Final Report

Project code: 21-304

Prepared by: Dr Amy Moss, Dr Thi Hiep Dao, Ms Nasima Akter

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Nutritional strategies to mitigate coccidiosis

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This project is supported by Poultry Hub Australia through funding from AgriFutures Australia as part of its AgriFutures Chicken Meat Program.

Researcher Contact Details

Dr Amy Moss
Department of Animal Science, School of Environmental and Rural Science, the University of New England, Armidale NSW 2351
Phone: 02 6773 5217
Email: amoss22@une.edu.au

In submitting this report, the researcher has agreed to Poultry Hub Australia publishing this material in an edited form.

Poultry Hub Australia Contact Details

Poultry Hub Australia

CJ Hawkins Homestead, Ring Road

University of New England

Armidale NSW 2350

02 6773 1855

poultryhub@une.edu.au

www.poultryhub.org

Project Summary

Project Title	Nutritional strategies to mitigate coccidiosis
Project No.	21-304
Date	Start: 01/01/2022 End: 30/04/2023
Project Leader(s)	Dr Amy Moss
Organisation	The University of New England
Email	amoss22@une.edu.au
Project Aim	The study aimed to determine if the nutritional strategies of post-pellet whole wheat, xylo-oligosaccharide, high fat (vegetable oil), high carbohydrate, supplementation of threonine and branched-chain amino acids (valine, isoleucine and leucine) and short chain fatty acid inclusions may reduce the severity of the coccidiosis challenge in broilers, in comparison to a ground grain, negative control (unchallenged) and positive control (challenged) diets, containing breed recommended nutrient levels. It is hypothesised that one or multiple of these strategies may return performance to that of the negative control treatment.
Background	Coccidiosis is currently the biggest disease challenge and one of the biggest challenges overall for broiler production in Australia, particularly as Australia has pushed to ban antibiotic growth promotants and coccidiostats. The economic impact of coccidiosis worldwide is estimated at approximately $14.5 billion USD (Blake et al., 2021). Vaccines are available, but they are expensive and birds may experience depressed performance. Additionally, newly identified strains are not mitigated with the current vaccines available. Therefore, this project was designed to explore the nutritional strategies to reduce coccidiosis burden within industry.
Research Outcome	The results of the current study showed that coccidiosis challenge increased intestinal lesion score, intestinal length, and faecal Eimeria oocyst count and altered the gut microbiota population resulting in reduced growth performance and carcass yield in birds. However, nutritional strategies such as xylo-oligosaccharide, high carbohydrate and short chain fatty acid inclusion could maintain bird growth performance and attenuate the severity of coccidiosis challenge on the gut microbiota population. Additionally, supplementation of high-fat, threonine and branched-chain amino acids were effective in increasing the number of beneficial bacteria such as *Bacteroides* spp. in the caeca and dietary xylo-oligosaccharide inclusion had the potential to reduce the number of Eimeria oocysts in ~~feces~~faeces of coccidiosis challenged birds.
Impacts and Outcomes	The outcomes of this study are directly relevant and beneficial to the Australian poultry industry. Coccidiosis affects all broiler farms within Australia, and has significant economic, environmental and social impacts for the Australian chicken meat industry. Thus, the nutritional strategies proposed in this study − particularly xylo-oligosaccharide, high carbohydrate and short chain fatty acid inclusion − may represent significant positive impact for the industry. Furthermore, all of the nutritional strategies explored

	in this study are readily implementable by the Australian poultry industry and would not be costly to include in a standard Australian broiler diet.
Publications	Manuscripts are in preparation, the following conference abstract was presented; N. Akter, T.H. Dao, A.A. Jahan, A. Kumar, S.B. Wu, Sukirno, E. Kim, G. Underwood, P. Young, M. Benham, M.R. Bedford, A.F. Moss (2023). Nutritional strategies to mitigate coccidiosis. Proceedings of 34th Australian Poultry Science Symposium, Sydney, Australia, 6-8th February 2023.

Project Status

Have the aims of the project been achieved?	Yes
Date final report was due	30/04/2023
Have any publications been released during this project?	Yes
Are there publications that are planned/in preparation that will be release after the completion of this project?	Yes
Has any IP arisen from this project?	No
Is there any reason to embargo this final report?	No

Executive Summary

Coccidiosis is a disease with substantial economic impact, particularly due to the push to ban anticoccidials. Vaccines are available but can be ~~expensive, and~~expensive and are often implemented for free range and breeder flocks only. Thus, it is imperative to find effective nutritional alternatives to reduce the impact of coccidiosis on broiler chickens. The aim of this experiment was to determine if the nutritional strategies of post-pellet whole wheat, xylooligosaccharide, high fat (vegetable oil), high carbohydrate, supplementation of threonine and branched-chain amino acids and short-chain fatty acid inclusions may assist broilers to combat the severity of coccidiosis challenge, in comparison to a ground grain, negative control (unchallenged) and positive control (challenged) diets, containing breed recommended nutrient levels. A total of 576 off-sex day-old male Ross 308 (six replicates, 12 birds/pen) were allocated to one of the eight dietary treatments on the basis of initial body weight. Birds were offered starter (days 1 – 10), grower (days 10 – 21) and finisher (days 21 – 35) diets. Birds in the challenge treatments were dosed with *E. maxima* and *E. acervulina* (Eimeria Pty Ltd.) in 1 mL sterile phosphate-buffered saline (PBS), while unchallenged birds were dosed with 1 mL PBS on day 14. Birds had unlimited access to feed and water in an environmentally controlled facility. Lighting and temperature followed Ross 308 guidelines. Fecal collection was performed daily from days 17 to 28 to determine coccidial oocyst count. Feed intake, weight gain and FCR were calculated for each feeding phase. The other measurements included intestinal lesion scores on day 21; carcass yield (breast, thigh, drumstick, abdominal fat) and bone morphology and ash content on day 35; weights of full and empty gizzard, intestinal length and diameter and caecal microbiota populations on days 21 and 35. The results of this study showed that coccidiosis challenge increased intestinal lesion score, intestinal length, and faecal Eimeria oocyst count and altered the gut microbiota population resulting in reduced growth performance and carcass yield in birds. However, nutritional strategies such as xylo-oligosaccharide, high carbohydrate and short chain fatty acid inclusion could maintain birds growth performance and attenuate the severity of coccidiosis challenge on the gut microbiota population. Additionally, supplementation of high-fat, threonine and branched-chain amino acids was effective in increasing the number of beneficial bacteria such as *Bacteroides* spp. in the caeca and dietary xylo-oligosaccharide inclusion had the potential to reduce the number of Eimeria oocysts in ~~feces~~faeces of coccidiosis challenged birds. The nutritional strategies proposed in this study particularly xylo-oligosaccharide, high carbohydrate and short chain fatty acid inclusion may represent a significant positive impact on the industry.

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Table of Contents

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Introduction

Coccidiosis is now one of the major challenges faced by the Australian poultry industry with the push to ban antibiotic growth promotants and the fact that vaccines are expensive and require further development and reduced cost before they can be widely implemented throughout the broiler industry. Furthermore, three new strains of coccidiosis have been identified that escape immune inhibition from current vaccines (Blake et al., 2021). Thus, in the removal of broad spectrum coccidiostats, there will be strains of coccidiosis that cannot be controlled. Therefore, any nutritional strategies which may be readily implemented to reduce coccidiosis burden would be of vital importance for the Australian poultry industry. These nutritional strategies may be seen within one of two categories; promoting oocyst destruction and improving the resilience of the gut during coccidiosis challenge.

The ability of whole grain (Liu et al., 2014), dietary fat inclusion (Mateos et al., 2002) and xylooligosaccharide (XOS, Craig et al., 2020) to reduce digesta passage rates and/or increase digesta retention within the gizzard may promote oocyst destruction. Australia practices whole grain feeding to generate improvements in FCR, reduce excreta moisture, and to reduce the amount of feed pelleted. It was originally reported (Cumming 1989) that whole grain feeding lessens the effect of coccidiosis due to improving gut integrity, acidifying the gut and the grinding action of the gizzard. This result is logical, but quite surprisingly, studies have shown the opposite findings where whole grain feeding appears to worsen cocci effects (Gabriel et al., 2003; Gabriel et al., 2006). Whether or not coccidiosis is improved or worsened by whole grain inclusion may have a significant impact on the Australian poultry industry once antibiotic growth promotants are banned, and thus the effect must be determined. Additionally, Craig et al. (2020) reported that XOS inclusion to diets of broilers under coccidiosis challenge reduced faecal egg counts within the excreta. The possibility is that XOS may reduce digesta passage rates via caecal fermentation and thereby increase digesta retention times within the acidic, grinding gizzard which could promote oocyst destruction. Recent research also indicated that combined supplementation of XOS and gamma-irradiated *astragalus* polysaccharides upregulated cytokine gene expression, enhanced IgA-producing cell production and modulated caecal microbiota populations resulting in improved intestinal mucosal immunity and barrier function in birds (Wang et al., 2022).

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Aside from promoting oocyst destruction, gut resilience can also be improved to preserve the functionality of the gut during coccidiosis challenge. Threonine (Thr) is an important amino

acid in the construction of mucin and therefore has importance for intestinal development and maintenance (Horn et al., 2009). Furthermore, the branched chain amino acids (BCAA) have been shown to maintain intestinal immune related function in pigs (Ren at al., 2015). Short-chain fatty acids (SCFA) have also been demonstrated to stimulate proliferation of crypt cells and enhance tissue turnover and maintenance of the gut (Frankel et al., 1994) and have antimicrobial properties. Thus, the inclusion of SCFA may stimulate get regeneration and thereby lessen the effects of coccidiosis challenge. Additionally, the antimicrobial effect may help to reduce the impact of secondary infection risk in a gut damaged by coccidiosis.

Nutritional strategies may be seen within one of two categories; promoting oocyst destruction and improve resilience of the gut during coccidiosis challenge. Therefore, the aim of this project was to employ nutritional strategies to promote oocyst destruction (whole wheat, XOS, high fat vs high carbohydrate axis) and improve gut resilience (XOS, Thr + BCAA, SCFA) to combat the severity of coccidiosis challenge in broilers.

Objectives

The study aimed to determine if the nutritional strategies of post-pellet whole wheat, XOS, high fat (vegetable oil), high carbohydrate, supplementation of Thr and BCAA (valine, isoleucine and leucine) and SCFA inclusions may assist broilers to combat the severity of the coccidiosis challenge, in comparison to a ground grain, negative control (unchallenged) and positive control (challenged) treatments, containing breed recommended nutrient levels. It is hypothesised that one or multiple of these strategies may return performance to that of the negative control treatment.

Methodology

Experimental design, animals and diets

The study was conducted at the University of New England broiler facility in Armidale, NSW, Australia. All experimental procedures were approved by the UNE Animal Ethics Committee and met the requirements of the Australian Code of Practice for Care and Use of Animals for Scientific Purposes (NHMRC, 2013). A total of 576 day-old male (off-sex) Ross 308 broilers (6 replicates of 12 birds/pen) were allocated to one of 8 dietary treatments on arrival at UNE. Dietary treatments consisted of a negative control (NC) standard industry wheat-soybean meal-based diet without coccidiosis challenge, a positive control (PC) standard industry wheat-

soybean meal-based diet with coccidiosis challenge and six wheat-soybean meal based diets with challenge plus a nutritional strategy to combat the severity of the coccidiosis challenge. Seven nutritional strategies included a post-pellet whole grain inclusion (5%, 10% and 20% in starter, grower and finisher phase respectively), inclusions of XOS, high vegetable oil, high carbohydrate, Thr and BCAA (2% above the breeder requirements), and SCFA. In the SCFA treatment, sodium butyrate was used as the SCFA additive at 0.08%. Birds were offered starter (0-10 days), grower (10-21 days) and finisher (21-35 days) diets. Broilers in the challenge treatments were dosed with E. maxima and E. acervulina (Eimeria Pty.) in 1 mL sterile phosphate-buffered saline (PBS) on day 14 using a crop needle, with care not to bypass the crop. Un-challenged broilers were dosed with 1 mL PBS. Birds had unlimited access to feed and water and were housed in floor pens on litter in an environmentally controlled facility. Birds had a '23-h-on-1-h-off' lighting regime for the first day and gradually transitioned to '18-h-on-6-h-off' lighting regime by day 7. An initial room temperature of 33 ± 1°C was maintained for the first day, and gradually decreased to 21 ± 1°C by the end of the third week and maintained at this temperature until experiment end. All feeds were formulated to meet the breed standard minimum nutrient requirements. Feedstuffs were analysed for nutrient content prior to diet formulation.

Excreta collection was performed daily from 17 to 28 days to determine total Eimeria oocyst count. Four birds per pen were sampled on day 21 to assess lesion score, intestinal length and diameter, and collect caecal content and on day 35 to assess gizzard weight, intestinal length and diameter, collect caecal content, measure carcass cut up and fat pad weight. Caecal digesta samples collected on days 21 and 35 were analysed for microbiota composition. The DNA of caecal content was extracted, then the relative amounts of *Bacillus* spp.*, Bacteroides* spp.*, Bifidobacterium* spp.*,* Enterobacteriaceae*, Lactobacillus* spp.*, Ruminococcus* spp., and total bacteria, expressed as $\log_{10}$ genomic DNA copies per gram of caecal digesta, were quantified as described by Kheravii et al. (2017). The quantitative real-time PCR (Rotorgene 6000 real-time PCR machine, Corbett, Sydney, Australia) was employed to determine the bacterial populations.

Statistical analysis

All data analyses were performed using R Commander (version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria). Data were tested for normal distribution and equal variances between the dietary treatments. A quantile comparison plot was employed to check the data distribution, then a Levene's test was used to test the homogeneity of variances

between the treatments. Depending on the results produced from the above 2 tests, either one-way ANOVA or the non-parametric ANOVA (Kruskal–Wallis test) was used to test statistical differences between the treatments. Tukey's post-hoc test was employed to identify pairwise differences between the treatments from significant ANOVA results. P-values were considered significant at ≤ 0.05.

Discussion of Results

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Diet composition and analysed nutrient content of the experimental treatments are given in Tables 1, 2, 3 and 4, respectively. The growth performance of experimental treatments in the starter, grower, finisher and overall period are shown in Tables 5, 6, 7 and 8, respectively. The trial has successfully been completed. Fecal oocyst counting from days 17 to 28 (Table 16) and intestinal lesion score on day 21 (Table 9) showed that the NC group was coccidiosis free, and the challenge was successful. Additionally, our aim for a mild challenge was successful, as mortality rates remained acceptable, which gives the nutritional strategies the best chance of having an effect. Though it was not significantly different, the challenge had the greatest impact on the birds growth performance during the finisher phase, where weight gain and FCR of the PC treatment were numerically worsened compared to the NC treatment (Table 7). Over the entire study, the XOS, high carbohydrate and SCFA treatments maintained weight gain and FCR in comparison to the NC treatment (Table 8). Whereases, the whole grain and high fat treatments exhibited the lowest weight gain and worst FCR compared to the other treatments from days 0 to 35 ($P < 0.001$, Table 8). Additionally, birds on the Thr and BCAA treatment had higher FCR compared to the NC treatment ($P < 0.001$, Table 8). This finding was consistent with the intestinal lesion score results where birds on the Thr and BCAA treatment had higher duodenal lesion score than those on the NC treatment ($P = 0.044$, Table 9). With the exception of these treatments, the nutritional strategies were successful at maintaining a similar performance to the NC treatment in the current study. The dietary XOS inclusion may reduce digesta passage rates via caecal fermentation resulting in increased digesta retention times within the acidic, grinding gizzard which could promote Eimeria oocyst destruction and increase growth performance in coccidiosis challenged birds (Craig et al., 2020). Whereas, the antimicrobial effects of SCFA might reduce the impacts of secondary infection risk in the gut and promote growth performance during coccidiosis challenge (Frankel et al., 1994; Wu et al., 2018). The findings of this study were in good agreement with those previously reported.

Whole grain feeding has been reported to increase digesta retention within the gizzard and promote gizzard functionality (Liu et al., 2014) that may promote Eimeria oocyst destruction and increase growth performance in coccidiosis challenged birds. This finding is logical, but quite surprisingly, other studies have shown the opposite findings that whole grain feeding appears to worsen cocci effects (Gabriel et al., 2003; Gabriel et al., 2006). In the current study, the whole grain treatment experienced some pellet quality issues, which may have led to a poorer feed intake and feed flicking within this treatment which is a common issue of whole grain feeding regimes. Similarly, additional dietary fat supplementation may slow the digesta passage rate and thereby mitigate the burden of coccidiosis challenge by increasing destruction of oocysts within the gizzard. The high fat treatment in the current study had a high feed intake, but which was not matched with a reciprocal high weight gain, and therefore FCR was worsened. It is notable that the NC treatment had no/negligible lesion scores, and some of the treatments numerically reduced lesion score in comparison to the PC treatment (Table 9).

The relative carcass yield on day 35 and relative weights of full and empty gizzard on days 21 and 35 of experimental treatments are shown in Tables 10 and 11, respectively. The XOS and Thr and BCAA treatments had similar breast weight while whole wheat and high fat treatments reduced breast weight compared to the PC and NC treatment on day 35 ($P < 0.001$; Table 10). Noticeably, high fat inclusion reduced abdominal fat weight compared to the NC treatment on day 35 ($P < 0.001$; Table 10). Whole wheat treatment had higher full and empty gizzard weight compared to the PC on both day 21 and day 35 ($P < 0.001$; Table 11). Additionally, birds on the Thr and BCAA treatment had higher full gizzard weight compared to the PC treatment on day 35 ($P < 0.001$; Table 11). The findings on carcass yield and gizzard weight of this study were consistent with the growth performance results. Ott et al. (2018) observed lower fat pad weights in coccidiosis challenged birds compared to the unchallenged birds. In contrast, increased fat pad weights in Eimeria challenged birds were reported by other research groups (Poudel et al., 2022). The reasons for this conflicting evidence are unclear. It has been reported that Eimeria challenge reduced carcass yield in birds (Laika and Jahanian, 2017; Poudel et al., 2022). In the current study, challenged birds received whole wheat and high fat treatments had lower breast weight compared to the PC and NC treatments. The proliferation of *Eimeria* in the intestine of challenged birds might impair gut osmolality and reduce glucose, sodium and potassium absorption resulting in reduced protein synthesis and subsequently, carcass weight as shown in the current study and others (Awad et al., 2009; Teng et al., 2020; Poudel et al., 2022).

Intestinal length and diameter on days 21 and 35 are given in Tables 12 and 13, respectively. Birds on the SCFA and Thr and BCAA treatments had higher jejunum length compared to the NC treatment on day 21 ($P = 0.005$; Table 12). Also, birds on the SCFA treatment tended to have a higher duodenum length compared to the NC treatment on day 21 ($P = 0.054$; Table 12). All coccidiosis challenged birds had higher ileum length compared to the NC treatment on day 21 ($P < 0.001$; Table 12). On day 35, all nutritional strategies except high carbohydrate treatment increased duodenal and jejunal length compared to the NC treatment ($P < 0.001$; Table 13). Also, whole wheat and SCFA treatment increased ileal length compared to the NC treatment on day 35 ($P = 0.021$; Table 13). The intestinal diameter was not different between the dietary treatments on days 21 and 35 (Tables 12 and 13). As high energy levels are required for maintaining intestinal homeostasis (Dao et al., 2021), the longer intestine in coccidiosis challenged birds might partly explain the lower growth performance observed in the respective treatments.

The caecal microbiota populations of treatment groups on days 21 and 35 are shown in Tables 14 and 15, respectively. The results showed that XOS and high carbohydrate treatments reduced the numbers of caecal *Bacillus* spp. compared to the PC group on day 21 ($P = 0.025$, Table 14). Coccidiosis challenge increased the number of *Bifidobacterium* spp. in the PC group compared to the NC group on day 21 ($P = 0.022$, Table 14). However, inclusions of Thr and BCAA and SCFA reduced the numbers of *Bifidobacterium* spp. to the levels of the NC group on day 21 ($P = 0.022$, Table 14). The other microbiota populations were not different between the treatments on day 21 ($P > 0.05$, Table 14). On day 35, challenged birds offered high-fat and Thr and BCAA diets had higher numbers of caecal *Bacteroides* spp. compared to the challenged PC birds ($P = 0.004$, Table 15). The other microbiota populations were not different between the treatments on day 35 ($P > 0.05$, Table 15). Bifidobacteria, Lactobacillus spp., *Bacillus* spp., and *Bacteroides* spp. have been considered beneficial bacteria in the gut (Torok et al., 2011; Latorre et al., 2015; Latorre et al., 2016). In this study, the number of *Bifidobacterium* spp. was increased in the challenged birds compared to the unchallenged birds on day 21. Increased Lactobacillus spp. count has been observed in coccidiosis-challenged birds (Faber et al., 2012; Moraes et al., 2019). The presence of proteins and the increased amount of mucus from coccidia-damaged cells in coccidiosis-challenged birds (Collier et al., 2008) might provide substrates for both beneficial and pathogenic bacteria to grow (Deplancke and Gaskins, 2001). Others have shown that coccidial vaccination did not affect the numbers of Bacteroidia and Bacilli in *Eimeria*-challenged birds (Wang et al., 2019). Thus,

the results of this study and others illustrated that coccidiosis challenge might alter the gut microbiota populations in birds. However, this study demonstrated that nutritional strategies such as XOS, high carbohydrate, Thr and BCAA, and SCFA supplementation could diminish the effects of coccidiosis challenge on the gut microbiota population.

Phyla *Bacteroidetes* are gram-negative gut-friendly bacteria responsible for fermenting polysaccharides and other indigestible carbohydrates and are related to fat accumulation in birds (Torok et al., 2011; Chen et al., 2020). Previous studies have indicated that the number of *Bacteroides* spp. in birds fed diets supplemented with plant essential oils was positively correlated with many lipid metabolites that might be associated with fatty acid biosynthesis and lipid metabolism in birds (Chen et al., 2020). Also, increased *Bacteroides* abundance was observed in birds supplemented with 400 mg/kg isoleucine compared to the un-supplemented group (Liu et al., 2023). These facts may explain the higher numbers of caecal *Bacteroides* spp. in birds offered diets containing high fat, Thr and BCAA compared to the challenged PC birds on day 35 in the current study.

The results on total Eimeria oocyst count from days 17 to 28, individual oocyst count from days 20 to 22, and tibia morphology and ash content on day 35 are presented in Tables 16, 17 and 18, respectively. Birds on the PC treatment had higher oocyst counts compared to the NC treatment from days 26 to 28 ($P = 0.002$; Table 16). Whole wheat feeding increased oocyst count compared to the PC treatment from days 17 to 19 ($P < 0.001$; Table 16). Similarly, individual oocyst count showed that coccidiosis challenge increased the numbers of *E. maxima* and *E. acervulina* in the feces on days 21 and 22 ($P < 0.05$, Table 17). Whole grain feeding increased the number of *E. acervulina* in the feces compared to the NC treatment on day 20 ($P = 0.018$, Table 17). Noticeably, high carbohydrate and SCFA treatments had lower numbers of fecal *E. maxima* ($P = 0.006$) and the XOS treatment had lower numbers of fecal *E. acervulina* ($P < 0.001$) compared to the PC treatment on day 22 (Table 17). These results might partly explain the lower growth performance in the challenged birds particularly those received whole wheat feeding compared to the unchallenged NC treatment in the current study. Tibia weight, length, diameter, breaking strength and ash content were not different between the dietary treatments on day 35 ($P > 0.05$; Table 18). Previous studies have indicated that Eimeria challenge might adversely affect bone health as it impairs the duodenum and upper jejunum - the main sites of mineral absorption (Van der Klis et al., 1990; Kakhki et al., 2019). Similarly, Shaw et al. (2011) observed that coccidiosis challenge decreased growth performance and absorption of calcium and phosphorous resulting in reduced bone breaking strength in birds.

The absence of coccidiosis effects on bone quality in the current study might be due to the mild challenge applied.

Implications

Based on the current findings, we have demonstrated that coccidiosis challenge increased intestinal lesion score, intestinal length, and faecal Eimeria oocyst count and altered the gut microbiota population resulting in reduced growth performance and carcass yield in birds. However, the nutritional strategies such as XOS, high carbohydrate and SCFA inclusion could maintain bird growth performance and attenuate the severity of coccidiosis challenge on the gut microbiota population. Additionally, supplementation of high-fat, Thr and BCAA was effective in increasing the number of beneficial bacteria such as *Bacteroides* spp. in the caeca and dietary XOS inclusion had potential to reduce the number of Eimeria oocysts in feces of coccidiosis challenged birds. The reduction of oocysts excreted into the environment may be particularly beneficial to prevent re-infection of subsequent flocks, particularly if the litter is reused. The impact of XOS inclusion on cocci oocyst environmental build-up through multiple flock cycles is therefore of interest for further study. The outcomes of this study are directly relevant and beneficial to the Australian poultry industry. Coccidiosis affects all broiler farms within Australia, and has significant economic (approximately $224 million AUD annually), environmental and social impacts for the Australian chicken meat industry. Thus, the nutritional strategies proposed in this study particularly XOS, high carbohydrate and SCFA inclusion may represent significant positive impact for the industry. Furthermore, all of the nutritional strategies explored in this study are readily implementable by the Australian poultry industry and would not be costly to include in a standard Australian broiler diet.

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Recommendations

As more is known how best to use the nutritional strategies to combat the negative effects of coccidiosis challenge in birds, the industry adoption of these strategies will increase. As XOS, high carbohydrate and SCFA inclusion are the most effective nutritional strategies observed in the current study, determining the effects of dietary XOS, carbohydrate and SCFA levels on the growth performance and gut health of broilers during coccidiosis challenge may be worthwhile for further studies and may help to expand the impacts of this study.

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Acknowledgments

We sincerely thank Poultry Hub Australia, AB Vista and Eimeria Pty Ltd., Australia, for supporting this project. We thank the University of New England for use of animal and laboratory facilities.

Media and Publications

Manuscripts are in preparation, the following conference abstract has arisen from this project;

- N. Akter, T.H. Dao, A.A. Jahan, A. Kumar, S.B. Wu, Sukirno, E. Kim, G. Underwood, P. Young, M. Benham, M.R. Bedford, A.F. Moss (2023). Nutritional strategies to mitigate coccidiosis. Proceedings of 34th Australian Poultry Science Symposium, Sydney, Australia, 6-8th February 2023.

Intellectual Property Arising

Not applicable- IP generated pertains to the know-how of formulating food waste based feeds and the knowledge described within the report.

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Appendices

Table 1. Diet composition and calculated nutrient values of experimental starter diets from days 0 to 10 (as-fed basis)

Ingredients, %	Basal control[1]	High fat	High Carbohydrate	Thr-BCAA[2]
Soybean meal	33.47	34.73	33.47	30.96
Canola oil	2.89	4.40	2.89	3.41
Wheat	60.14	51.31	60.14	57.26
Canola meal	0.00	5.00	0.00	5.00
Limestone	1.52	1.44	1.52	1.46
Salt	0.173	0.010	0.173	0.179
Mono-dicalcium phosphate	0.562	0.534	0.562	0.529
Sodium bicarbonate	0.130	0.630	0.130	0.125
L-lysine	0.275	0.161	0.275	0.267
DL-methionine	0.336	0.284	0.336	0.305
L-threonine	0.135	0.493	0.135	0.137
L-valine	0.011	0.000	0.011	0.012
Choline chloride 75%	0.025	0.686	0.025	0.025
Vitamin and mineral premix[3]	0.175	0.175	0.175	0.175
Filler (bentonite)	0.100	0.100	0.100	0.100
Xylanase[4]	0.025	0.025	0.025	0.025
Phytase[5]	0.030	0.030	0.030	0.030
Total	100	100	100	100
Calculated nutrient, % (Otherwise, as stated)				
Dry matter	90.5	90.7	90.5	90.5
AME (Kcal/kg)	3005	3009	3005	3006
Crude protein	23.39	25.30	23.39	23.75
Cude fibre	2.62	3.02	2.62	3.01
Crude fat	4.17	5.67	4.17	4.74
Ash	5.14	5.31	5.14	5.18
Dig[6] lysine	1.28	1.28	1.28	1.28
Dig methionine	0.623	0.603	0.623	0.611
Dig methionine+cystine	0.950	0.950	0.950	0.950
Dig cysteine	0.323	0.342	0.323	0.335
Dig threonine	0.860	1.275	0.860	0.880
Dig tryptophan	0.300	0.317	0.300	0.303
Dig glycine	0.778	0.847	0.778	0.806
Dig arginine	1.370	1.461	1.370	1.370
Dig serine	0.778	0.847	0.778	0.806
Dig histidine	0.507	0.541	0.507	0.512

Dig isoleucine	0.877	0.938	0.877	0.883
Dig leucine	1.465	1.564	1.465	1.480
Dig phenylalanine	0.979	1.038	0.979	0.983
Dig tyrosine	0.823	0.857	0.823	0.815
Dig valine	0.960	1.021	0.960	0.980
Calcium	0.960	0.960	0.960	0.960
Available phosphorus	0.480	0.480	0.480	0.480
Sodium	0.160	0.230	0.160	0.160
Chloride	0.230	0.230	0.230	0.230
Potassium	0.944	1.000	0.944	0.945
Linoleic acid	1.215	1.377	1.215	1.261
Choline (mg/kg)	1867	5875	1867	2088
Dietary electrolyte balance (mEq/kg)	246	291	246	246

[1]The other diets which were not included in the table were made by adding respective additives on top of the basal control diet.
[2]Diet with increased threonine (Thr) and branched chain amino acid (BCAA) supplementation
[3]Vitamin and mineral premix per kg diet: vitamin A, 12 MIU; vitamin D, 5 MIU; vitamin E, 75 mg; vitamin K, 3 mg; nicotinic acid, 55 mg; pantothenic acid, 13 mg; folic acid, 2 mg; riboflavin, 8 mg; cyanocobalamin, 0.016 mg; biotin, 0.25 mg; pyridoxine, 5 mg; thiamine, 3 mg; antioxidant, 50 mg; Cu, 16 mg as copper sulfate; Mn, 60 mg as manganese sulfate; Mn, 60 mg as manganous oxide; I, 0.125 mg as potassium iodide; Se, 0.3 mg; Fe, 40 mg, as iron sulfate; Zn, 50 mg as zinc oxide; Zn, 50 mg as zinc sulfate.
[4]Xylanase 8000 L, Danisco
[5]Axtra PHY 5000L, Danisco
[6]Digestible amino acid coefficients for raw ingredients were determined by Near-Infra Red spectroscopy (Foss NIR 6500, Denmark) standardized with Evonik AMINONIR® Advanced calibration.

Table 2. Diet composition and calculated nutrient values of experimental grower diets from days 10 to 21 (as-fed basis)

Ingredients, %	Basal control[1]	High fat	High carbohydrate	Thr-BCAA[2]
Soybean meal	25.88	26.27	28.40	26.11
Canola oil	4.41	5.80	3.89	4.44
Wheat	61.75	54.31	64.60	61.49
Canola meal	5.00	10.00	0.00	5.00
Limestone	1.35	1.27	1.41	1.35
Salt	0.185	0.000	0.179	0.187
Mono-dicalcium phosphate	0.334	0.304	0.367	0.333
Sodium bicarbonate	0.112	0.641	0.117	0.109
L-lysine	0.258	0.167	0.266	0.251
DL-methionine	0.264	0.217	0.295	0.262
L-threonine	0.097	0.043	0.115	0.109
L-valine	0.000	0.000	0.007	0.004
Choline chloride 75%	0.025	0.642	0.025	0.025
Vitamin and mineral premix[3]	0.175	0.175	0.175	0.175
Filler (bentonite)	0.100	0.100	0.100	0.100
Xylanase[4]	0.025	0.025	0.025	0.025
Phytase[5]	0.030	0.030	0.030	0.030
Total	100	100	100	100
Calculated nutrient, % (Otherwise, as stated)				
Dry matter	90.6	90.8	90.6	90.6
AME (Kcal/kg)	3109	3112	3108	3109
Crude protein	21.77	23.15	21.43	21.86
Cude fibre	2.93	3.33	2.53	2.93
Crude fat	5.75	7.14	5.17	5.77
Ash	4.62	4.76	4.59	4.63
Dig[6] lysine	1.150	1.150	1.150	1.150
Dig methionine	0.549	0.530	0.562	0.548
Dig methionine+cystine	0.870	0.870	0.870	0.870
Dig cysteine	0.316	0.334	0.304	0.317
Dig threonine	0.770	0.770	0.770	0.785
Dig tryptophan	0.278	0.292	0.275	0.279
Dig glycine	0.739	0.798	0.710	0.742
Dig arginine	1.230	1.301	1.230	1.236
Dig serine	0.739	0.798	0.710	0.742
Dig histidine	0.466	0.494	0.460	0.468
Dig isoleucine	0.796	0.845	0.790	0.800
Dig leucine	1.343	1.423	1.329	1.349
Dig phenylalanine	0.893	0.940	0.889	0.897
Dig tyrosine	0.743	0.767	0.751	0.746
Dig valine	0.882	0.942	0.870	0.890
Calcium	0.870	0.870	0.870	0.870

Available phosphorus	0.435	0.435	0.435	0.435
Sodium	0.160	0.230	0.160	0.160
Chloride	0.230	0.213	0.230	0.230
Potassium	0.856	0.900	0.855	0.860
Linoleic acid	1.422	1.572	1.377	1.425
Choline (mg/kg)	1963	5709	1741	1968
Dietary electrolyte balance (mEq/kg)	224	270	223	225

[1]The other diets which were not included in the table were made by adding respective additives on top of the basal control diet.
[2]Diet with increased threonine (Thr) and branched chain amino acid (BCAA) supplementation
[3]Vitamin and mineral premix per kg diet: vitamin A, 12 MIU; vitamin D, 5 MIU; vitamin E, 75 mg; vitamin K, 3 mg; nicotinic acid, 55 mg; pantothenic acid, 13 mg; folic acid, 2 mg; riboflavin, 8 mg; cyanocobalamin, 0.016 mg; biotin, 0.25 mg; pyridoxine, 5 mg; thiamine, 3 mg; antioxidant, 50 mg; Cu, 16 mg as copper sulfate; Mn, 60 mg as manganese sulfate; Mn, 60 mg as manganous oxide; I, 0.125 mg as potassium iodide; Se, 0.3 mg; Fe, 40 mg, as iron sulfate; Zn, 50 mg as zinc oxide; Zn, 50 mg as zinc sulfate.
[4]Xylanase 8000 L, Danisco
[5]Axtra PHY 5000L, Danisco
[6]Digestible amino acid coefficients for raw ingredients were determined by Near-Infra Red spectroscopy (Foss NIR 6500, Denmark) standardized with Evonik AMINONIR® Advanced calibration.

Table 3. Diet composition and calculated nutrient values of experimental finisher diets from days 21 to 35 (as-fed basis)

Ingredients, %	Basal control[1]	High fat	High carbohydrate	Thr-BCAA[2]
Soybean meal	20.76	26.23	22.95	20.23
Canola oil	5.18	6.80	4.75	5.29
Wheat	66.48	54.54	68.93	65.86
Canola meal	5.00	10.00	0.67	6.07
Limestone	1.24	1.15	1.29	1.23
Salt	0.193	0.120	0.188	0.194
Mono-dicalcium phosphate	0.137	0.082	0.166	0.129
Sodium bicarbonate	0.097	0.469	0.101	0.096
L-lysine	0.249	0.003	0.256	0.247
DL-methionine	0.233	0.146	0.260	0.227
L-threonine	0.077	0.000	0.093	0.088
Choline chloride 75%	0.020	0.137	0.020	0.020
Vitamin and mineral premix[3]	0.175	0.175	0.175	0.175
Filler (bentonite)	0.100	0.100	0.100	0.100
Xylanase[4]	0.025	0.025	0.025	0.025
Phytase[5]	0.030	0.030	0.030	0.030
Total	100	100	100	100
Calculated nutrient, % (Otherwise, as stated)				
Dry matter	90.6	90.7	90.6	90.6
AME (Kcal/kg)	3198	3198	3198	3198
Crude protein	19.84	22.69	19.53	19.92
Cude fibre	2.84	3.33	2.50	2.93
Crude fat	6.53	8.14	6.05	6.65
Ash	4.07	4.42	4.03	4.08
Dig[6] lysine	1.020	1.020	1.019	1.020
Dig methionine	0.497	0.460	0.508	0.494
Dig methionine+cystine	0.800	0.800	0.800	0.800
Dig cysteine	0.298	0.335	0.292	0.301
Dig threonine	0.680	0.727	0.679	0.695
Dig tryptophan	0.253	0.292	0.251	0.254
Dig glycine	0.671	0.798	0.766	0.677
Dig arginine	1.090	1.300	1.089	1.090
Dig serine	0.671	0.798	0.646	0.677
Dig histidine	0.419	0.494	0.414	0.420
Dig isoleucine	0.710	0.845	0.704	0.711
Dig leucine	1.207	1.423	1.194	1.211
Dig phenylalanine	0.804	0.940	0.799	0.805
Dig tyrosine	0.672	0.768	0.678	0.670
Dig valine	0.796	0.942	0.779	0.800
Calcium	0.780	0.780	0.780	0.780
Available phosphorus	0.390	0.390	0.390	0.390

Sodium	0.160	0.230	0.160	0.160
Chloride	0.230	0.160	0.230	0.230
Potassium	0.767	0.900	0.765	0.768
Linoleic acid	1.550	1.724	1.514	1.559
Choline (mg/kg)	1810	2868	1616	1857
Dietary electrolyte balance (mEq/kg)	201	285	200	201

[1]The other diets which were not included in the table were made by adding respective additives on top of the basal control diet.
[2]Diet with increased threonine (Thr) and branched chain amino acid (BCAA) supplementation
[3]Vitamin and mineral premix per kg diet: vitamin A, 12 MIU; vitamin D, 5 MIU; vitamin E, 75 mg; vitamin K, 3 mg; nicotinic acid, 55 mg; pantothenic acid, 13 mg; folic acid, 2 mg; riboflavin, 8 mg; cyanocobalamin, 0.016 mg; biotin, 0.25 mg; pyridoxine, 5 mg; thiamine, 3 mg; antioxidant, 50 mg; Cu, 16 mg as copper sulfate; Mn, 60 mg as manganese sulfate; Mn, 60 mg as manganous oxide; I, 0.125 mg as potassium iodide; Se, 0.3 mg; Fe, 40 mg, as iron sulfate; Zn, 50 mg as zinc oxide; Zn, 50 mg as zinc sulfate.
[4]Xylanase 8000 L, Danisco
[5]Axtra PHY 5000L, Danisco
[6]Digestible amino acid coefficients for raw ingredients were determined by Near-Infra Red spectroscopy (Foss NIR 6500, Denmark) standardized with Evonik AMINONIR® Advanced calibration.

Table 4. Analysed nutrient values of experimental diets (as-is basis)

Feeding phase	Treatment	Dry matter (%)	Gross energy (MJ/kg)	Crude protein (%)
Starter	Negative control	87.08	16.52	22.52
	Positive control	87.08	16.52	22.52
	Whole wheat	86.58	16.37	22.03
	Xylo-oligosaccharide	86.24	16.39	22.86
	High fat	88.37	17.17	24.89
	High carbohydrate	87.08	16.52	22.52
	Threonine + BCAA	86.47	16.63	23.25
	Short chain fatty acids	86.70	16.47	23.81
Grower	Negative control	86.47	16.77	21.17
	Positive control	86.47	16.77	21.17
	Whole wheat	86.70	16.77	21.05
	Xylo-oligosaccharide	87.24	16.96	22.45
	High fat	88.52	17.55	22.49
	High carbohydrate	86.69	16.69	21.30
	Threonine + BCAA	86.70	16.71	21.76
	Short chain fatty acids	84.59	16.74	20.41
Finisher	Negative control	87.14	17.01	20.74
	Positive control	87.14	17.01	20.74
	Whole wheat	86.93	17.01	19.15
	Xylo-oligosaccharide	87.56	17.08	19.43
	High fat	87.23	17.46	21.54
	High carbohydrate	87.05	16.85	19.69
	Threonine + BCAA	85.84	16.97	19.48
	Short chain fatty acids	87.01	16.97	19.23

Table 5. Growth performance of experimental treatments during the starter phase (days 0-10)

Treatment	Weight gain (g)	Feed intake (g)	FCR
Negative control	258[ab]	342[a]	1.298[a]
Positive control	257[ab]	375[bc]	1.436[ab]
Whole wheat	237[a]	377[c]	1.596[b]
Xylo-oligosaccharide	260[b]	359[abc]	1.385[a]
High fat	257[ab]	345[a]	1.337[a]
High carbohydrate	251[ab]	355[ab]	1.421[a]
Threonine + BCAA	262[b]	355[ab]	1.356[a]
Short chain fatty acids	262[b]	370[bc]	1.383[a]
SEM	4.90	4.56	0.04
P-value	**0.015**	**< 0.001**	**< 0.001**

[a,b,c]*Means within columns not sharing a common suffix are significantly different at the 5% level of probability.*

Table 6. Growth performance of experimental treatments during the grower phase (days 10-21)

Treatment	Weight gain (g)	Feed intake (g)	FCR
Negative control	739[b]	1079[ab]	1.463[a]
Positive control	747[b]	1096[ab]	1.469[a]
Whole wheat	665[a]	1130[b]	1.703[b]
Xylo-oligosaccharide	728[b]	1062[a]	1.438[a]
High fat	666[a]	1111[ab]	1.811[b]
High carbohydrate	731[ab]	1069[ab]	1.456[a]
Threonine + BCAA	744[b]	1089[ab]	1.456[a]
Short chain fatty acids	721[ab]	1118[ab]	1.545[a]
SEM	13.17	14.03	0.03
P-value	**< 0.001**	**0.018**	**< 0.001**

[a,b]*Means within columns not sharing a common suffix are significantly different at the 5% level of probability.*

Table 7. Growth performance of experimental treatments during the finisher phase (days 21-35)

Treatment	Weight gain (g)	Feed intake (g)	FCR
Negative control	1443[c]	2348[a]	1.606[a]
Positive control	1421[bc]	2422[abc]	1.735[ab]
Whole wheat	1334[ab]	2703[c]	2.023[d]
Xylo-oligosaccharide	1422[c]	2464[abc]	1.740[ab]
High fat	1330[a]	2641[abc]	1.986[cd]
High carbohydrate	1326[a]	2370[ab]	1.751[abc]
Threonine + BCAA	1418[bc]	2681[bc]	1.897[bcd]
Short chain fatty acids	1408[abc]	2465[abc]	1.756[abc]
SEM	30.21	69.87	0.05
P-value	**0.032**	**0.002**	**< 0.001**

[a,b,c,d]*Means within columns not sharing a common suffix are significantly different at the 5% level of probability.*

Table 8. Growth performance of experimental treatments over the entire study (days 0-35)

Treatment	Weight gain (g)	Feed intake (g)	FCR	Mortality (%)
Negative control	2431[bc]	3721[a]	1.510[a]	4.17
Positive control	2433[c]	3887[abc]	1.590[ab]	2.78
Whole wheat	2235[a]	4211[c]	1.884[c]	5.56
Xylo-oligosaccharide	2410[bc]	3898[abc]	1.622[ab]	4.17
High fat	2252[ab]	4144[bc]	1.840[c]	0.00
High carbohydrate	2311[abc]	3794[ab]	1.614[ab]	4.17
Threonine + BCAA	2424[bc]	4126[bc]	1.705[bc]	8.33
Short chain fatty acids	2394[abc]	3943[abc]	1.650[ab]	0.00
SEM	38.06	83.23	0.04	1.89
P-value	**< 0.001**	**< 0.001**	**< 0.001**	0.147

[a,b,c]*Means within columns not sharing a common suffix are significantly different at the 5% level of probability.*

Table 9. Intestinal lesion scores at day 21

	Lesion Scores		
Treatment	Duodenum	Jejunum	Ileum
Negative control	0.38[a]	0.21	0.00
Positive control	1.90[ab]	0.83	2.54
Whole wheat	2.38[ab]	0.79	0.46
Xylo-oligosaccharide	2.13[ab]	0.58	1.04
High fat	1.95[ab]	0.80	2.13
High carbohydrate	1.40[ab]	0.30	1.46
Threonine + BCAA	2.38[b]	0.88	1.58
Short chain fatty acids	1.63[ab]	0.55	1.46
SEM	0.43	0.18	0.74
P-value	**0.044**	0.098	0.298

[a,b]Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

Table 10. Relative carcass yield on day 35 (g/kg body weight)

Treatment	Breast	Thigh	Drumstick	Abdominal Fat
Negative control	179^{c}	101	87.0	10.43^{bc}
Positive control	173^{bc}	101	87.1	9.14^{ab}
Whole wheat	157^{a}	105	88.9	10.02^{bc}
Xylo-oligosaccharide	175^{bc}	105	88.6	9.68^{abc}
High fat	150^{a}	105	90.2	7.34^{a}
High carbohydrate	169^{b}	103	87.0	11.71^{c}
Threonine + BCAA	171^{bc}	102	87.8	9.66^{abc}
Short chain fatty acids	169^{b}	104	86.1	11.00^{bc}
SEM	2.03	1.54	0.99	0.51
P-value	**< 0.001**	0.299	0.153	**< 0.001**

^{a,b,c}*Means within columns not sharing a common suffix are significantly different at the 5% level of probability.*

Table 11. Relative weights of full and empty gizzard on days 21 and 35 (g/kg body weight)

Treatment	Day 21		Day 35	
	Gizzard full	Gizzard empty	Gizzard full	Gizzard empty
Negative control	25.0^{a}	19.0^{b}	16.6^{a}	12.6^{a}
Positive control	27.1^{ab}	18.4^{ab}	16.8^{ab}	12.6^{a}
Whole wheat	35.8^{c}	22.8^{c}	24.8^{d}	16.8^{b}
Xylo-oligosaccharide	26.7^{ab}	17.9^{ab}	16.9^{ab}	12.9^{a}
High fat	29.7^{b}	19.3^{b}	19.5^{abc}	13.6^{a}
High carbohydrate	27.1^{ab}	17.7^{ab}	18.8^{abc}	13.2^{a}
Threonine + BCAA	25.1^{a}	16.4^{a}	20.0^{c}	13.6^{a}
Short chain fatty acids	28.6^{ab}	18.6^{ab}	19.7^{bc}	13.5^{a}
SEM	0.84	0.57	0.62	0.42
P-value	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**

^{a,b,c}*Means within columns not sharing a common suffix are significantly different at the 5% level of probability.*

Table 12. Intestinal length and diameter on day 21 (cm)

Treatment	Duodenum		Jejunum		Ileum	
	Length	Diameter	Length	Diameter	Length	Diameter
Negative control	25.8[a]	1.83	58.8[a]	1.90	60.5[a]	1.59
Positive control	27.0[ab]	1.78	67.8[ab]	2.23	76.6[b]	1.79
Whole wheat	27.4 [ab]	1.78	65.8[ab]	2.21	71.4[b]	1.73
Xylo-oligosaccharide	27.2 [ab]	1.82	67.2[ab]	2.14	70.3[b]	1.85
High fat	26.5 [ab]	1.91	66.0[ab]	2.18	70.0[b]	1.78
High carbohydrate	27.6 [ab]	1.73	65.0[ab]	2.34	73.3[b]	1.86
Threonine + BCAA	27.7 [ab]	1.82	72.9[b]	2.18	72.2[b]	1.83
Short chain fatty acids	28.8[b]	1.77	68.7[b]	2.14	72.3[b]	1.82
SEM	0.54	0.08	2.04	0.09	1.55	0.19
P-value	**0.054**	0.812	**0.005**	0.075	**< 0.001**	0.082

[a,b]Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

Table 13. Intestinal length and diameter on day 35 (cm)

Treatment	Duodenum		Jejunum		Ileum	
	Length	Diameter	Length	Diameter	Length	Diameter
Negative control	28.5[a]	2.29	68.0[a]	2.26	68.9[a]	1.89
Positive control	31.3[ab]	2.25	80.7[b]	2.50	75.6[ab]	1.92
Whole wheat	32.8[b]	2.22	79.9[b]	2.34	81.3[b]	1.88
Xylo-oligosaccharide	33.8[b]	2.22	78.1[b]	2.39	79.2[ab]	1.85
High fat	33.0[b]	2.23	82.8[b]	2.45	75.9[ab]	1.89
High carbohydrate	30.4[ab]	2.20	75.8[ab]	2.43	77.7[ab]	1.98
Threonine + BCAA	32.2[b]	2.15	80.8[b]	2.27	78.5[ab]	1.74
Short chain fatty acids	33.2[b]	2.25	81.5[b]	2.44	80.9[b]	1.87
SEM	0.73	0.06	2.07	0.08	2.40	0.05
P-value	**< 0.001**	0.874	**< 0.001**	0.289	**0.021**	0.133

[a,b]Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

Table 14. Caecal microbiota of broiler chickens on day 21 (log_{10} [genomic DNA copies/g of caecal contents]) content)

Treatment	*Lactobacillus* spp.	*Ruminococcus* spp.	*Bacteroides* spp.	*Bacillus* spp.	*Bifidobacterium* spp.	Enterobac teriaceae	Total bacteria
Negative control	9.18	10.07	7.73	8.32^{bc}	9.08^{a}	8.96	11.4
Positive control	9.33	10.14	7.59	8.54^{c}	9.74^{cd}	8.84	11.5
Whole wheat	9.34	10.06	7.56	8.40^{c}	9.78^{cd}	8.84	11.4
Xylo-oligosaccharide	9.27	10.11	7.62	7.99^{ab}	9.59^{bcd}	8.65	11.4
High fat	9.44	9.98	7.55	8.36^{c}	9.82^{d}	9.00	11.4
High carbohydrate	9.30	10.14	7.62	7.97^{a}	9.75^{cd}	8.61	11.4
Threonine + BCAA	9.31	10.10	7.69	8.29^{abc}	9.31^{abc}	8.97	11.4
Short chain fatty acids	9.26	10.08	7.72	8.29^{abc}	9.27^{ab}	9.04	11.4
SEM	0.07	0.04	0.05	0.12	0.16	0.15	0.05
P-value	0.374	0.306	0.066	**0.025**	**0.022**	0.322	0.876

^{a,b,c,d}*Means within columns not sharing a common suffix are significantly different at the 5% level of probability.*

Table 15. Caecal microbiota of broiler chickens on day 35 (log_{10} [genomic DNA copies/g of caecal contents]) content)

Treatment	*Lactobacillus* spp.	*Ruminococcus* spp.	*Bacteroides* spp.	*Bacillus* spp.	*Bifidobacterium* spp.	Enterobac teriaceae	Total bacteria
Negative control	9.15	10.02	7.62^{ab}	8.65	10.28	8.66	11.42
Positive control	9.07	9.99	7.52^{a}	8.47	10.30	8.70	11.47
Whole wheat	9.09	10.02	7.62^{ab}	8.65	10.53	8.78	11.48
Xylo-oligosaccharide	9.06	9.97	7.52^{a}	8.62	10.42	8.80	11.45
High fat	9.09	10.00	7.65^{bc}	8.82	10.18	8.56	11.45
High carbohydrate	9.05	10.03	7.58^{ab}	8.57	10.41	8.44	11.49
Threonine + BCAA	9.13	9.97	7.73^{c}	8.62	10.29	8.68	11.42
Short chain fatty acids	9.08	10.05	7.54^{a}	8.68	10.18	8.53	11.49
SEM	0.08	0.03	0.04	0.11	0.10	0.19	0.04
P-value	0.986	0.718	**0.004**	0.617	0.260	0.907	0.697

^{a,b,c}*Means within columns not sharing a common suffix are significantly different at the 5% level of probability.*

Table 16. Oocyst count per gram of feces from days 17 to 28

Treatment	Oocyst count per gram of feces			
	Day 17-19	Day 20-22	Day 23-25	Day 26-28
Negative control	0^{a}	0^{a}	0^{a}	0^{a}
Positive control	$115,585^{ab}$	$57,939^{ab}$	$24,854^{abcd}$	$147,326^{b}$
Whole wheat	$337,982^{c}$	$95,981^{b}$	$27,533^{bcd}$	$152,157^{b}$
Xylo-oligosaccharide	$108,987^{a}$	$81,631^{ab}$	$19,260^{abc}$	$70,799^{ab}$
High fat	$141,157^{ab}$	$89,629^{b}$	$46,604^{cd}$	$180,128^{b}$
High carbohydrate	$137,983^{ab}$	$86,864^{b}$	$18,254^{ab}$	$122,431^{ab}$
Threonine + BCAA	$112,781^{ab}$	$88,936^{b}$	$44,675^{cd}$	$120,439^{ab}$
Short chain fatty acids	$271,111^{bc}$	$85,676^{b}$	$46,781^{d}$	$148,518^{b}$
SEM	33,502	18,139	5,665	28,275
P-value	**< 0.001**	**0.013**	**< 0.001**	**0.002**

^{a,b,c,d}*Means within columns not sharing a common suffix are significantly different at the 5% level of probability.*

Table 17. Individual oocyst count from days 20 to 22

Treatment	Day 20		Day 21		Day 22	
	E. maxima	*E. acervulina*	*E. maxima*	*E. acervulina*	*E. maxima*	*E. acervulina*
Negative control	0^{a}	0^{a}	0^{a}	0	0^{a}	0^{a}
Positive control	767^{b}	$5,620^{ab}$	650^{b}	11,467	450^{b}	$4,183^{c}$
Whole wheat	367^{ab}	$13,017^{b}$	480^{ab}	2,900	250^{ab}	$1,800^{abc}$
Xylo-oligosaccharide	633^{b}	$4,550^{ab}$	280^{ab}	4,680	183^{ab}	$1,167^{ab}$
High fat	820^{b}	$12,350^{ab}$	500^{ab}	5,417	133^{ab}	$1,480^{abc}$
High carbohydrate	620^{b}	$8,717^{ab}$	400^{ab}	8,533	0^{a}	$1,933^{abc}$
Threonine + BCAA	667^{b}	$11,800^{ab}$	600^{b}	7,767	283^{ab}	$3,925^{bc}$
Short chain fatty acids	280^{ab}	$3,017^{ab}$	340^{ab}	9,567	20^{a}	$1,650^{abc}$
SEM	172	2,869	121	2,932	85	570
P-value	**0.033**	**0.018**	**0.017**	0.1632	**0.006**	**< 0.001**

^{a,b,c}*Means within columns not sharing a common suffix are significantly different at the 5% level of probability.*

Table 18. Tibia morphology and ash content on day 35

Treatment	Fresh weight (g)	Air-dry weight (g)	Length (mm)	Diameter (mm)	Breaking strength (N)	Ash as is (%)
Negative control	13.1	7.00	92.2	7.97	400	39.8
Positive control	11.9	6.23	91.8	7.13	371	38.9
Whole wheat	12.1	6.42	90.8	7.02	394	39.4
Xylo-oligosaccharide	11.8	6.28	89.4	7.00	430	40.3
High fat	12.0	6.31	90.0	6.89	378	39.2
High carbohydrate	11.4	6.08	90.8	6.93	392	39.8
Threonine + BCAA	12.8	6.80	90.4	7.06	375	39.8
Short chain fatty acids	12.6	6.73	91.7	7.07	396	40.0
SEM	0.56	0.30	0.78	0.31	22.17	0.37
P-value	0.428	0.341	0.174	0.305	0.676	0.226