenave et al., 2020), as well as can regulate gastrointestinal tissue responsible for motility (Delesalle et al., 2008).

According to Almeida Paz et al. (2019), a healthier intestine due to probiotic supplementation may increase serotonin production, also modulating fear-related behaviors in broiler chickens. Likewise, evaluating chickens under heat stress and supplemented with symbiotics, Mohammed et al. (2018) observed a good response for growth and improved welfare by natural behavior expression. There are few other studies supporting the present findings (Wang, 2018; Cheng at al., 2019), however, no literature on chicken-fed prebiotics was found. Another important fact in this matter was stated by

Haller (2013), who confirmed that stressful conditions could lead to low levels of serotonin in the brain, which can stimulate aggressive behavior of animals, e.g., feather pecking (van Hierden et al., 2004). Evaluating thermal stress impact on performance and health of broiler chickens, Ahmed-Farid et al. (2021) found that low serotonin levels in the brain negatively affected physiological and metabolic responses of the birds. Simitzis et al. (2013) reported that stock density is associated with low well-being and performance of the birds, which especially affects locomotor activity. In order to reduce heat stress impact on broiler chickens, Salah et al. (2021) evaluated phytogenic supplementation in the diet and found better serotonin levels in the brain, leading to better health and performance of the birds.

Despite the fact that some behavioral parameters (latency to lie and approximation test) did not differ statistically in the present study, gait score showed a trend that yeast-based additives associated with postbiotic, in especial ACW+P250, improved locomotion condition of the birds. Although the challenge had a significant impact on the walkability of the birds, at the end of the trial about 70% of the chickens showed normal walking (GS0), especially ACW+P250 (about 75%), inferring in acceptable welfare conditions according to Nääs et al. (2009) and Fernandes et al. (2012). These findings associated with the previous described suggested good effects from prebiotic supplementation in the diet of broiler chickens. Due to the complexity of understanding the interactions between the gut-brain axis, cell modulation, and well-being responses, serotonin modulation is not yet clear and needs to be explored. To the authors' knowledge, our study is the first literature associating intestinal health and integrity, serotonin production, and welfare for chickens supplemented with yeast-based additives.

2.5. CONCLUSION AND APPLICATIONS

1. All parameters of performance were affected by the challenge, however salinomycin supplementation resulted in better production and yeast-based additives showed promising results compared to negative control.
2. The association of prebiotic with postbiotic in the diet of broiler chickens was able to provide similar results to the antibiotic group for the intestinal morphology parameters, diminishing oocytes invasion and cell disruption in the mucosa.

3. Concerning welfare, serotonin blood level was increased for chickens fed yeast-based additives, which can indicate better well-being and life quality of the birds.
4. Increased serotonin production might have improved physiological and metabolic responses for intestinal health, leading to improved response against *Eimeria* trough intestinal level.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest to disclose.

ACKNOWLEDGMENTS

The author would like to thank FAPESP, São Paulo Research Foundation for the financial support (No 2020/12641-4) given to this project.

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Table 1. Ingredient and nutritional composition of the reference diet, as fed basis

Ingredients (g/kg, unless noted)	**Prestarter (1-7d)**	**Starter (7-21d)**	**Grower (21-35d)**	**Finisher (35-41d)**
Corn	390.2	414.4	449.1	541.5
Soybean meal	465.5	438.7	403.1	322.1
Wheat	50.0	50.0	50.0	50.0
Soybean oil	52.9	58.9	63.4	57.5
Dicalcium phosphate	18.7	16.2	13.8	10.3
Limestone	10.1	9.2	8.7	7.4
Salt	5.4	5.2	4.9	4.7
DL-Methionine	3.3	3.3	3.1	2.5
L-Lysine HCl	1.1	1.1	1.2	1.6
Choline chloride 60%	0.8	0.8	0.6	0.4
Inert	0.6	0.6	0.6	0.6
Vitamin premix [1]	0.5	0.5	0.5	0.5
Mineral premix [2]	0.5	0.5	0.5	0.5
L-Threonine	0.4	0.6	0.5	0.4
Total (kg)	1,000	1,000	1,000	1,000
Nutritional composition (calculated)				
Metabolizable energy	3,000	3,075	3,150	3,225
Crude protein	253.1	243.0	229.4	199.9
Calcium	10.1	9.1	8.2	6.6
Available Phosphorus	4.8	4.3	3.8	3.1
Sodium	2.3	2.2	2.1	2.0
Potassium	10.3	9.9	9.4	8.2
Digestible lysine	13.6	13.1	12.2	10.7
Digestible met + cyst	9.9	9.7	9.1	7.9
Digestible threonine	8.8	8.6	8.1	7.0
Digestible tryptophan	2.9	2.8	2.6	2.2

[1] Salus Group, Composition per kg of product: Vit. A - 20,000,000 UI; Vit. D3 - 8,000,000 UI; Vit. E – 44,000 UI; Vit. K3 – 6,000 mg; Vit. B1 – 4,400 mg; Vit. B2 – 14,000 mg; Vit. B6 – 7,000 mg; Vit. B12 – 32,000 µg; Nicotinic acid – 90 g; Pantothenic acid – 32 g; Biotin - 240 mg; Folic acid – 3,200 mg; Selenium – 1,000 mg.

[2] Salus Group, Composition per kg of product: Manganese – 160,000 mg; Iron – 100,000 mg; Zinc – 140,000 mg; Copper – 20,000 mg; Iodine – 2,000 mg.

Table 2. ISI histological alterations evaluated in intestine segments[1]

Organ	Alteration	Impact Factor		Score	Final Score	Maximum Score
Intestine	Lamina propria thickness	2	X	3	6	45
	Epithelial thickness	1	X	3	3	
	Enterocytes proliferation	1	X	3	3	
	Epithelial plasma cell infiltration	1	X	3	3	
	Lamina propria Infl. infiltration	3	X	3	9	
	Goblet cells proliferation	2	X	3	6	
	Congestion	2	X	3	6	
	Presence of oocysts	3	X	3	9	

[1]Adapted from Belote et al. (2018)

Table 3. Productive performance of broiler chickens supplemented with yeast-based additives and challenged with *Eimeria* ssp.

Item	Treatment						Statistic		
	NC	PC	YCW	YCW+N	ACW+P500	ACW+P250	SEM	CV	p-value
1-21 days									
BW 21, g	786 bc	860 a	794 bc	775 bcd	756 d	758 cd	6.39	5.2	< 0.001
FI, g	968 b	1011 a	967 b	946 b	952 b	952 b	5.02	3.3	< 0.001
BWG, g	724 bc	819 a	752 b	734 bc	716 c	718 bc	6.36	5.4	< 0.001
FCR	1.34 c	1.23 a	1.29 bc	1.29 bc	1.33 c	1.32 bc	0.01	3.2	< 0.001
1-28 days									
BW 28, g	1516 b	1655 a	1553 b	1488 b	1482 b	1520 b	11.42	4.8	< 0.001
FI, g	1942 b	2045 a	1961 b	1906 b	1920 b	1932 b	10.47	3.4	< 0.001
BWG, g	1469 b	1614 a	1511 b	1447 b	1441 b	1480 b	11.39	4.9	< 0.001
FCR	1.32 b	1.27 a	1.30 b	1.32 b	1.33 b	1.30 b	0.01	2.1	< 0.001
1-41 days									
BW 41, g	2978 b	3195 a	3020 b	3017 b	2991 b	3020 b	15.93	3.3	< 0.001
FI, g	4512 b	4715 a	4546 ab	4495 b	4499 b	4535 ab	21.09	3.0	0.0176
BWG, g	2955 b	3166 a	2988 b	2979 b	2952 b	3001 b	15.94	3.4	< 0.001
FCR	1.53	1.49	1.52	1.51	1.52	1.51	0.01	2.5	0.3945
Livability, %	94.6 b	96.2 ab	95.6 ab	97.4 ab	98.7 a	95.0 b	0.46	3.1	0.0310
PEF	449.9 b	504.2 a	457.5 b	476.0 ab	477.2 ab	460.0 b	4.33	5.8	0.0007
EFE	1.29 a	0.91 d	1.18 b	1.01 c	1.00 c	1.14 b	0.02	12.2	< 0.001

Means followed by different letters in the same column differed by Tukey test ($P < 0.05$). NC=negative control; PC=positive control; YCW=NC + 500 mg/kg of yeast cell wall; YCW+N=NC + 455 mg/kg of yeast cell wall and 45 mg/kg of free nucleotides; ACW+P500=NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic; and ACW+P250=NC + 250 mg/kg of autolyzed yeast cell associated with postbiotic; BW= body weight; BWG= body weight gain; FI= feed intake; FCR= feed conversion ratio; PEF= production efficiency factor; EFE= economic factor efficiency; SEM = standard error of the mean; CV (%) = Coefficient of variation.

Table 4. Serum FITC-d determination of broiler chickens supplemented with yeast-based additives and challenged with *Eimeria* ssp. at 20d

Treatment	FITC-d (µg/mL)
NC	0.3261 ab
PC	0.3200 ab
YCW	0.3006 a
YCW+N	0.3386 bc
ACW+P500	0.3564 c
ACW+P250	0.3557 c
SEM	0.004
CV	10.61
P-value	<0.0001

Means followed by different letters in the same column differed by Tukey test ($P < 0.05$). NC=negative control; PC=positive control; YCW=NC + 500 mg/kg of yeast cell wall; YCW+N=NC + 455 mg/kg of yeast cell wall and 45 mg/kg of free nucleotides; ACW+P500=NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic; and ACW+P250=NC + 250 mg/kg of autolyzed yeast cell associated with postbiotic; SEM = standard error of the mean; CV (%) = Coefficient of variation.

Table 5. Gait score of broiler chickens supplemented with yeast-based additives and challenged with *Eimeria* ssp.

Treatment	20d			27d			34d			40d		
	0	1	2	0	1	2	0	1	2	0	1	2
NC	93.1 b	6.6 a	0.3	92.7	5.6 a	1.7 b	90.4 b	6.8 a	2.7 ab	72.7	22.4 a	4.9 bc
PC	93.7 b	6.3 a	0.0	93.0	5.0 ab	2.0 b	92.5 a	5.1 b	2.4 b	73.1	18.7 c	8.2 a
YCW	92.3 b	6.7 a	1.0	90.9	5.7 a	3.4 a	88.9 c	6.9 a	4.1 a	71.9	22.6 a	5.6 b
YCW+N	95.1 a	4.9 b	0.0	94.1	4.6 b	1.3 b	92.5 a	4.7 b	2.7 ab	73.1	21.4 a	5.4 b
ACW+P500	95.8 a	3.6 b	0.6	94.1	3.6 c	2.3 b	92.7 a	3.7 c	3.7 a	74.2	20.1 b	5.7 b
ACW+P250	95.3 a	4.7 b	0.0	94.3	5.4 a	0.3 c	93.7 a	5.2 b	1.0 c	75.1	20.7 ab	4.2 c
P-value	0.0001	0.0001	0.2320	0.2410	0.0300	0.0100	0.0001	0.0020	0.0020	0.0651	0.0230	0.0020

Means followed by different letters in the same column differed by Tukey test ($P < 0.05$). NC=negative control; PC=positive control; YCW=NC + 500 mg/kg of yeast cell wall; YCW+N=NC + 455 mg/kg of yeast cell wall and 45 mg/kg of free nucleotides; ACW+P500=NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic; and ACW+P250=NC + 250 mg/kg of autolyzed yeast cell associated with postbiotic; SEM = standard error of the mean; CV (%) = Coefficient of variation.

Table 6. Latency to lie (based on gait score) and approximation test of broiler chickens supplemented with yeast-based additives and challenged with *Eimeria* ssp.

Treatment	Latency to lie (sec.)		Touch test (%)
	GS0	GS1	
NC	117	56	43.4
PC	120	58	48.7
YCW	115	55	47.9
YCW+N	131	52	49.7
ACW+P500	122	58	46.7
ACW+P250	137	52	47.1
SEM	8.93	4.71	1.25
CV	46.79	57.12	16.06
P-value	0.9791	0.932	0.5903

Means followed by different letters in the same column differed by Tukey test ($P < 0.05$). NC=negative control; PC=positive control; YCW=NC + 500 mg/kg of yeast cell wall; YCW+N=NC + 455 mg/kg of yeast cell wall and 45 mg/kg of free nucleotides; ACW+P500=NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic; and ACW+P250=NC + 250 mg/kg of autolyzed yeast cell associated with postbiotic; SEM = standard error of the mean; CV (%) = Coefficient of variation.

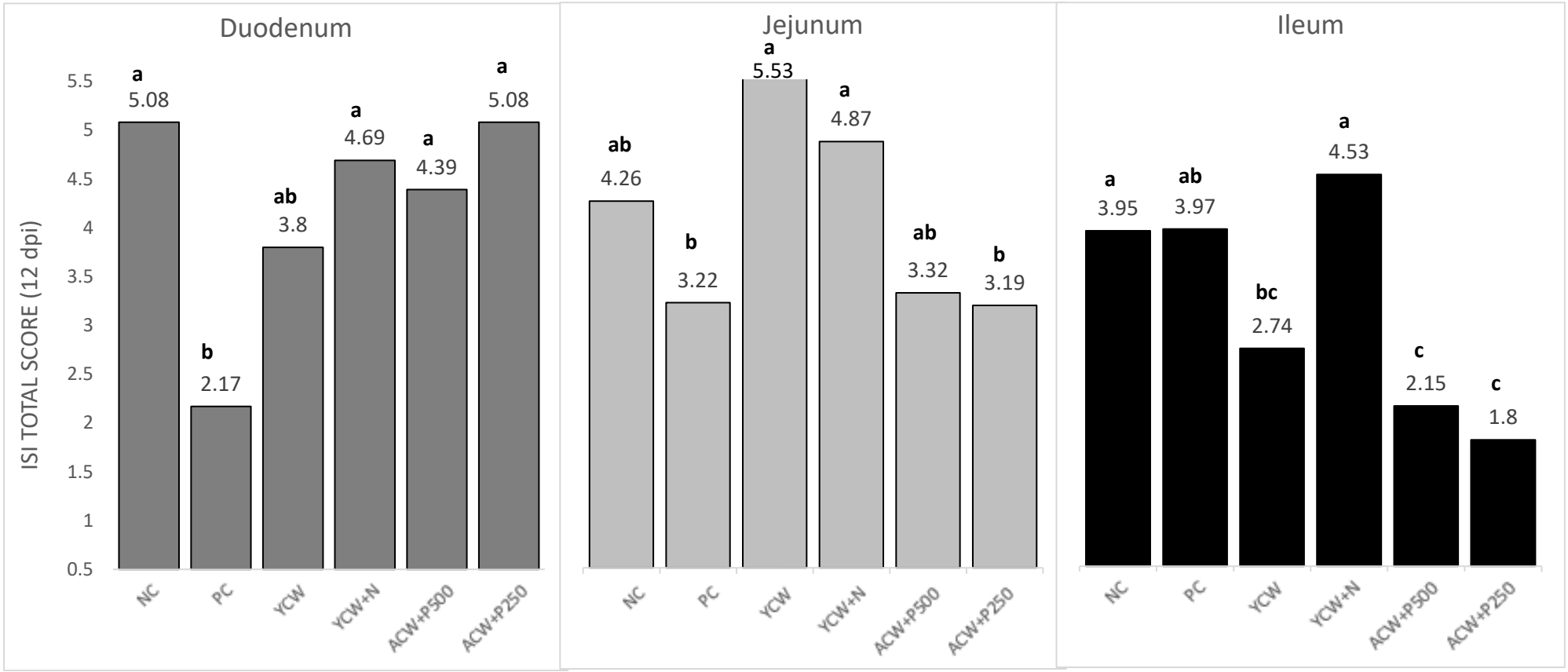


Figure 1. ISI total histological scores in duodenum, jejunum, and ileum in different groups at 12-days post infection (dpi). NC=negative control; PC=positive control; YCW=NC + 500 mg/kg of yeast cell wall; YCW+N=NC + 455 mg/kg of yeast cell wall and 45 mg/kg of free nucleotides; ACW+P500=NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic; and ACW+P250=NC + 250 mg/kg of autolyzed yeast cell associated with postbiotic. 12 dpi= 1st collection (d15); 26 dpi= 2nd collection (d29). Means followed by different letters between treatments in the same column differed by Tukey test ($P < 0.05$). Error bars represent standard error of the mean

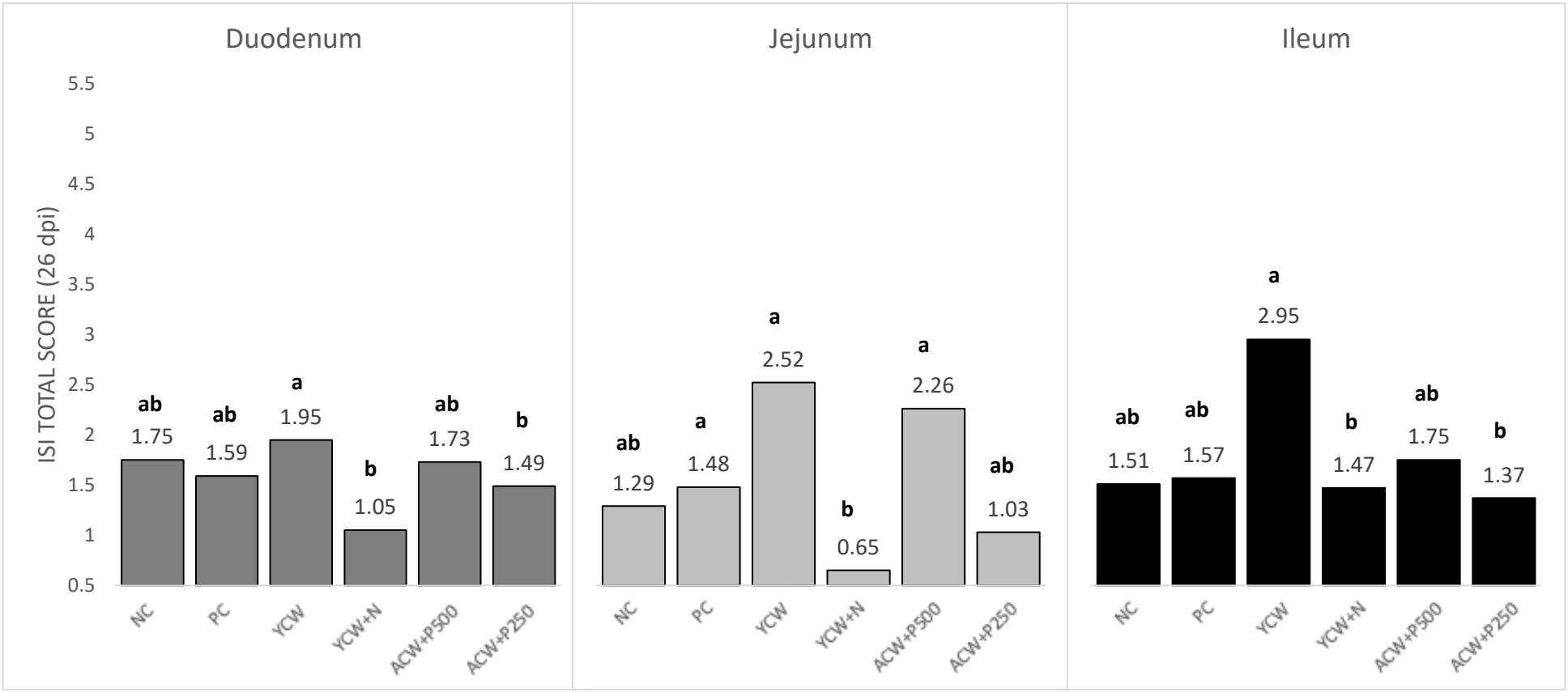


Figure 2. ISI total histological scores in duodenum, jejunum, and ileum in different groups at 26-days post infection (dpi). NC=negative control; PC=positive control; YCW=NC + 500 mg/kg of yeast cell wall; YCW+N=NC + 455 mg/kg of yeast cell wall and 45 mg/kg of free nucleotides; ACW+P500=NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic; and ACW+P250=NC + 250 mg/kg of autolyzed yeast cell associated with postbiotic. 12 dpi= 1st collection (d15); 26 dpi= 2nd collection (d29). Means followed by different letters between treatments in the same column differed by Tukey test ($P < 0.05$). Error bars represent standard error of the mean

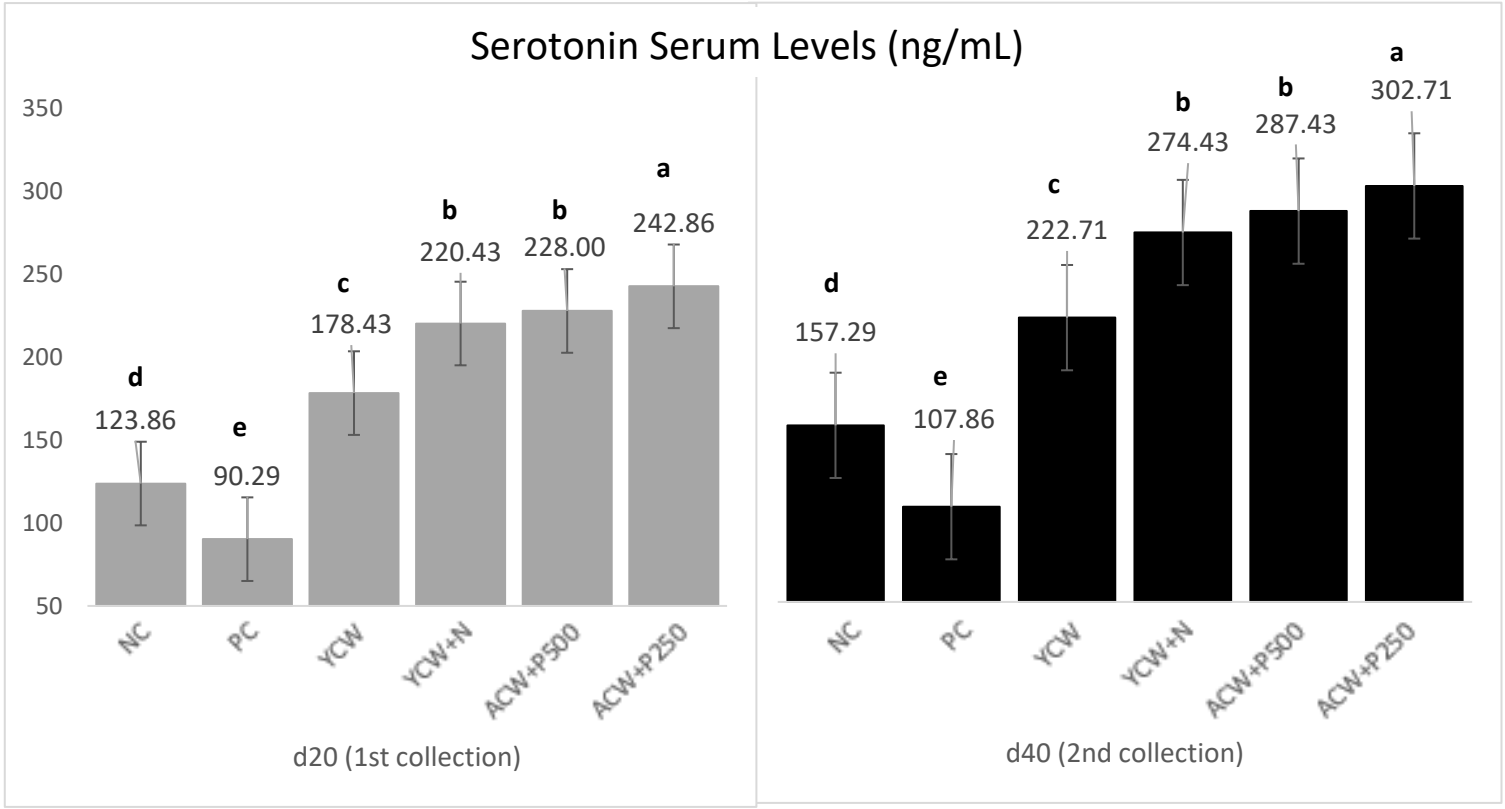


Figure 3. Serotonin serum levels of broiler chickens supplemented with yeast-based additives and challenged with *Eimeria* ssp. at 20 and 40 days of age. NC=negative control; PC=positive control; YCW=NC + 500 mg/kg of yeast cell wall; YCW+N=NC + 455 mg/kg of yeast cell wall and 45 mg/kg of free nucleotides; ACW+P500=NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic; and ACW+P250=NC + 250 mg/kg of autolyzed yeast cell associated with postbiotic. Means followed by different letters in the same column differed by Tukey test ($P < 0.05$). Error bars represent standard error of the mean

CAPÍTULO III

EFFECTS OF YEAST-BASED ADDITIVES ON INTESTINAL MICROBIOTA, HEALTH, AND PRODUCTIVE PARAMETERS OF BROILER CHICKENS CHALLENGED WITH *SALMONELLA* HEIDELBERG

Manuscript prepared according to the guidelines of Poultry Science

ABSTRACT

The current study aimed to investigate the effects of dietary supplementation of *Saccharomyces cerevisiae* yeast-based additives on growth performance, microbiota parameters, and immune system responses of chickens challenged with *Salmonella* Heidelberg (SH). A total of 1000 d-old male chicks were placed into 40 pens (25 birds/pen and 8 replicates/treatment), in a completely randomized design. Dietary treatments were as follows: negative control; positive control (halquinol); NC + 500 mg/kg of yeast cell wall; NC + 455 mg/kg of yeast cell wall and 45 mg/kg of free nucleotides; and NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic. At 3 and 15 days of age, half of the birds (4 replicates/treatment) were challenged with SH strain by gavage and non-challenged chickens received PBS in order to expose all birds to the same procedure. The performance was evaluated and analyses for *Salmonella* quantification, short chain fatty acids (SCFA), immune parameters, and microbiota modulation were conducted. Performance of the birds was affected by the challenge ($p<0.05$), and non-challenged group showed the best results. In general, YCW improved feed conversion ratio and feed intake of the chickens at 14 and 21 days compared to CN and CP, and YCW+N and ACW+PB improved FCR at 35 days compared to the NC ($p<0.05$). Regarding *Salmonella* contamination, no difference was observed for bacteria count in the cecum ($p>0.05$), however, chickens supplemented with yeast-based prebiotics showed lower *Salmonella* counting in the liver and litter compared to NC. For fatty acids quantification, challenged group showed higher values compared to non-challenged birds. NC had higher value of acetate and total SCFA content compared to YCW+N and ACW+PB, while propionate concentration was lower compared to all treatments ($p<0.05$) of the challenged group. Immune system was modulated at 10 days of age on blood parameters comparing specially challenged and non-challenged birds. Immunoglobulins were significantly increased for YCW supplementation compared to NC ($p<0.05$). Challenged birds also presented higher levels of immunoglobulins compared to non-challenged birds. Regarding microbiota, alpha diversity analysis showed a higher bacterial richness in cecum of challenged birds, however no differences were observed between treatments. The composition of microbiota regulated by yeast-based additives had the abundance of *Turicibacter*, a serotonin biomarker responsible to well-being. Overall, yeast-based additives reduce the impact of SH by regulating immune responses and maintaining performance, in which can be a valuable feed additive to broiler chickens.

3.1. INTRODUCTION

The poultry industry is a solid and dynamic sector in the world due to its constant adaptation from contemporary systems to new production models to ensure protein to feed the population. Although improvements in the production systems have been done to guarantee food safety from the farm to the market, zoonotic pathogens still represent a challenge to the poultry industry (Hafez and Attia, 2020). The antibiotic misusage remains as a critical issue point because residue can affect the quality and safety of the final product leading to the development and transmission of antibiotic-resistant pathogens to human health (Khan and Rahman, 2022).

Considered a public concern regarding food contamination for human consumption, *Salmonella* is the most incident bacteria and an etiological agent that causes a wide range of enteric and systemic diseases (Broz et al., 2012). Highly resistant to different environmental conditions, this gram-negative pathogen is transmitted through the fecal-oral route and has multifactorial virulence (e.g., toxin production, resistance to the immune system, cell disruption, unbalance of the intestinal microbiota). Individuals colonized by *Salmonella* may still be asymptomatic, which aggravates the impact of this foodborne disease responsible for high economic losses in the poultry industry (Foley et al., 2013). Concerning about the different serotypes, *Salmonella* Heidelberg has been gaining prominence in recent years as one of the most common serotypes in humans due to the high prevalence in poultry products (Voss-Rech, et al., 2019; Santos et al., 2022), however in-depth understanding about this serovar is needed (Kaldhone et al., 2017).

Nutrition strategies has been proposed to improve production safety and animal health and the use of yeast-based additives is considered a promising alternative to antibiotic-free systems (Bilal et al., 2021). Derived from the ethanol sugarcane fermentation process, yeast cell components are release as active metabolites (e.g., mannanoligosaccharides, β-glucans and nucleotides) that stimulate the immune system and promote animal performance (Brummer et al., 2010; Bortoluzzi et al., 2018; Kim et al., 2022). Yeast cell is classified as a prebiotic additive used to modulate the intestinal microbiota and control pathogens such as *Salmonella* (Pourabedin and Zhao, 2015; Pourabedin et al., 2017; Girgis, et al., 2022). In addition, the production of short-chain fatty acids can stimulate beneficial effects such as cell function and the expression of

enzymes associated with serotonin synthesis, produced mainly in the intestine (Silva et. al., 2020).

Several studies have shown positive results in immunomodulatory responses to inflammatory mechanisms, reducing pathogenic agents (Pourabedin et al., 2016; Xue et al., 2017; Teng and Kim, 2018), as well as changing positively metabolite concentrations in the intestinal microbiota of broilers supplemented with yeast-based products (Corrigan et al., 2015; Kiros et al., 2019; Alkhulaifi et al., 2022). In a study conducted by Bonato et al. (2020), the authors verified that broilers challenged with *Salmonella* Enteritidis and supplemented with yeast cell wall presented better intestinal integrity and positive effects on the cecum microbiota and immunological parameters compared to birds without supplementation. The interaction between the microbiota and immune system of chickens for the maintenance of homeostasis is complex. However, it is known that microbiota imbalance may provoke to the development of dysbiosis to the organism, leading to metabolic and immunological disorders (Jeurissen et al., 2002).

Due to the importance of pathogen control to one health and its economic impact on poultry production systems, yeast-based additives are considered as natural alternatives in nutrition to replace antimicrobial growth promoters still used in commercial production systems. Thus, the objective of this study was to investigate the effect of supplementation of *Saccharomyces cerevisiae* yeast-based additives on performance, modulation of the intestinal microbiota, and immune system responses of broiler chickens challenged with *Salmonella* Heidelberg.

3.2. MATERIAL AND METHODS

The experimental procedures were approved by the Institutional Animal Care and Use Committee, of the School of Veterinary Medicine and Animal Sciences of the São Paulo State University (FMVZ/UNESP), Botucatu, SP, Brazil (protocol number: 0133/2020).

3.2.1. Animals, Diets, and Housing

The study was conducted at the facilities of the FMVZ/UNESP. One-day-old male broiler chicks (1000) of a commercial strain (Ross AP95) were used in the experiment. Chicks were weighed (average live weight of 37.0 g) and placed into 40 pens (25 birds/pen and 4 replicates/treatment), in a completely randomized design. The birds were housed in

floor pens (1,00 m wide × 2.50 m long) with new wood shaving litter and equipped with a tube feeder and a bell drinker. Additionally, there was an internal area in the facility, separating the rooms to increase biosecurity during experimental challenge.

The nutritional program consisted of three diets: pre-starter (1–7 d), starter (7–21 d), and grower (21–35 d), fed from 1 to 35 days of age. Chickens were fed an isonutritive and isoenergetic diet based on corn and soybean meal (Table 1) to meet nutritional requirements for medium-superior performance, according to Rostagno et al. (2017). Feed and water were available *ad libitum*. The experimental treatments were divided into challenged and non-challenged groups as follow: basal diet unsupplemented negative control (NC); basal diet supplemented with 50 mg/kg of halquinol, positive control (PC); NC + 500 mg/kg of yeast cell wall; NC + 455 mg/kg of yeast cell wall and 45 mg/kg of free nucleotides; and NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic. Prebiotic additives (*Saccharomyces cerevisiae*) were added in powder form, with the guarantee levels: Yeast cell wall: Mannanoligosaccharides: 17%; β-glucans: 28%; Crude protein (CP): max. 35%. Free nucleotides: min. 15% nucleotides; min. 50% CP. Autolyzed yeast cell associated with functional compounds: Postbiotics and *Bacillus subtilis* min. 1.0 x 10^7 CFU/g; CP: min. 25% (the association of strong and active components such as mannans and beta-glucans with any substance released by or produced through the metabolic activity of inactivated microbial cells (Kouhounde et al., 2022). For the concentration of free nucleotides, the process consisted of extracting and concentrating RNA up to 80-90% and then it was hydrolyzed with enzymes. Additives were added by replacing an inert substance in the basal diet.

Table 1. Ingredient and nutritional composition of the reference diet, as fed basis

Ingredients (g/kg, unless noted)	**Prestarter (1-7d)**	**Starter (7-21d)**	**Grower (21-35d)**
Corn	444.3	462.7	496.6
Soybean meal	473.6	447.8	408.7
Soybean oil	40.6	51.4	59.6
Dicalcium phosphate	19.7	17.2	15.0
Limestone	9.5	8.6	8.1
Salt	5.4	5.2	5.0
DL-Methionine	3.3	3.3	3.1
L-Lysine HCl	1.0	1.0	1.3
Choline chloride 60%	0.8	0.8	0.6
Vitamin premix [1]	0.5	0.5	0.5
Mineral premix [2]	0.5	0.5	0.5
Inert	0.5	0.5	0.5
L-Threonine	0.3	0.5	0.5
Total (kg)	1,000	1,000	1,000
Nutritional composition (calculated)			
Metabolizable energy	3,000	3,100	3,200
Crude protein	253.1	243.0	228.1
Calcium	10.1	9.1	8.2
Available Phosphorus	4.8	4.3	3.8
Sodium	2.3	2.2	2.1
Potassium	10.1	9.7	9.1
Digestible lysine	13.6	13.1	12.3
Digestible met + cyst	9.9	9.7	9.1
Digestible threonine	8.8	8.6	8.1
Digestible tryptophan	3.0	2.8	2.6

[1] Salus Group, Composition per kg of product: Vit. A - 20,000,000 UI; Vit. D3 - 8,000,000 UI; Vit. E – 44,000 UI; Vit. K3 – 6,000 mg; Vit. B1 – 4,400 mg; Vit. B2 – 14,000 mg; Vit. B6 – 7,000 mg; Vit. B12 – 32,000 µg; Nicotinic acid – 90 g; Pantothenic acid – 32 g; Biotin - 240 mg; Folic acid – 3,200 mg; Selenium – 1,000 mg.

[2] Salus Group, Composition per kg of product: Manganese – 160,000 mg; Iron – 100,000 mg; Zinc – 140,000 mg; Copper – 20,000 mg; Iodine – 2,000 mg.

3.2.2. Salmonella Heidelberg and Experimental Challenge

The selected strain of *Salmonella* Heidelberg (SH) was resistant to nalidixic acid (Nal) and rifampicin (Rif), developed by means of successive cell culture media in brilliant green agar (BGA) containing Nal (100µg/mL of medium) and Rif (100µg/mL of medium), according to Andreatti et al. (1997), to facilitate subsequent bacterial enumeration. The inoculum used as a challenge consisted of the SH cultures grown in 250 mL brain-heart-infusion (BHI) broth and incubated at 41ºC for 18 hours. The number of colony-forming units (CFU) was determined by means of serial decimal dilutions in

phosphate-buffered saline (PBS) solution at pH 7.2. The bacteria count was performed by plating 0.1 mL of the culture (BHI) and serial decimal dilutions (PBS) in duplicate, on BGA (Nal/Rif). The plates were incubated at 41°C for 24 hours, and the CFU number of SH was determined.

Birds were confirmed to be *Salmonella*-free by fecal culture upon arrival and euthanasia of 10 chicks to subsequent tests for *Salmonella* spp. described by Mallinson and Snoeyenbos (1989). Aiming to promote an imbalance of the intestinal microbiome and immune system response, at the age of 3 and 15 days, experimental birds of each challenged group (4 replicates/treatment) were orally inoculated with SH strain by gavage at the dose of 1mL and concentration of 10^8CFU/mL/bird. Non-challenged chickens received 1mL of PBS in order to expose all birds to the same procedure. Sanitary management between treatment groups was periodized during the experiment to avoid cross-contamination.

3.2.3. Salmonella Quantification and Sample Collection

At 7 days post the first and second infection (dpi), i.e., 10 and 22 days of age, 2 birds per experimental unit (8 birds per treatment) from the challenged group were selected randomly, euthanized by cervical dislocation, and aseptically necropsied for ceca and liver collection. The organs samples were removed and immediately placed in sterile plastic tubes and frozen at -20°C. Pooled samples were macerated and diluted in PBS at the proportion of 1:10. Serial decimal dilutions were plated on BGA containing nalidixic acid (100µg/mL of medium) and rifampicin (100µg/mL of medium), incubated at 37°C for 24 hours, and characteristic SH colonies were counted. Results are expressed in CFU/mL (adapted from Desmidt et al., 1998). The number of CFU per mL of organ was converted to a $\log_{10}$ scale to interpret the results. Samples of the bedding were also collected 7 dpi and followed the same analytical procedures for SH counting.

For microbiological assessments, a pool of the ceca contents and ceca tissue were obtained during necropsies of the chickens for both challenged and non-challenged groups at 7 days post the first infection. The samples were carefully homogenized and frozen at −80°C for subsequent analysis of microbiota characterization and short chain fatty acids.

At 10 and 21 days of age, blood samples were collected from 4 broiler chickens per treatment, challenged and non-challenged group (total of 80 birds), from the brachial vein

into 4 mL clot accelerator and EDTA tubes with disposable needles and syringes. Whole blood samples were placed at room temperature for 2 hours and centrifuged at 1000 × g for 20 min. Supernatant was collected and transferred to micro tubes and stored at -80 ºC for further analyses. Plasma samples from the EDTA treated tubes were refrigerated to cell counting.

The chickens' growth performance was weekly measured by body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and viability.

3.2.4. Quantification of Short Chain Fatty Acids

To measure short-chain fatty acids (SCFA) in the cecum of broiler chickens, one gram of cecal content were diluted in Milli-Q water in the ratio of 1:1 (volume:mass) and then centrifuged for 60 minutes at 15,000 × g. Subsequently 400 μL of the supernatant were transferred to a chromatographic vial, adding 100 μL of metaphosphoric acid solution + formic acid at a 3:1 ratio, and 50 μL of 2-ethyl-butyric acid was included as an internal standard. The samples were injected into a gas chromatographer with a total running time of 16.5 min, divided into 3 heating cycles as follow: 80°C (1 min), 120°C (3 min), and 205°C (2 min). The concentration of SCFA (μM) was calculated based on an external calibration chromatogram curve of acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids.

3.2.5. Blood Biochemistry and Immune Response

Hematocrit (Ht) was determined by the microhematocrit method. Capillary tubes were filled with 2/3 blood sample, sealed, and centrifuged at 12,000×g for 5 minutes. The capillaries were read on a standardized scale and their values were obtained by percentage. Additionally, total plasma protein was determined by the refractometry method. Hemoglobin concentration was measured by the cyanmet hemoglobin method and the quantification of hemoglobin cyanide was obtained by spectrophotometry (Bioplus – 2000S, Barueri, São Paulo, Brazil), according to fabricant instructions.

The red blood cells (RBC), leukocytes and thrombocytes were determined optically, using the manual method with a Neubauer chamber. For dilution, 1.8mL of 0.9% saline solution and 200μL 0.1% toluidine blue solution (final solution at 0.01%) were added in 20μL of blood. Dilution was incubated for 10 minutes for cell staining and subsequently placed at the Neubauer chamber. Another 10 minutes were waited for the sedimentation

of the cells and reading was performed. RBC were counted in five central quadrants in 400x magnification under optical microscope, and the value was multiplied by the factor 5,050, in order to compensate the amount of dilution. Leukocytes and thrombocytes were counted in the four large lateral quadrants in 10x magnification, and the obtained value was multiplied by the factor 252.5 to obtain the number of cells per microliter. Furthermore, a classification count was also performed for lymphocytes, hemophiles, eosinophils, monocytes and basophils, calculating the percentage of each cell type in 100 counted cells (Onbasilar and Aksoy, 2005).

The hematimetric indices of mean cell volume (MCV) and the mean cell hemoglobin concentration (MCHC) were calculated using the following formulas:

Formula 1: MCV (fL) = Ht (%) / RBC (x106 / uL) * 10

Formula 2: MCHC (g/dL) = hemoglobin (g/dL) / Ht (%) * 100

The serum immunoglobulin concentration (IgA and IgY) of chickens was determined by means of a commercial enzyme-linked immunosorbent Assay (ELISA) kits (ECH0083 and ECH0032, Wuhan Fine Biotech Co., Ltd.).

3.2.6. Microbiota Characterization

Intestinal microbiota was evaluated by sequencing the 16S rRNA gene. Zymo Research's "ZR Fecal DNA MiniPrep®" commercial kit was used to extract DNA from the samples following the protocol recommended by the manufacturer.

The extracted DNA was quantified by spectrophotometry at 260nm. To assess the integrity of the extracted DNA, all samples were run by 1% agarose gel electrophoresis. A 460-base segment of the V3V4 hypervariable region of the 16S rRNA gene was amplified using the universal primers described by the methodology, and the following PCR conditions: 95°C for 3 min; 25 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s, followed by 72°C for 5 min. The metagenomic library was constructed from these amplicons using the commercial kit Illumina® "Nextera DNA Library Preparation Kit". The amplified samples were gathered in pools and sequenced on the Illumina® MiSeq sequencer (Degnan and Ochman, 2012). The reads obtained from the sequencer were analyzed on the QIIME 2 (Quantitative Insights Into Microbial Ecology) platform (Caporaso et al., 2010 and 2011), followed by a workflow of the removal of sequences from low quality, filtration, removal of chimeras, and taxonomic classification. The

sequences were classified into genera recognition of Amplicon Sequence Variants (ASVs), in this case, the homology between sequences when compared against a database. The last update (SILVA 138) of the year 2019 of the ribosomal RNA genes sequences database SILVA was used to compare the sequences (Yilmaz et al., 2014). To generate the classification of bacterial communities by identification of ASVs, 16,133 and 18,308 reads per sample were used for the analyses of 09/09/2021 and 21/09/2021, respectively, in order to normalize the data, avoiding sample comparison with different number of reads.

3.2.7. Statistical Analysis

The data were analyzed by ANOVA with procedures appropriate for a completely randomized design using the PROC MIXED of SAS 9.4 (2013). The homogeneity of variances was assessed by Levene's test and data normality was verified by Shapiro-Wilk test. The bacterial enumeration data were transformed to $\log_{10}$ and reported as means with their standard errors. For performance data, the pen was considered as the experimental unit, and for bacterial count, SCFA concentration, blood biochemical, and microbiota parameters the single birds from each pen were considered the experimental unit. The means for all treatments were tested using contrast analysis and main results were presented to assess the effects of *Salmonella* infection (contrast 1 - challenged vs. non-challenged); the effects of antibiotic (contrast 2 - NC vs. PC); the effects of yeast-based additives (contrast 3 - NC vs. YCW; contrast 4 - NC vs. YCW+N; contrast 5 - NC vs. ACW+PB); and the effect of yeast-based additives replacing the antibiotic (contrast 6 - PC vs. YCW; contrast). All contrasts were performed for the challenged group, except contrast 1.

Microbiological diversity of the cecum bacterial communities was compared between the groups in the alpha diversity analyses through Wilcoxon test (Wilcoxon, 1992), accepting statistically results below 0.05. Statistical analyses for beta diversity were performed through PERMANOVA present in the Qiime2 pipeline, using number of 10,000 permutations. All figures and analyses were performed using R version 4.2.0 and R Studio software (2022). Alpha diversity analyses were carried out using the packages phyloseq (McMurdie and Holmes, 2013), vegan (Oksanen et al., 2007) and microbiome (Lahti and Shetty, 2018). The Wilcoxon rank sum test was applied to determine significant

differences between taxa relative abundance of all experimental groups. Statistical significance for all variables were considered at 5% of significance (Littell et al., 2002).

3.3. RESULTS

3.3.1. Performance

Productive performance of the birds was affected by the *Salmonella* challenge ($p<0.05$) along the experiment (Table 2 and 3). The main effects were seen for LW and WG of the broiler chickens from 21 to 28 days of age, in which the non-challenged group showed the best results (approximately 20 to 60 grams heavier). Similarly, the FCR of challenged birds was affected from 28 to 35 days of age on average 0.05 points higher than non-challenged group. Regarding the viability, SH infection had a significant impact during the entire life cycle of the birds, increasing by average approximately 3% mortality of the challenged group ($p<0.05$).

Table 2. Productive performance of broiler chickens supplemented with yeast-based additives and challenged with *Salmonella* Heidelberg at 14 and 21 days of age

	Treatments [1]	LW (g)	WG (g)	FI (g)	FCR	Viab. (%)	LW (g)	WG (g)	FI (g)	FCR	Viab. (%)
		1-14 days					1-21 days				
CHALLENGED	NC (1)	534	497	541	1.09	90.0	1104	1081	1220	1.13	89.0
	PC (2)	541	504	552	1.10	96.0	1107	1070	1246	1.16	96.0
	YCW (3)	542	506	518	1.03	95.0	1098	1087	1199	1.10	92.0
	YCW+N (4)	547	510	536	1.05	82.0	1125	1088	1224	1.13	92.0
	ACW+PB (5)	538	499	549	1.10	91.0	1111	1072	1246	1.16	91.0
NON-CHALLENGED	NC (6)	554	517	551	1.06	94.0	1142	1096	1252	1.14	94.0
	PC (7)	542	505	539	1.07	96.0	1111	1086	1233	1.14	94.0
	YCW (8)	549	512	556	1.09	96.0	1142	1105	1257	1.14	96.0
	YCW+N (9)	534	497	534	1.08	95.0	1122	1085	1233	1.14	95.0
	ACW+PB (10)	556	519	552	1.06	96.0	1149	1112	1259	1.13	96.0
	SEM	2.04	2.09	3.20	0.01	0.62	4.29	3.27	5.42	0.004	0.71
		Probability level of the contrasts									
CONSTRASTS	1 (1_5 vs. 6_10)	0.09	0.08	0.26	0.88	0.0359	0.0032	0.0062	0.07	0.47	0.0391
	2 (1 vs. 2)	0.41	0.42	0.46	0.93	0.0307	0.84	0.37	0.31	0.24	0.0318
	3 (1 vs. 3)	0.30	0.31	0.10	0.0264	0.07	0.73	0.69	0.40	0.08	0.34
	4 (1 vs. 4)	0.12	0.13	0.72	0.18	0.46	0.22	0.62	0.87	0.24	0.34
	5 (1 vs. 5)	0.63	0.77	0.60	0.73	0.71	0.68	0.45	0.30	0.30	0.52
	6 (2 vs. 3)	0.83	0.83	0.0211	0.0217	0.71	0.58	0.22	0.0515	0.0054	0.21

[1] NC=negative control; PC=positive control; YCW=NC + 500 mg/kg of yeast cell wall; YCW+N=NC + 500 mg/kg of yeast cell wall and 250 mg/kg of free nucleotides; and ACW+PB=NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic; LW= live weight; BW= body weight; FI= feed intake; FCR= feed conversion ratio; Viab. = viability; SEM=standard error of the mean.

Table 3. Productive performance of broiler chickens supplemented with yeast-based additives and challenged with *Salmonella* Heidelberg at 28 and 35 days of age

	Treatments [1]	LW (g)	WG (g)	FI (g)	FCR	Viab. (%)	LW (g)	WG (g)	FI (g)	FCR	Viab. (%)
		1-28 days					1-35 days				
CHALLENGED	NC (1)	1763	1726	2268	1.31	85.0	2520	2483	3567	1.44	85.0
	PC (2)	1764	1727	2229	1.29	92.0	2621	2584	3503	1.36	92.0
	YCW (3)	1727	1690	2240	1.33	87.0	2527	2490	3525	1.42	87.0
	YCW+N (4)	1772	1734	2221	1.28	87.0	2640	2603	3514	1.35	87.0
	ACW+PB (5)	1793	1754	2233	1.27	87.0	2641	2603	3525	1.35	87.0
NON-CHALLENGED	NC (6)	1790	1753	2226	1.27	90.0	2667	2630	3488	1.33	90.0
	PC (7)	1811	1767	2202	1.25	90.0	2649	2605	3488	1.34	90.0
	YCW (8)	1815	1767	2148	1.22	92.0	2626	2580	3396	1.32	92.0
	YCW+N (9)	1825	1788	2237	1.25	90.0	2638	2600	3521	1.36	90.0
	ACW+PB (10)	1877	1840	2267	1.23	92.0	2689	2651	3573	1.35	91.0
	SEM	11.13	11.28	10.27	0.01	0.70	17.06	17.07	15.54	0.01	0.70
		Probability level of the contrasts									
CONSTRASTS	1 (1_5 vs. 6_10)	0.0206	0.0342	0.29	0.0139	0.0241	0.14	0.16	0.28	0.0035	0.0354
	2 (1 vs. 2)	0.99	0.99	0.41	1.00	0.0270	0.15	0.20	0.37	0.0043	0.0286
	3 (1 vs. 3)	0.47	0.48	0.55	0.21	0.51	0.98	0.92	0.55	0.39	0.52
	4 (1 vs. 4)	0.87	0.87	0.32	0.73	0.51	0.13	0.15	0.46	0.0012	0.52
	5 (1 vs. 5)	0.55	0.59	0.46	0.55	0.51	0.13	0.13	0.56	0.0021	0.52
	6 (2 vs. 3)	0.42	0.43	0.82	0.21	0.11	0.16	0.20	0.76	0.0338	0.11

[1] NC=negative control; PC=positive control; YCW=NC + 500 mg/kg of yeast cell wall; YCW+N=NC + 500 mg/kg of yeast cell wall and 250 mg/kg of free nucleotides; and ACW+PB=NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic; LW= live weight; BW= body weight; FI= feed intake; FCR= feed conversion ratio; Viab. = viability; SEM=standard error of the mean.

Evaluating each treatment, a better performance of birds supplemented yeast-based additives is suggested at 14 to 21 days of age, in which yeast cell wall (YCW) reduced significantly the FI ($p=0.02$, 14d; $p=0.05$, 21d), improving FCR ($p=0.02$, 14d; $p=0.01$, 21d) compared to PC. In addition, better FCR was also observed to YCW compared to NC ($p=0.03$) treatment at 14d of age. Throughout the experiment, statistical difference was observed for FCR between treatments at 35 days of age ($p<0.05$), however no difference was observed for LW ($p>0.05$). Chickens supplemented with yeast-based additives (YCW+N and ACW+PB) improved feed conversion compared to NC ($p<0.03$) and showed similar results to PC, indicating a good alternative to replace this antimicrobial growth promoter. Unexpectedly, YCW showed a significantly reduction in growth at 35 days of age which similar results as NC. Viability between challenged treatments had significant difference only for PC compared to NC along the entire period ($p<0.05$).

3.3.2. Salmonella Heidelberg Infection

Salmonella Heidelberg counts in the cecum, liver, and litter are presented in Table 4. Overall, *Salmonella* was detected predominantly in the cecum at 10 days of age; however, no difference for bacteria count between treatments was observed throughout the experiment ($p>0.05$). Reduced number of SH was found in the liver of chickens supplemented with yeast-based prebiotics when compared to NC ($p<0.05$), in which the concentration of *Salmonella* was lower for ACW+PB ($P<0.03$) at 10 days of age and YCW ($p<0.02$), YCW+N ($p<0.01$) and ACW+PB ($p<0.02$) at 22 days of age. For litter contamination, higher SH count was observed for NC compared to PC and YCW+N at day 10 ($p=0.0016$). Additionally, PC showed better results compared to YCW ($p=0.0135$). At day 22, no differences were observed between contrasts.

Table 4. Mean log_{10} of the colony-forming units per gram of cecum and liver content, and litter of broiler chickens inoculated orally with 5×10^8 CFU/mL of *Salmonella* Heidelberg on the 3th and 15th day of life

	Treatments [1] (log_{10} CFU/g)	CECUM	LIVER	LITTER	CECUM	LIVER	LITTER
		10 days of age			22 days of age		
CHALLENGED	NC (1)	5.25	3.99	3.83 b	2.50	2.50	4.47
	PC (2)	4.46	2.37	1.00 a	1.73	1.51	3.52
	YCW (3)	4.09	2.39	3.06 ab	1.74	1.25	2.65
	YCW+N (4)	5.52	2.88	1.00 a	1.58	1.00	4.09
	ACW+PB (5)	5.02	1.57	2.57 ab	3.10	1.25	2.85
	SEM	0.32	0.35	0.35	0.21	0.19	0.37
	Probability level of the contrasts						
CONSTRASTS	2 (1 vs. 2)	0.47	0.14	0.0016	0.19	0.07	0.43
	3 (1 vs. 3)	0.29	0.15	0.31	0.19	0.0255	0.14
	4 (1 vs. 4)	0.81	0.31	0.0016	0.12	0.0094	0.75
	5 (1 vs. 5)	0.83	0.0350	0.11	0.30	0.0255	0.19
	6 (2 vs. 3)	0.74	0.99	0.0135	0.98	0.61	0.47

[1] NC=negative control; PC=positive control; YCW=NC + 500 mg/kg of yeast cell wall; YCW+N=NC + 500 mg/kg of yeast cell wall and 250 mg/kg of free nucleotides; and ACW+PB=NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic; SEM=Standard error of the mean. CV=Coefficient of variation.

3.3.3. Short Chain Fatty Acids

Fatty acids and total SCFA in the cecal digesta (μM/L) are shown in Table 5. It was observed that the challenged group showed increased values compared to non-challenged group. The significant effects were observed for acetate (p=0.0013), butyrate (p=0.0068), and total SCFA concentration (p=0.0012) in the ceca. Additionally, evaluating the challenged group, there was statistical difference between treatments contrasted. Overall, NC showed higher value of acetate and total lip content compared to YCW+N and ACW+PB ($p<0.04$), while propionate concentration was lower compared to all treatments ($p<0.01$).

Table 5. Concentration of short-chain fatty acids in the cecal content of broiler chickens challenged with *Salmonella* Heidelberg

	Treatments [1]	ACETIC	PROPIONIC	ISOBUTYRIC	BUTYRIC	ISOVALERIC	VALERIC	TOTAL
CHALLENGED	NC (1)	31.10	0.09	0.02	1.81	0.10	0.13	39.63
	PC (2)	32.32	0.31	0.02	1.46	0.05	0.34	37.69
	YCW (3)	29.31	0.13	0.00	2.17	0.02	0.82	31.15
	YCW+N (4)	28.01	0.23	0.00	2.02	0.01	0.41	27.50
	ACW+PB (5)	28.09	0.72	0.00	2.17	0.03	0.18	27.65
NON-CHALLENGED	NC (6)	28.75	0.01	0.01	1.50	0.01	0.19	30.19
	PC (7)	29.63	0.35	0.02	1.48	0.06	0.38	31.91
	YCW (8)	18.77	0.08	0.00	0.99	0.04	0.15	20.03
	YCW+N (9)	27.76	0.23	0.03	1.50	0.18	0.12	22.74
	ACW+PB (10)	15.75	0.27	0.00	0.95	0.00	0.31	17.01
	SEM	1.40	0.05	0.00	0.14	0.02	0.06	1.54
	Probability level of the contrasts							
CONSTRASTS	1 (1_5 vs. 6_10)	0.0013	0.08	0.70	0.0068	0.71	0.21	0.0012
	2 (1 vs. 2)	0.91	0.0034	0.89	0.44	0.50	0.42	0.72
	3 (1 vs. 3)	0.10	0.0006	0.36	0.67	0.37	0.0150	0.13
	4 (1 vs. 4)	0.0349	0.0013	0.32	0.41	0.30	0.27	0.0330
	5 (1 vs. 5)	0.0368	0.0002	0.32	0.65	0.38	0.86	0.0349
	6 (2 vs. 3)	0.12	0.38	0.43	0.26	0.79	0.08	0.23

[1] NC=negative control; PC=positive control; YCW=NC + 500 mg/kg of yeast cell wall; YCW+N=NC + 500 mg/kg of yeast cell wall and 250 mg/kg of free nucleotides; and ACW+PB=NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic; SEM=Standard error of the mean.

3.3.4. Immune System

The results of immune system parameters are presented in Table 6 and 7. At day 10, there was a difference between the challenged and non-challenged groups for MCV ($p=0.0232$) and PT ($p=0.0330$), and a tendency for MCHC ($p=0.0786$). Contrasting each treatment, it was possible to observe that the YCW challenged group had greater ($p<0.0270$) lymphocytes count than NC and PC. Moreover, there was a tendency of YCW challenged group to present lower ($p<0.065$) heterophils than NC and PC.

At day 22, it was not observed significant difference of the treatments on blood parameters that could indicate immune system chances. Tendencies were observed for thrombocytes ($p=0.0581$) between challenged and non-challenged birds, eosinophils ($p=0.0959$) between NC and YCW+N, and basophils ($p=0.0735$) for NC compared to PC and ACW+PB treatments.

Regarding immunoglobulins (Table 8), on day 10, the level of IgA was significantly increased following YCW diet compared with the NC ($p=0.0220$). Serum IgY levels had a tendency to increase for challenged birds compared to non-challenged birds ($p=0.0864$) and for PC compared to YCM ($P=0.0879$). On day 22, serum IgA ($p<0.0480$) and IgY ($p=0.0447$) levels were increased for broiler chickens fed YCW as compared with broilers fed NC diet. In addition, challenged group presented higher IgY levels ($p=0.0121$) than non-challenged group. No significant difference was recorded between other contrasts.

Table 6. Mean of blood biochemical parameters at day 10 of age of broiler chickens challenged and non-challenged with *Salmonella* Heidelberg

	Treatments [1]	RBC	HBG	HCT	MCV	MCH	TP	TC	WBC	H	LYM	EOS	BASO	MONO
	1st measurement (10 days of age)													
CHALLENGED	NC (1)	1.56	6.70	30.75	197.60	21.83	3.00	70050	18072	29.75	61.00	2.50	2.25	4.50
CHALLENGED	PC (2)	1.61	6.95	32.25	200.65	21.53	3.00	75182	13167	29.00	59.75	1.25	3.00	5.00
CHALLENGED	YCW (3)	1.51	6.70	30.75	203.80	21.80	3.10	83847	16901	17.25	74.50	2.00	1.00	5.25
CHALLENGED	YCW+N (4)	1.49	6.78	30.25	202.85	22.45	2.65	86066	14429	22.25	66.75	3.75	1.50	4.50
CHALLENGED	ACW+PB (5)	1.59	6.73	31.25	197.05	21.55	3.05	61098	16925	26.75	58.25	4.25	3.00	7.75
NON-CHALLENGED	NC (6)	1.67	6.93	30.75	184.73	22.60	2.80	58165	13167	22.75	65.00	1.00	3.50	7.75
NON-CHALLENGED	PC (7)	1.53	6.70	29.25	191.80	22.93	2.55	70647	12931	32.50	64.50	2.00	2.75	3.25
NON-CHALLENGED	YCW (8)	1.49	6.80	30.25	202.55	22.55	2.65	75861	16933	31.75	58.75	2.00	2.25	4.50
NON-CHALLENGED	YCW+N (9)	1.58	6.83	30.25	193.08	22.60	2.75	73505	13987	31.00	56.75	3.50	1.75	7.00
NON-CHALLENGED	ACW+PB (10)	1.48	6.55	29.75	200.75	22.13	2.75	69967	15252	22.00	68.25	0.50	1.50	5.75
	SEM	0.02	0.06	0.34	1.40	0.19	0.05	3318.22	738.23	1.43	1.41	0.35	0.30	0.47
	Probability level of the contrasts													
CONSTRASTS	1 (1_5 vs. 6_10)	0.97	0.94	0.18	0.0232	0.08	0.0330	0.42	0.32	0.28	0.59	0.17	0.76	0.79
CONSTRASTS	2 (1 vs. 2)	0.61	0.44	0.36	0.58	0.74	1.00	0.74	0.23	0.90	0.83	0.42	0.61	0.82
CONSTRASTS	3 (1 vs. 3)	0.63	1.00	1.00	0.26	0.98	0.70	0.38	0.74	0.0503	0.0269	0.75	0.40	0.73
CONSTRASTS	4 (1 vs. 4)	0.49	0.81	0.76	0.34	0.49	0.19	0.31	0.30	0.23	0.33	0.42	0.61	1.00
CONSTRASTS	5 (1 vs. 5)	0.74	0.94	0.76	0.92	0.76	0.85	0.57	0.74	0.63	0.64	0.26	0.61	0.14
CONSTRASTS	6 (2 vs. 3)	0.32	0.44	0.36	0.57	0.76	0.70	0.58	0.38	0.06	0.0164	0.63	0.18	0.91

[1] NC=negative control; PC=positive control; YCW=NC + 500 mg/kg of yeast cell wall; YCW+N=NC + 500 mg/kg of yeast cell wall and 250 mg/kg of free nucleotides; and ACW+PB=NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic; SEM=Standard error of the mean. RBC=red blood cells, HBG=hemoglobin, HCT=hematocrit, MCV=mean corpuscular volume, MCH= mean corpuscular hemoglobin, TP=total protein, TC=thrombocytes, WBC=white blood cells, H=heterophils, LYM=lymphocytes, EOS=eosinophils, BASO=basophils, MONO=monocytes.

Table 7. Mean of blood biochemical parameters at day 21 of age of broiler chickens challenged and non-challenged with *Salmonella* Heidelberg

	Treatments	RBC	HBG	HCT	MCV	MCH	TP	TC	WBC	H	LYM	EOS	BASO	MONO
	2nd measurement (21 days of age)													
CHALLENGED	NC (1)	2.24	7.05	30.50	137.85	23.18	2.55	31409	14168	31.75	53.00	7.25	5.25	2.50
	PC (2)	2.16	7.18	30.25	141.35	23.75	2.60	32381	15468	33.75	55.75	4.50	2.75	3.25
	YCW (3)	2.15	6.93	30.50	143.08	22.73	2.70	34441	16690	24.00	61.25	7.50	4.25	3.00
	YCW+N (4)	2.26	6.75	29.75	132.38	22.70	2.35	32656	13678	29.00	44.50	21.00	3.25	2.25
	ACW+PB (5)	2.11	6.80	29.75	142.30	22.85	2.70	33366	17260	22.25	56.00	13.75	2.75	3.50
NON- CHALLENGED	NC (6)	2.00	7.05	30.00	142.67	23.73	2.70	40979	15203	27.75	60.00	6.00	2.75	3.50
	PC (7)	2.10	6.70	28.25	141.33	23.70	2.60	38913	17395	26.25	52.50	14.75	4.25	2.25
	YCW (8)	2.10	7.05	30.00	142.68	23.55	2.75	32675	19845	25.00	45.75	24.00	3.00	2.25
	YCW+N (9)	2.25	7.45	31.50	140.33	23.63	3.10	37359	19201	32.50	45.75	16.50	1.75	3.50
	ACW+PB (10)	2.14	6.90	27.75	139.33	23.25	2.40	36394	17389	25.25	53.25	16.00	2.50	3.00
	SEM	0.03	0.07	0.24	1.71	0.19	0.05	1096.09	728.28	1.30	1.89	1.86	0.31	0.32
	Probability level of the contrasts													
CONSTRASTS	1 (1_5 vs. 6_10)	0.28	0.45	0.61	0.62	0.21	0.16	0.0581	0.13	0.76	0.49	0.20	0.19	1.00
	2 (1 vs. 2)	0.50	0.68	0.82	0.68	0.54	0.80	0.85	0.70	0.74	0.75	0.73	0.07	0.64
	3 (1 vs. 3)	0.48	0.68	1.00	0.54	0.63	0.46	0.55	0.46	0.20	0.34	0.98	0.46	0.75
	4 (1 vs. 4)	0.87	0.33	0.49	0.52	0.61	0.33	0.81	0.89	0.65	0.33	0.10	0.15	0.87
	5 (1 vs. 5)	0.24	0.42	0.49	0.60	0.73	0.46	0.70	0.37	0.12	0.73	0.42	0.07	0.53
	6 (2 vs. 3)	0.98	0.42	0.82	0.84	0.27	0.62	0.68	0.72	0.11	0.52	0.71	0.27	0.87

[1] NC=negative control; PC=positive control; YCW=NC + 500 mg/kg of yeast cell wall; YCW+N=NC + 500 mg/kg of yeast cell wall and 250 mg/kg of free nucleotides; and ACW+PB=NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic; SEM=Standard error of the mean. RBC=red blood cells, HBG=hemoglobin, HCT=hematocrit, MCV=mean corpuscular volume, MCH= mean corpuscular hemoglobin, TP=total protein, TC=thrombocytes, WBC=white blood cells, H=heterophils, LYM=lymphocytes, EOS=eosinophils, BASO=basophils, MONO=monocytes.

Table 8. Mean of IgY and IgA blood levels at day 10 and 21 of age of broiler chickens on day 10 and 21 of age

	Treatments [1]	IgY	IgA	IgY	IgA
		10 days of age		21 days of age	
CHALLENGED	NC (1)	6.38	5.45	3.86	6.50
	PC (2)	7.64	6.51	6.19	6.88
	YCW (3)	4.46	7.78	8.00	8.10
	YCW+N (4)	5.06	6.26	6.84	7.23
	ACW+PB (5)	5.37	6.53	5.57	7.26
NON-CHALLENGED	NC (6)	4.31	6.78	5.41	6.99
	PC (7)	4.42	8.12	2.31	7.94
	YCW (8)	1.92	5.82	4.41	7.58
	YCW+N (9)	5.17	6.82	3.22	8.12
	ACW+PB (10)	5.98	7.50	3.27	8.17
	SEM	0.42	0.23	0.48	0.17
	Probability level of the contrasts				
CONSTRASTS	1 (1_5 vs. 6_10)	0.09	0.25	0.0121	0.11
	2 (1 vs. 2)	0.49	0.28	0.25	0.62
	3 (1 vs. 3)	0.30	0.0220	0.0449	0.0447
	4 (1 vs. 4)	0.47	0.41	0.14	0.34
	5 (1 vs. 5)	0.22	0.98	0.76	0.62
	6 (2 vs. 3)	0.09	0.20	0.37	0.12

[1] NC=negative control; PC=positive control; YCW=NC + 500 mg/kg of yeast cell wall; YCW+N=NC + 500 mg/kg of yeast cell wall and 250 mg/kg of free nucleotides; and ACW+PB=NC + 500 mg/kg of autolyzed yeast cell associated with postbiotic; SEM=Standard error of the mean.

3.3.5. Microbiota

Bacterial community in the ileal digesta of broiler chickens on day 10 was estimated by Chao and Shannon indexes of richness and diversity. Assessing the taxonomic composition data, which measures microbial population within individual samples, the most predominant genus in the intestine of challenged and non-challenged birds were: *Novosphingobium*, *Lactobacillus*, and *Romboutsia* (Figure 1). The bacteria taxonomic composition of challenged birds showed a numerically higher diversity of identified species (more 12,4%) compared to non-challenged birds, which indicates that the *Salmonella* inoculation affected the microbiota community of the chickens. Based on Shannon's diversity ($p=0.249$), challenged and non-challenged birds did not show significant difference for bacterial communities between groups (Figure 2a). Regarding the richness observed in the microbiota

of these two groups we were able to evaluate that the challenged group also had a higher species richness than the non-challenged group (Figure 2b). When Chao1 was applied to this data set, the species richness was non-significant following the same behavior as the diversity index.

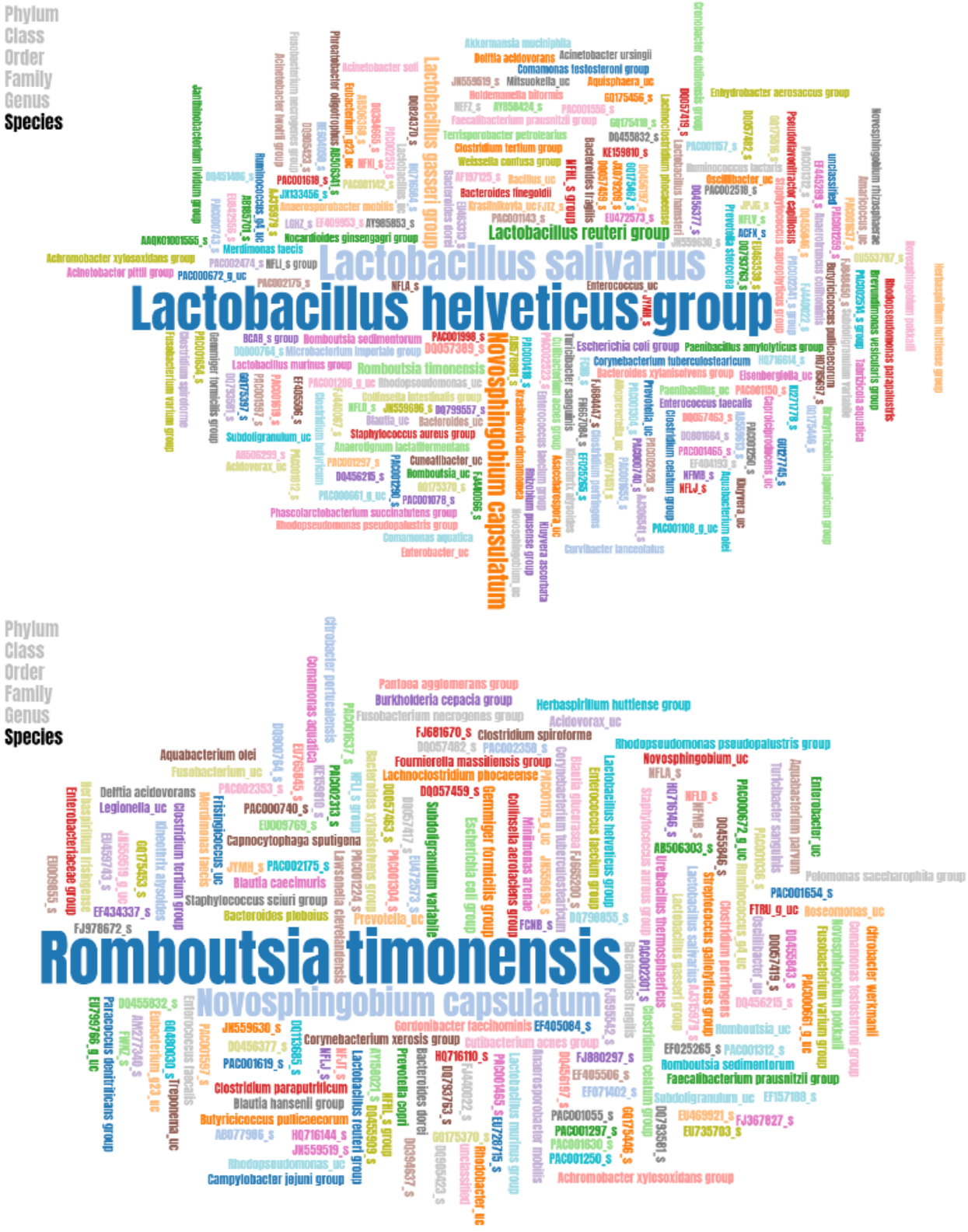


Figure 1. Non-challenge and challenge word clouds (from up to bellow) of microbiota taxonomic composition of birds at 10 days of age

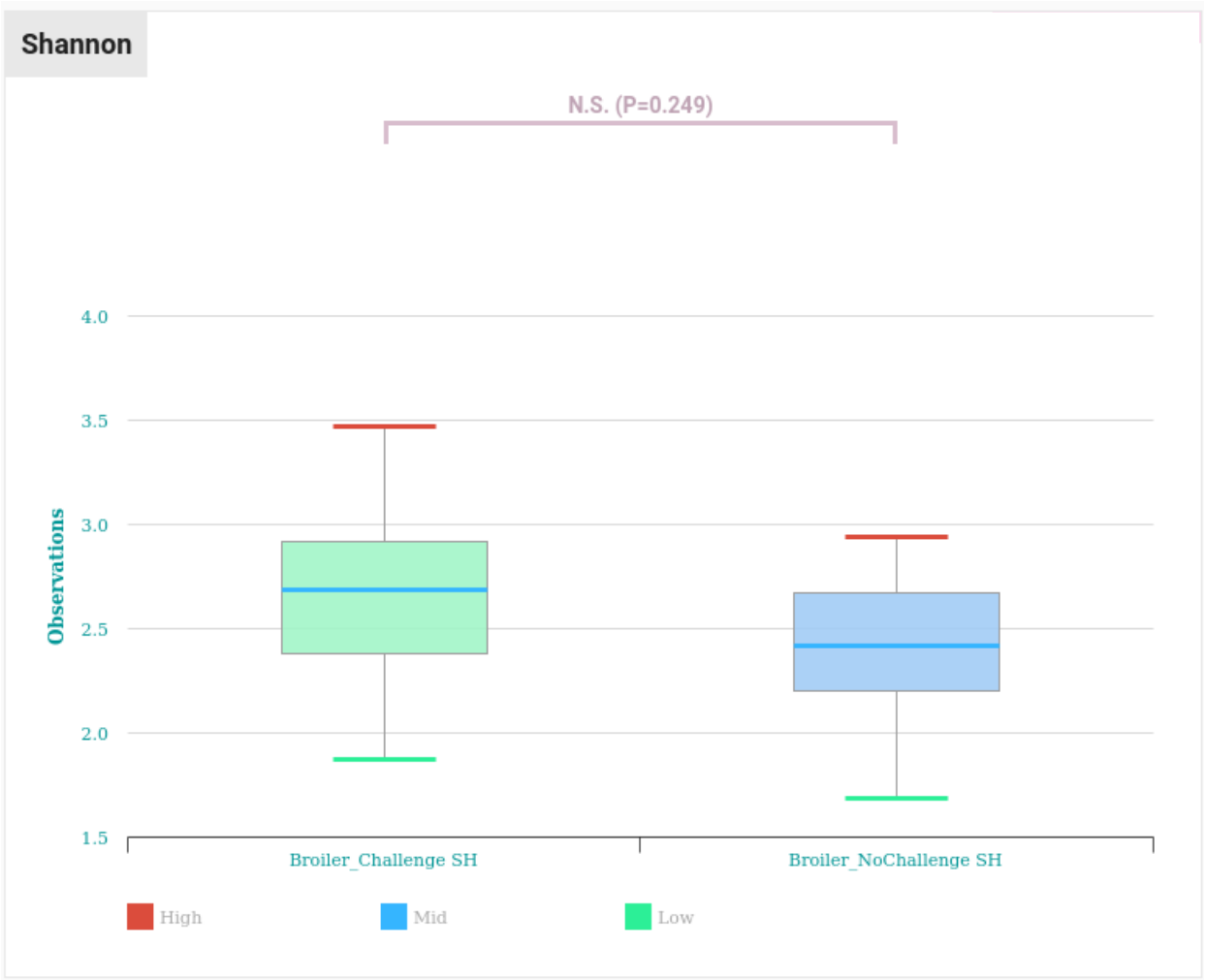


Figure 2a. Alpha diversity measured through the Shannon diversity index. Box plots correspond to the challenged group (green) and non-challenged group (blue) of birds at 10 days of age

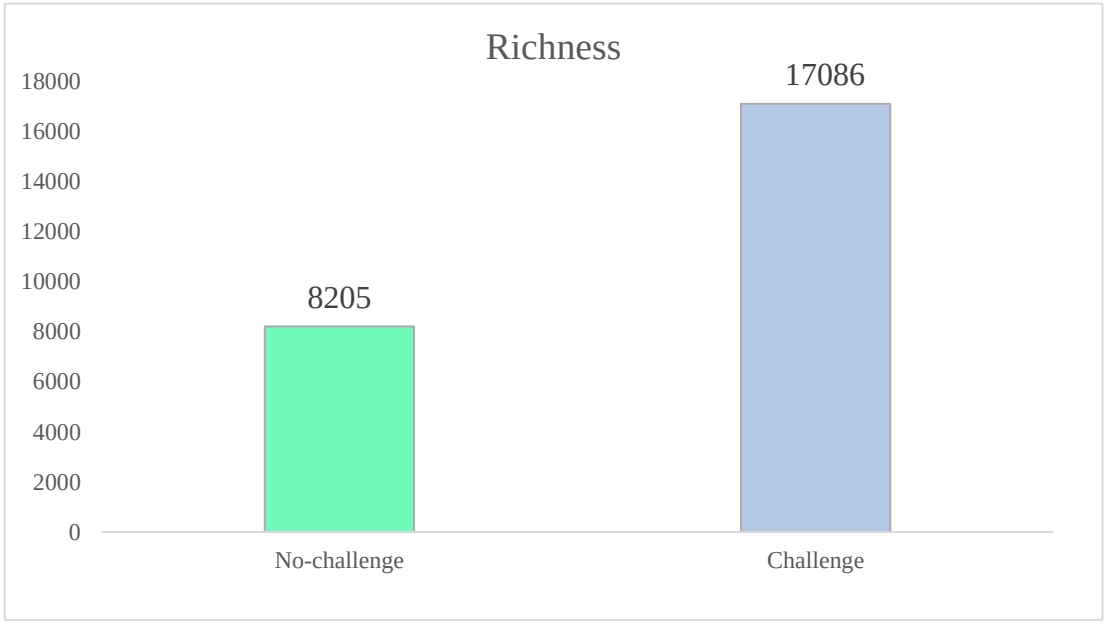
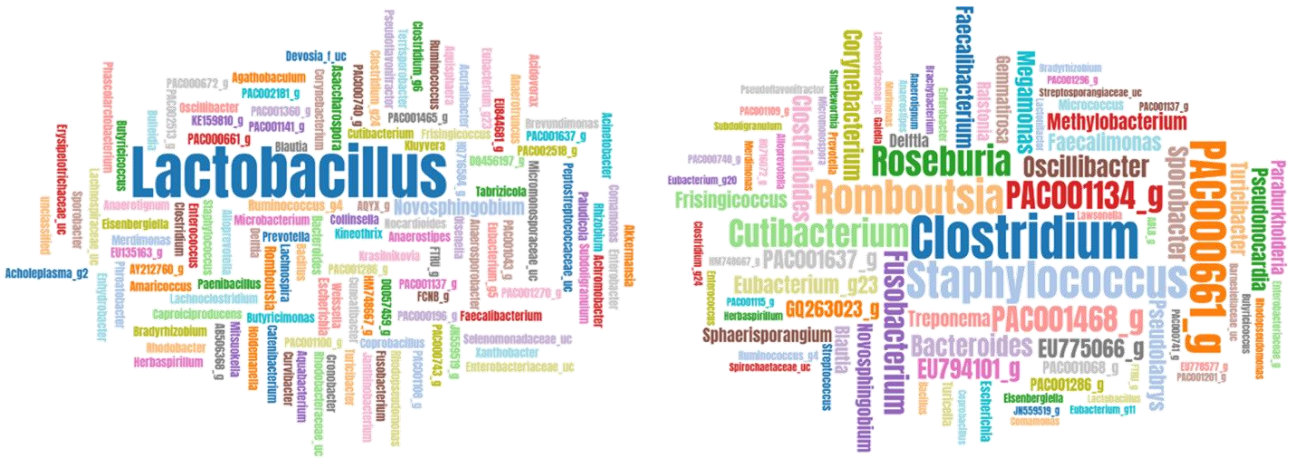


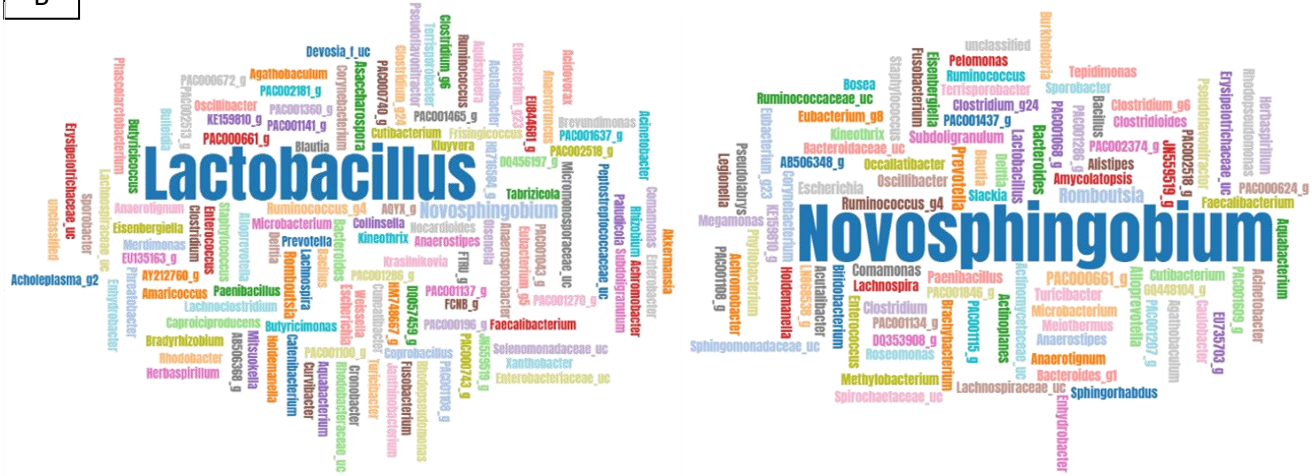
Figure 2b. Observed richness of non-challenge and challenge groups

Evaluating the alpha diversity between challenged chickens without supplementation and fed yeast-based additives or antimicrobial (Figure 3), we can infer that by taxonomic composition *Lactobacillus* is the most predominant genus in NC treatment while yeast-based additives had higher presence of *Novosphingobium*. Besides these genera, we can observe, there is a relevant presence of the genus *Clostridium* and *Staphylococcus* in the microbiota of PC birds, *Turicibacter* for birds of the YCW and YCW+N, and *Faecalibacterium* for birds of ACW+PB. Overall, the supplementation strategy did not influence relative abundance of bacteria, diversity, and richness of the microbial community through Chao1 and Shannon's indexes ($p>0.05$). The three most abundant genera of bacteria presented in the cecum of challenged birds from each treatment are shown in Table 9.

A



B



C

D

Figure 3. Comparison of microbiota taxonomic composition between challenged birds at 10 days of age. The assigned letters correspond to the following: (A) NC vs. PC; (B) NC vs. YCW; (C) NC vs. YCW+N; (D) NC vs. ACW+PB

Table 9. The three most prevalent bacterial genera observed in the cecum of challenged broiler chickens

Treatments	1st	2nd	3th
NC	*Lactobacillus* (69.7%)	*Novosphingobium* (10.3%)	*Romboutsia* (3.3%)
PC	*Clostridium* (7.4%)	*Staphylococcus* (6.3%)	*Romboutsia* (5.6%)
YCW	*Novosphingobium* (62.8%)	*Romboutsia* (6.2%)	*Prevotella* (3.4%)
YCW+N	*Novosphingobium* (43.8%)	*Lactobacillus* (6.5%)	*Romboutsia* (5.2%)
ACW+PB	*Novosphingobium* (45.9%)	*Lactobacillus* (22.1%)	*Faecalibacterium* (4.6%)

Median relative abundances (%) were used to determine the rank of each taxon

Comparing specifically the microbiota of challenged birds from NC and YCW, analysis of the beta diversity determined by the Bray–Curtis dissimilarity revealed that the bacterial microbiota of the yeast-based prebiotic group clustered apart from that of a NC group, demonstrating that the microbial taxonomic diversity is different for both groups (Figure 4).

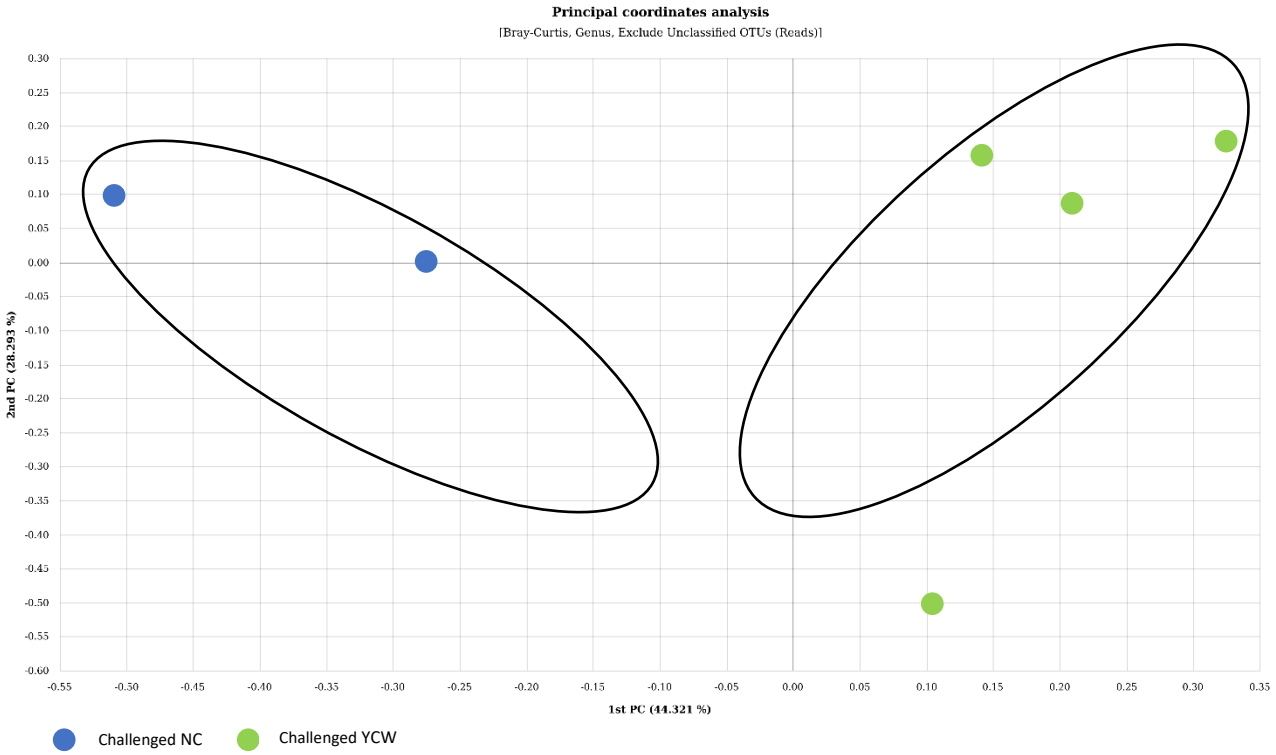


Figure 4. Beta diversity measured by Bray-Curtis distance based between community diversity analysis for challenged birds between NC (blue points) and YCW (green points) treatments

3.4. DISCUSSION

The present study has been conducted in a similar experimental protocol to that of some other reports (Shao et al., 2016; Fazelnia et al., 2020; Cirilo et al., 2023). The supplementation of yeast-based additives was efficient to support the growth of the birds, showing similar results as the antibiotic. Additionally, it was observed better results for FI and FCR at 14, 21 and 35 days of age compared to the antibiotic supplementation. According to Fazelnia et al. (2020), the supplementation of synbiotics and probiotics can reduce negative effects of *Salmonella* infection on growth and immune responses of broiler chickens. Similarly, dietary *Saccharomyces cerevisiae* supplementation stimulate performance of chickens which may be associated to anti-inflammatory responses (Lin et al., 2023).

Indeed, our findings showed that *Salmonella* Heidelberg infection was able to impair growth of the broiler chickens during the experimental period. The decrease in performance in challenged birds could be attributed to the pathogen effect on digestion due to disruption of the intestinal mucosa and energy requirements for immune system response boosting against the pathogens (Shao et al., 2016). However, it is likely that functional components of yeasts have the capacity to reduce the colonization

of bacteria in the intestine, reducing the competition of pathogens and host cells for nutrients (Fathima et al., 2022).

Mannanoligosaccharides and β-glucans are the main components of yeast cells, responsible to modulate immune functions and prevent or reduce enteric pathogens in the animal intestine (Fathima et al., 2023). Due to the high efficiency of mannanoligosaccharides to bind to pathogen receptors (Spring et al., 2000) and β-glucans to modulate immune system, activating defense cells (Rajapakse et al., 2010), the supplementation of yeast-based additives are known to help the organism self-defense and consequently the production performance of broiler chickens (Pascual et al., 2020; Fathima et al., 2023). Kiros et al. (2019) did not find difference in performance between challenged and non-challenged chickens, once *Salmonella* Heidelberg was not considered a critical pathogen to birds' health.

The infection of *Salmonella spp*. in the digestive tract of broilers chickens may be influenced by age, immune system status, shape particles, and type of diet, material used as litter in the broiler facility, and intestinal microbiota diversity, which in turn may be modulated by feed additives, such as prebiotics (Jung et al., 2008; Vellano et al. 2019). In the current study, unexpectedly, there were no difference among treatments for both collection (10 and 22-d old) in *Salmonella* Heidelberg count in cecum and liver. The highest concentrations of bacteria in the broiler gastrointestinal tract are in the cecum (Danzeisen et al., 2011), which is the main reservoir of *Salmonella* spp. in birds (Fanelli et al., 1971; Dunkley et al., 2007; Qu et al., 2008). *Saccharomyces cerevisiae* is a rich oligosaccharide substance composed by mannanoligosaccharides (MOS), which is expected to reduce pathogens that utilize mannose-specific type 1 fimbriae, such as *Salmonella* (Lee et al., 2016; Park et al., 2017). Spring et al. (2000) and Stanley et al. (2016) observed a reduction in *Salmonella* ceca population at day 10 and 21, respectively, in birds fed a diet added prebiotic.

According to the gastrointestinal tract of birds, the first anatomical portion that ingested food and microorganisms stop is the crop, which is used for fermentation, hydrolysis of starch, food storage, and has a relatively acid environment (pH ≅ 4.5) (Micciche et al., 2018). This characteristic can be a barrier against pathogens. Despite the absence of difference among treatments for both collection (10 and 22-d old) in *Salmonella* Heidelberg count in cecum and liver, a numerical decrease in the count of both organs were observed according to the collection age. Oro et al. (2023) determining *Salmonella* Heidelberg count in cecum of broilers, also observed a reduction according to the broiler age, even without any additive added to the diet.

The first gender of bacteria detected to colonize the cecum within the first hour of life are the streptococci and enterobacteria, being distributed throughout the gastrointestinal tract of birds within 24 h (Aruwa et al., 2021). At 3-d old, it is already possible to find other bacteria gender such as lactobacilli, streptococci, enterococci, and coliforms in other portions of the gastrointestinal tract (Coloe et al., 1984; Aruwa et al., 2021). Some of these bacteria, such as *Lactobacillus spp*., beside produce volatile fatty acid, which make the environmental not favorable for *Salmonella* developing due to its acidity, can also produce substances with protein origin, such as bacteriocins, which also corroborate to inhibit the growth of pathogens, such as *Salmonella* spp. (Rumjanek et al., 2004).

Although differences among treatments were not observed in *Salmonella* count in organs, the litter of NC treated animals presented higher *Salmonella* Heidelberg count than PC and YCW+N. Previous studies reported that *Salmonella* type 1 fimbriae is susceptible to adhere to some oligosaccharides present in the yeast cell wall, such as MOS, eliminating them via excreta, and thus preventing adhesion to the intestinal mucosa and causing damage to the host (Finucane et al., 1999). The pathogen microorganism count in litter is an important topic in view broilers can be infected by *Salmonella* via ingestion of litter material, and thus increase *Salmonella* count in crop, being a source of bacterial contamination during the slaughter process (Malone et al., 1983). In this study, no statistical difference was observed among NC treatment and YCW and ACW+PB for *Salmonella* count. Both yeast-based additives were also not different from PC and YCW+N treatments, which were different from NC, corroborating that some yeast-based prebiotics have the capacity to reduce *Salmonella* infection. This same behavior of data was not observed to the litter count at day 22, which may be related to the age of the birds, microbiota development, maturation of the immune system, and resistance against bacteria (Berchieri Júnior, 2000).

In the present study, higher total short-chain fatty acids (SCFA) content for challenged group may indicate that birds were affected over time following SH infection. SCFA have an important role in intestinal regulation, acting as an energy source where a percentage of the acids produced by the fermentation of carbohydrates is used by the host (Bergman, 1990; Hofacre et al., 2020). It is well known that SCFAs, especially butyrate, propionate and valerate, act directly on the intestine promoting an anti-inflammatory effect due to the development of regulatory T cells (Lucas López et al., 2017). These fatty acids help epithelial cell integrity by reducing the impact of pathogens and maintaining homeostasis status to the chickens (Onrust et al., 2018) Following infection, birds challenged with *Salmonella* had higher production of butyrate compared to non-challenged group, and propionate concentration was lower to NC compared to all treatments. Interestingly, only challenged birds from YCW showed higher valerate concentration compared to NC, while all other

treatments presented similar values. Due to the mode of action of these acids, which is based on their ability to penetrate the bacterial cell membrane and acidify the cell cytoplasm, inhibiting bacterial growth (Gomez-Osorio et al., 2021), the SCFA have similar antimicrobial activity against gram-negative and gram-positive bacteria. It may explain the maintained performance of chickens supplemented with yeast-based additives. According to Van Immerseel et al. (2006), the presence of SCFA such as butyrate may down regulate *Salmonella* contamination in chickens, while propionate can inhibit epithelial cell invasion. Additionally, butyric acid may also down-regulate *Salmonella* virulence by direct action on virulence gene expression (Gantois et al. 2006). For Jacobson et al. (2018), the host microbiota composition is responsible to control *Salmonella* infection due to production of propionate by disrupting intracellular pH homeostasis.

For acetate production, our findings demonstrated that the challenged group had higher concentration compared to non-challenged birds. Acetate is the most abundant SCFA in the cecum of the chickens, produced from acetyl-CoA derived from glycolysis (Liu et al., 2021). The higher level of acetate in response to the challenge conducted shows that the SH infection was able to stimulate its production. The acetate produced by the microbiota plays an important role to host enterocytes providing energy, regulating luminal pH, and stimulating the immune system of the birds against pathogens (LeBlanc et al., 2017; Khan and Chousalkar, 2020). For the contrasts evaluated of the challenged group, the acetate level was higher in NC treatment compared to YCW+N and ACW+PB. It seems that certain commensal bacteria that increased in abundance in response to *Salmonella* infection may have produced acetate (Khan and Chousalkar, 2020). Additionally, the same authors described that pathogen can degrade a variety of compounds and consequently produce short chain fatty acids, such as acetate. However, the interactions between microbiota and SCFA need further investigation.

Regarding blood parameters, *Salmonella* spp. is responsible to stimulate different subsets of immune system cells, which produce cytokines, such as interleukins, which in turn participate of the induction and regulation of the immune response (Okamura et al., 2004). Interleukins can reduce humoral response by stimulating the adrenocorticotropic hormone release, which in turn stimulates corticosterone production, that has been found to inhibit the production and actions of antibodies, acting as an immunosuppressive (Gross, 1992; Sadeghi et al., 2015). Heterophils are cells of defense against microbial infections of natural immunity, while lymphocytes are cells which produce antibodies (Sadeghi et al., 2015). In the present study, at day 10, the contrast test indicated YCW supplemented birds had lower serum levels of heterophils and higher serum levels of lymphocytes than PC and NC, resulting in a lower H:L ratio, which is associated with less stress load (Scholz et al., 2008). This also may explain the better FCR observed for YCW supplemented birds in the first

phase, which presented better health status, and thus were able to have a greater digestive and absorption efficiency. The lower serum level of lymphocytes can be related to the higher corticosterone levels, which can inhibit its population and function. On the other hand, it indicates less inflammatory process, which is associated with increased levels of white blood cells, a condition that was not observed among treatments in the present study (Post et al., 2003; Sadeghi et al., 2015).

The MCV is a parameter to infer the erythrocyte size, while the MCH is used to indicate the amount of hemoglobin relative to the size of the cell per red blood cell (Odunitan-Wayas et al., 2018). Total protein concentration is made up 40 to 50% by albumin, and globulin is the second most prevalent protein on its composition (Schmidt et al., 2007). In the current study, the challenged groups presented greater MCV ($p=0.0232$), a tendency to lower MCHC ($p=0.0786$), and higher total protein ($p=0.0330$) compared to non-challenged birds at 10 days of age. Antimicrobial mechanisms within the mature phagosome have generally been divided into oxygen dependent, that are located within primary granules, and are initiated by the process of phagocytosis or by perturbation of the cell membrane (Babior et al., 2002; Weiss and Wardrop, 2010). The requirements of these oxygen-cells related according to the challenge may be one of the reasons for these finds. Albumin is an important protein which binds and transports anions, cations, fatty acids, hormones, and generally, high blood levels indicate dehydration (Kaneko et al. 1997; Odunitan-Wayas et al., 2018). Despite in the present study the albumin levels were not directly measured, due to its association with the total protein concentration, this correlation could indicate higher microbial unbalance to SH challenged birds, corroborating to this result.

At day 22, a tendency ($p=0.0581$) for thrombocytes serum levels between challenged and non-challenged group was observed. This result was expected, in view thrombocytes may be related to innate immunity due to their phagocytosis capacity, the participation in blood coagulation, as well as the role to participate in the removal of foreign material from the blood (Schmidt et al., 2007; Nagasawa et al., 2014; Campbell, 2015). The basophils levels tended to be higher ($p=0.0735$) to NC than PC and ACW+PB, which can be explained by the function of these cells, in which basophils are one of the first leukocytes to enter tissue as part of the early inflammatory response in birds (Weiss and Wardrop, 2010). However, Al-Khalifa and Al-Nasser (2019) feeding two prebiotics MOS and FOS for broilers did not observe any difference for MCV, MCH, total protein, heterophils, lymphocytes, basophils, and thrombocytes levels compared to broilers fed a control diet.

The intestinal tract produces a large amount of IgA by its activated mucosal B cells, which is responsible for the first-line immune defense. In the present study, YCW group presented higher

levels of IgA compared to the NC at 10 and 22-d old, which can be correlated to the lower SH count in liver found in this same day for YCW compared to NC. It indicates that the immunity of broilers fed the prebiotic was enhanced by regulating the intestinal microbiota, helping to resist the infection stress, also reflecting in the FCR improvement observed for this treatment compared to the NC group. A similar behavior of the immunoglobulins data from this study was observed by Yin et al. (2008), which found increased IgA and IgG levels in early waned piglets supplemented with an oligosaccharide galacto-mannan prebiotic compared to animals receiving the antibiotic lincomycin.

The SH challenged tended ($p=0.0864$) to present higher IgY levels at day 10 and showed higher ($p=0.0121$) IgY levels at day 22 than non-challenged groups. Baptista et al. (2013) observed higher IgA in intestinal fluid in birds challenged with *Salmonella* Typhimurium at 21-d old than a control group, but no difference for IgY was observed between the treatments. Al-Khalifa and Al-Nasser (2019) feeding two prebiotics (MOS and FOS) for broilers observed higher IgY concentration for chickens fed FOS than control and MOS group but did not find differences for IgA levels among control, MOS, and FOS groups. On the other hand, Kim et al. (2011) did not observed differences for IgG blood concentrations between broilers chickens fed prebiotics (FOS and MOS) and control group.

The *Salmonella* serotypes that cause avian paratyphus have varied pathogenicity, differing about invasiveness, and may remain in the digestive tract without causing severe systemic infection (Gast et al., 2013), which can explain the absence of differences among some immune parameters in the current study. Furthermore, it is reported in some studies that the effectiveness of antimicrobial growth promoters and some feed additives are more evident under poor management, extreme temperatures, high flock density, and characteristics of disease and stress conditions (Hooge, 2004; Sims et al., 2004). Despite the SH challenged used to simulate a disease clinical condition, this study was carried out in controlled experimental facility, with biosecurity measures, and new litter, which may not have required the maximum response of the feed additives to the birds.

It is well known that health status is straight correlated to microbiota composition (Hooper et al., 2012), which modulate gut-brain axis via several direct and indirect pathways (Cryan et al., 2019). The gut microbiota consists of a diverse community of bacteria which play an important role to maintain intestinal homeostasis, protecting the body through intestinal barrier defense, antibacterial compounds release, competitive exclusion, and signalizing the immune system to combat pathogens and undesirable substances (Kogut, 2019).

Prior to hatching, the chicken GIT is considered sterile once environmental bacteria have not been in contact to the chicken gut (Apajalahti and Kettunen, 2006; Pan and Yu, 2014). When colonized by aerobic and anaerobic bacteria, the microbiota starts to stable in several communities to support body functions such as energy demand, nutrient digestion, and immune system (Yadav and Jha, 2019). The diversity of bacterial communities is extremely important because each bacterium has a specific ecological niche and synergism with other bacterial species to maintain homeostasis (Kogut, 2022). Understanding the diversity (number of species present in that microbiota) is very important but cannot be the only parameter to be taken into consideration to reach a conclusion regarding eubiosis and dysbiosis. To compose a more complete picture, identifying who are the groups that, in fact, may be influencing that ecosystem and consequently the animal is crucial.

According to Mirza et al. (2018), Firmicutes is the most abundant phylum in the cecum of chickens, followed by *Bacteroidetes*. The authors mentioned that *Proteobacteria* is not characteristically found in the cecum, however the present study showed the *Proteobacteria* as the main phylum observed in chickens fed yeast-based additives and antimicrobial growth promoter at 10 days of age. This may be a strong indication of the success of the sanitary challenge applied in this work since *Salmonella* belongs to the phylum Proteobacteria. Interestingly, only NC challenged treatment is in accordance with the literature. Understanding the genera of the bacteria found between treatments, it is possible to note that we have two main genera: *Novosphingobium* and *Lactobacillus*.

The genus *Novosphingobium* is characterized as gram negative bacteria classified into the subclass *Proteobacteria* and family *Erythrobacteraceae* (Liu et al., 2021). The literature mentioned that these bacteria are often associated with the biodegradation of aromatic compounds such as phenol, pyrene, estrogen, etc (Gan et al., 2013) as well as under groundwater remediation systems (Tiirola et al., 2002). There is some literature citing the presence of *Novosphingobium* in chicken microbiota (Bekele-Yitbarek, 2019; Zou et al., 2022), however no discussion has been reported about interactions in the host. While the species and strains measured in this experiment may differ from those used in the water treatment study, the increased presence of *Novosphingobium* in the cecum of chickens fed yeast-based additives may be due to high levels of aromatic protein fermentation end products (e.g., phenols, indoles) associated with protein content in the diet (Lubbs et al., 2009). This hypothesis has a positive effect, as these bacteria may decrease the concentration of these harmful compounds in the host.

The genera *Lactobacillus* is a gram-positive bacterium which produces lactic acid, responsible to inhibit the growth of several species of harmful bacteria (Makarova et al., 2006). It is one of the main

dominant bacteria in the cecum together with *Bacteroides* and *Ruminococcus* (Qu et al., 2008; Wang et al., 2016). Although *Lactobacillus* species have been studied mostly as positive bacteria in the microbiota, due to its high abundance, *Lactobacillus* are a resilient bacterium in the microbial environment and are not suggested as an indicator for eubiosis. If we observe the NC, the most abundant bacteria were *Lactobacillus*. This treatment did not receive any additive or growth promoter, so the dysbiosis caused by *Salmonella* inoculation was not able to reduce this specific group. We can infer that the genus *Lactobacillus* could not be considered a good biomarker of intestinal health because it is an extremely resilient bacteria in this challenging situation, failing to promote consistently the dysbiosis in the present study. According to Juricova et al. (2022), there are questionable effects of *Lactobacillus* against *Salmonella* in *in vivo* experiments, but it does not exclude that by some conditions a protective response can occur against the infection. For the present study, it was rather unexpected to find the microbiota of NC chickens mostly colonized by *Lactobacillus* compared to the other treatments.

Although the alpha diversity analyses did not differ statistically for richness and diversity between treatment comparison, some particularities in taxonomic composition were found to the additives' supplementation. Yeast-based additives showed the presence of the genera *Turicibacter*, a benefic microorganism to the intestine. According to Hoffman and Margolis (2020), *Turicibacter* have been described as a novel serotonin sensor through the expression of the protein CUW 0748, which has homology and sequence to the serotonin transporter (SERT) expressed on the host enterocytes. The bacteria interact with the host enterochromaffin cells in the lumen, increasing serotonin availability in the enterocytes and posterior absorption to the blood (Fung et al., 2019). In addition, under conditions of increased serotonin availability, *Turicibacter* may lead to growth colonization and produce steroids to lipid metabolism.

For antibiotic growth promoter, the presence of *Clostridium* and *Staphylococcus* indicated that the microbiota may be under imbalance condition, once the ascendence of pathogenic bacteria, such as some strains of *Clostridium perfringens* can cause opportunistic secondary infections in broiler chickens (Aruwa et al., 2021). Furthermore, other bacteria found in the cecum of chickens supplemented with antibiotic growth promoter was the genera *Romboutsia*. According to Han et al. (2022), *Romboutsia* is a gram-positive coccus that is found in human mucosa and may be related to the host health. A recent study reported that *Romboutsia* is positively correlated with short chain fatty acid formation and negatively correlated with uric acid and serotonin production (Song et al., 2022). Thereby, it can upregulate and downregulate some metabolites and produce beneficial effects to the

host. Additionally, it may explain the absence of the genera *Turicibacter* in the microbiota of challenged chickens supplemented with antibiotic growth promoter.

In general, the results give us an insight of the microbiota behavior for broiler chickens fed yeast-based additives in a *Salmonella* challenge condition. Some authors mentioned that microbiota diversity changes depending on the age, diet, intestinal segment, health status, management, and environmental factors (Kers et al., 2018), which could influence to a different response of the present study. To better understand how these microorganisms affect the birds' health, further studies about bacteria-specific function in the microbiota need to be explored.

3.5. CONCLUSIONS

The overall results indicate that yeast-based additives were able to maintain performance similar to that of the growth promoter of *Salmonella*-challenged chickens. In addition, lower contamination of *Salmonella* Heidelberg in the litter and liver was observed for birds supplemented with yeast additives compared to NC, with emphasis to YCW+N at 10 days and all treatments at 22 days, respectively. Concerning immune responses and microbiota, yeast-based additives were able to stimulate the production of immunoglobulins and the concentration of some short-chain fatty acids, indicating an increased abundance in response to maintain homeostasis against pathogen. The microbiota richness was higher in the cecum of challenged birds, yet challenged chickens fed yeast-based prebiotics have the presence of *Turicibacter* bacterium, a serotonin biomarker responsible to well-being. Therefore, yeast-based additives can be considered as important natural alternatives to broiler chicken nutrition, nevertheless, further research is needed to investigate pathways of interaction between microbiota and immune system in challenge condition.

ACKNOWLEDGMENTS

The author would like to thank FAPESP, São Paulo Research Foundation for the financial support (No 2020/12641-4) given to this project.

DISCLOSURES

The author declares that there is no conflict of interest to disclose.

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4. CONSIDERAÇÕES FINAIS

Os benefícios dos prebióticos, em especial os produtos à base de leveduras são notórios, por isso, estes aditivos vem sendo utilizados pela indústria avícola substituindo o uso de antimicrobianos melhoradores de desempenho a fim de atender as demandas mundiais em segurança alimentar e qualidade do produto final. Todavia, estes aditivos são responsáveis por diversos mecanismos de ação, muitos deles ainda pouco elucidados que demandam mais estudos, como por exemplo as interações da microbiota intestinal e os produtos da fermentação bacteriana, os mecanismos de ação do organismo para as respostas anti-inflamatórias e a expressão de comportamentos de bem-estar associados nutrição das aves.

Este estudo disponibilizou novas informações para o uso dos aditivos à base de levedura *Saccharomyces cerevisiae* em rações para frangos de corte. A relevância dos parâmetros avaliados teve como objetivo fornecer uma visão holística do sistema biológico para compreender melhor as respostas internas e externas relacionadas ao bem-estar, saúde e desempenho das aves nos sistemas de produção atuais.

Embora os resultados desta pesquisa demonstraram que os desafios aplicados (*Eimeria* e *Salmonella*) afetaram negativamente o desempenho dos frangos de corte, a alta produção de serotonina pelos frangos de corte suplementados com os aditivos à base de levedura no experimento 1 indicam melhor bem-estar associado a respostas metabólicas positivas no intestino das aves, que puderam ser verificadas por meio dos parâmetros morfológicos avaliados. Da mesma forma, foi verificada a produção de imunoglobulinas e aumento da concentração de alguns ácidos graxos de cadeia curta das aves suplementadas com aditivos à base de leveduras no experimento 2, indicando assim o aumento das respostas do sistema imune para manter a homeostase do organismo. Vale destacar que nesse experimento o desempenho das aves desafiadas e suplementadas com aditivos à base de leveduras foi similar ao das aves que receberam antimicrobiano melhorador de desempenho, e ainda, a microbiota dessas aves apresentou o gênero *Turicibacter* em sua composição, bactéria esta considerada um biomarcador de serotonina.

Em geral, os resultados demonstraram que a suplementação de aditivos à base de levedura na dieta de frangos de corte mostra-se interessante na formulação de rações da indústria avícola, visto que o uso desses aditivos naturais pode promover efeitos benéficos para a saúde, desempenho e bem-estar das aves.