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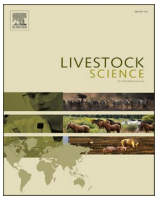

Supplementing functional amino acids and polyphenols at low dose can restore performance and amino acid digestibility in broilers challenged with coccidiosis

Tristan Chalvon-Demersay [a,*], Thanan Yamphet [b], Saksit Srinongkote [b], Hiroyuki Ohara [c], William Lambert [a]

[a] METEX NOOVISTAGO, 32 rue Guersant, 75017 Paris, France
[b] BARC. Bangkok Animal Research Center Co., Ltd., 74/4 Mu 7 Tambon Naiklong Bangplakod, Phrasamutjedi, Samut Prakan 10290 Thailand
[c] Ajinomoto Co., Inc.15-1, Kyobashi 1-chome, Chuo-ku, Tokyo 104-8315, Japan

HIGHLIGHTS

- Coccidiosis challenge impairs performance and nutrient digestibility.
- Functional amino acids and polyphenols mitigate the effect of coccidiosis on performance.
- Functional amino acids and polyphenols mitigate the effect of coccidiosis on nutrient digestibility.

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ABSTRACT

In broiler chickens, commercial intensive practices exacerbate the pressure of gut heath challenges such as coccidiosis. Coccidiosis impairs nutrient digestion, performance, livability of broiler chickens and reduces overall farm profitability. Literature reports that functional amino acids (AA; arginine, threonine, glutamine) and polyphenols could exert beneficial effects in broilers facing this challenge. The current study aimed at evaluating the efficiency of the supplementation of arginine, threonine and glutamine together with grape extract polyphenols (MIX) on performance, AA digestibility and gut morphology in broiler chickens challenged with coccidiosis.

Two hundred eighty-eight (288) newly hatched male broiler chicks of commercial strain (Ross 308) were allocated to 3 treatments with 8 replications. For all except one treatment (Uninfected Untreated Control – UUC), birds were artificially challenged with ADVENT® coccidiosis vaccine 10x dose on day 14. Broiler chickens from the two challenged groups received either no supplementation (Infected Untreated Control – IUC) or a mix of arginine, threonine and glutamine together with grape extract polyphenols at a dose of 1 kg/Ton (IUC+MIX) from day 0 to day 35. Performance and livability were followed during the 35 days of the trial. Lesion scoring, duodenum morphology and fecal oocyst count were assessed on day 21. On day 25 nutrient digestibility was measured on a subset of birds. All data were subjected to an ANOVA and differences among means were detected by multiple range tests.

During the course of the trial, coccidiosis challenge reduced growth (-3 g/d, $P = 0.008$) and feed conversion ratio (-3 pts, $P = 0.013$) without altering feed intake. It also increased total and species related lesion scores and oocyst counts in feces at d 21 ($P < 0.0001$). The supplementation of the additive mix restored performance to the same level as the UUC treatment but had no effect on lesion scores and fecal oocyst count. Interestingly, the coccidiosis challenge reduced villous height in duodenum ($P = 0.016$) which translated into lower digestibility of all AA ($P < 0.05$), except phenylalanine. The addition of the mix mitigated partially the effect of the challenge on digestibility and to a lesser extent on gut morphology. This study reports that the supplementation of a selected combination of functional amino acids and grape extract polyphenols can fully restore performance of coccidiosis-challenged birds with an improvement of AA digestibility. The results of this study suggest that the

* Corresponding author.
E-mail address: tristan.chalvon-demersay@metex-noovistago.com (T. Chalvon-Demersay).

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mode of action of the mix does not rely on a direct effect on lesion scores, oocyst count or gut morphology and still needs to be determined.

1. Introduction

In broiler chickens, coccidiosis is one of the main challenges at the gut level costing when uncontrolled an average of 0.21 euros per bird (Jones et al., 2019). This challenge is caused by the infection of Eimeria species which colonize different parts of the intestinal tract. Coccidiosis is associated with impairment of gut health marked by decreased villus height, number of goblet cells, AA transporters and digestive enzymes activity leading to a lower AA digestion (Tan et al., 2014). In line with these observations, Rochel et al. (2016) have shown that coccidiosis decreases the digestibility of nearly all AA and lowers plasma concentration of arginine, asparagine, glutamine, aspartate. Consistent effects concerning reduced plasma arginine were reported by Allen et al. in parallel of an increase in plasma nitric oxide (Allen and Fetterer, 2000).

In parallel, coccidiosis is associated with an increase in inflammation as indicated by the higher blood levels of cytokines, nitric oxide and IgA production used to fight the parasites (Allen, 1999; Tan et al., 2014). Crypt cell proliferation occurs to replace damaged enterocytes and mucus production is enhanced to form a physical barrier against the pathogens (Bortoluzzi et al., 2018). This latter effect could potentiate Clostridium perfringens colonization as this bacterium can use intestinal mucus as a source of nutrients (Dahiya et al., 2006). All these changes result in decreased feed intake and growth and increase the susceptibility of necrotic enteritis leading to a further decrease in performance and increase in mortality (Dahiya et al., 2006).

The effects of the functional AA glutamine, arginine and threonine when supplemented as a combination have been investigated in Eimeria and E. coli challenged broilers. This study revealed that the supplementation of these amino acids can positively contribute to the regeneration of intestinal mucosa of broilers which translates into better feed efficiency (Gottardo et al., 2016). The promising effects of these amino acids on gut health in broilers have been recently reviewed (Bortoluzzi et al., 2018; Chalvon-Demersay et al., 2021). In addition, re-analysis of existing transcriptomic data from chicken cecal epithelia upon infection by Eimeria tenella (Guo et al., 2013) revealed that the expression of genes encoding for enzymes involved in threonine and arginine catabolism were increased during coccidiosis infection in the cecum confirming the high demand for these amino acids in this context. This observation is related to the versatility of amino acids having the ability to be diverted from growth towards other functions (Brosnan and Rooyackers, 2013). Other studies have reported that grape extract polyphenols could exert beneficial effects in broilers through the modulation of oxidative stress (Farahat et al., 2017; Wang et al., 2008). Indeed, in broilers inoculated with E. tenella Houghton strain, the supplementation of low dose of grape seed extract polyphenols reduced lesion score, plasma and mucosal nitric oxide and increased plasma superoxide dismutase activity. These changes translated into partial restoration of performance (Wang et al., 2008). Similarly, it was reported that supplementation of grape seed extract increased glutathione level in liver tissues, synonym of lesser oxidative stress (Farahat et al., 2017). The ability of AAs to play a role on gut integrity and the one of polyphenols to reduce oxidative stress suggest good synergies between these molecules. A recently published study in corticosterone-challenged chickens seems to confirm this synergy (Barekatain et al., 2021a).

To our best knowledge, no study has investigated the effect of dietary supplementation of these AAs in combination with grape extract polyphenols in chickens facing coccidiosis. This study therefore aimed at investigating the effects of the supplementation of an arginine, threonine, glutamine and grape extract polyphenols mix (MIX) on performance, gut health and nutrient digestibility in broilers challenged with different Eimeria strains.

2. Materials and methods

2.1. Animals and housing

Experiment was conducted in compliance with national Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Two hundred and eighty-eight (288) newly hatched male broiler chicks of commercial strain (Ross 308) were randomly allocated in 24 pens (12 birds per pen). The experiment was conducted in a close-sided house with concrete floor pens using rice hull litter as bedding material. Each pen measured 1.0 m x 1.5 m and was equipped with a tubular feeder and 3 nipple water drinkers to be provide feed and water ad libitum. Birds were maintained under the lighting and management programs according to the Ross 308 broiler management manual. All birds were vaccinated for Newcastle and Infectious Bronchitis diseases at 7 days and Gumboro disease at 14 days of age.

2.2. Feed preparation

Feeding was done in three phases: starter (0–10 days), grower (10–25 days) and finisher phases (25–35 days). The composition and calculated nutrient content of basal diets are presented in Table 1. Diets were formulated in accordance to Rostagno et al. recommendations (2005). Premix composition is available in supplementary material. Diets were processed at a temperature of 85 °C and pelleted with a 3 mm diameter size for all feeding phases.

2.3. Treatments

The trial was arranged with three treatment groups with eight replications using 12 birds in a pen. For all except one treatment (Uninfected Untreated Control – UUC), birds were artificially challenged with ADVENT® (Huvepharma, Sofia, Bulgaria) coccidiosis vaccine 10x dose at day 14 by applying two droplets in the eyes, one in each eye. Broiler chickens from the two challenged treatments received either no supplementation (Infected Untreated Control – IUC) or an L-arginine, L-threonine, L-glutamine and grape extract polyphenols mix (MIX) at a dose of 1 kg/Ton (IUC+MIX) during the whole duration of the trial. MIX contained 95% of L-arginine, L-threonine and L-glutamine and 5% grape extract polyphenols on a weight basis (the exact composition is available upon request). The composition of the mix is similar to the one tested recently (Barekatain et al., 2021a). The AA are considered as “functional AA” since they are provided on top of a diet covering the AA requirement of broiler chickens. The grape extract polyphenols were obtained from seeds and skins and contains around 70% polyphenols on dry material. Addition of the MIX was checked by analyzing free amino acid contents in the feeds. The report and comparison between expected and analyzed values are available in supplementary material.

2.4. Performance and gut morphology

Total pen feed consumption and bird weights were measured as pen basis for the periods: day 0 to day 10, day 10 to day 25 and day 25 to day 35. Dead and culled birds were monitored and recorded daily. Feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were calculated for the periods: day 0 to day 10, day 10 to day 25 and day 25 to day 35 and day 0 to day 35. At 21 days of age, fecal samples were collected and analyzed for coccidia oocyst count and intestinal lesion scoring was measured on two random chickens per pen following

Table 1
Composition and calculated nutrient content of diets.

Ingredients, g/kg	STARTER 0–10d	GROWER 10–25d	FINISHER 25–35d
CORN	553.38	597.71	644.81
DEHULLED SOLVENT-EXTRACTED SOYBEAN MEAL	374.20	326.30	274.80
OIL, SOYA	33.90	41.20	48.40
DICALCIUM PHOSPHATE	21.50	17.90	15.15
DL-MET 99%	3.91	3.36	2.77
SALT	3.76	3.8	2.65
L-LYS HCL 99%	2.70	2.56	2.40
PREMIX*	2.00	2.00	2.00
L-THR 98.5%	1.38	1.2	1.01
CALCIUM CARBONATE	1.06	1.75	2.06
L-VAL 96.5%	1.01	0.83	0.64
COCCIDIOSTAT	0.50	0.50	0.50
CHOLINE CHLORIDE 60%	0.49	0.67	0.88
L-ARG 96.5%	0.11	0.12	0.13
PHYTASE**	0.10	0.10	0.10
SODIUM BICARBONATE			1.70
Total	1000	1000	1000
Characteristic	Value	Value	Value
CRUDE PROTEIN	22.83 (22.6)***	20.82 (21.0)	18.65 (18.6)
CRUDE FAT	6.15	6.95	7.75
CRUDE FIBER	3.47	3.28	3.07
STARCH	35.47	38.31	41.33
Ca	0.96	0.87	0.79
PHOS available POULTRY	0.48	0.44	0.4
K	0.97	0.88	0.79
Na	0.16	0.16	0.16
Cl	0.32	0.33	0.26
CHOLINE	1502.49	1497.96	1501.36
AMEn broiler (kCal / kg)	3000	3100	3200
LYS	1.42 (1.33)	1.27 (1.27)	1.12 (1.13)
THR	0.96 (0.91)	0.87 (0.85)	0.76 (0.76)
MET	0.69 (0.57)	0.61 (0.56)	0.53 (0.49)
CYS	0.35 (0.34)	0.32 (0.32)	0.3 (0.29)
M + C	1.04 (0.9)	0.94 (0.88)	0.83 (0.78)
TRP	0.29 (0.28)	0.26 (0.25)	0.23 (0.22)
ILE	1.13 (1.12)	1.02 (1.03)	0.90 (0.92)
VAL	0.95 (0.98)	0.85 (0.89)	0.75 (0.79)
ARG	1.48 (1.46)	1.33 (1.34)	1.17 (1.19)
GLY+SER	2.22 (2.02)	2.00 (1.84)	1.77 (1.66)
TD THR / LYS	0.67	0.67	0.67
TD MET / LYS	0.51	0.51	0.50
TD CYS / LYS	0.24	0.24	0.25
TD M + C / LYS	0.75	0.75	0.75
TD TRP / LYS	0.20	0.20	0.20
TD VAL / LYS	0.80	0.80	0.80
TD ILE / LYS	0.67	0.67	0.67
TD ARG / LYS	1.05	1.05	1.05
TD GLY+SER / LYS	1.34	1.35	1.37

TD: true digestibility
* Premix composition is available in supplementary data
** Quantum Blue Phytase, AB Vista, Marlborough, UK, 500 FTU, 0.15% available PHOS.
*** XX (XX): Expected values (Analyzed values).

the Johnson and Reid scoring system (Johnson and Reid, 1970) in all groups. Briefly, in this system, lesion scores are noted from zero to four (zero for a normal appearance of the intestine, four for very severe damages) in different parts of the gut for each of the three coccidial species likely to induce lesions in chickens (Eimeria acervulina in duodenum, Eimeria maxima in ileum, Eimeria tenella in cecum). In addition, for the UUC, IUC and IUC+MIX treatments, duodenum was collected from one bird per pen. The collected samples were kept in 10% formalin solution until microscopic examination and measurements of villi heights and crypt depths as previously described (Prakatur et al., 2019).

2.5. Nutrient digestibility

Another subset of two-hundred sixteen (216) birds from UUC, IUC and IUC+MIX treatments were allocated to 18 pens. The growth performance of birds was recorded on days 10, 21, 24 of age. On day 21, all floor pens were modified to grille floor and birds in each group were fed for three days the experimental diets supplemented with 0.3% chromic oxide. On day 25, all birds were sacrificed for ileal digesta collection. The ileal digesta obtained from birds in each cage were pooled, freeze dried and ground. Feed samples and dried ileal digesta were analyzed for crude protein by Kjeldahl method (Sáez-Plaza et al., 2013). Amino acids from the samples were analyzed according to NF EN ISO13903 with the exception of total tryptophan which was measured using MOD.0094 – Repealed standard AFNOR XP V18-114 (Bertocchi et al., 2019).

The ileal digestibility was then calculated based on dry matter using the equation:

$$AIDnutri\ (\%) = \frac{\frac{NUTRId}{CRd} - \frac{NUTRIi}{CRi}}{\frac{NUTRId}{CRd}} * 100$$

Where; AID*nutri* = *Apparent ileal digestibility of nutrient*$NUTRId$ = *Nutrient concentration in diet*$NUTRIi$ = *Nutrient concentration in ileal digesta*CRd = *Chromic oxide concentration in diet*CRi = *Chromic oxide concentration in ileal digesta*

2.6. Statistical analysis

Performance data were analyzed using generalized linear mixed model with treatment as fixed factor and block as a random factor. The barn was divided into 8 blocks, each block including a pen/replication from the 3 treatment groups. This was followed by post-hoc Tukey tests. Pen was used as the experimental unit. For digestibility and gut morphology, data were analyzed using general linear model with treatment as a fixed factor. Oocyst count in fecal samples and lesion scoring were analyzed with Kruskall-Wallis tests. For oocyst count, pen was used as the experimental unit. For lesion scoring, individual chickens (two randomly selected per pen) were used as the experimental unit. Statistical tests were performed using Minitab. The level of significance was set at $P < 0.05$.

3. Results

3.1. Performance

Prior to the challenge, from day 0 to day 10, performance was similar across the three treatments (Table 2). On the contrary, during the following periods (day 10 to day 25 and day 25 to day 35) and over the whole course of the trial, the coccidiosis challenge reduced BWG ($P < 0.05$) and increased FCR ($P < 0.05$) with small or no effect on FI. The supplementation of the MIX fully reversed the negative effect of the challenge (Table 2) so that the performance of these supplemented treatments is similar to the UUC group. No difference across treatment was noticed in terms of mortality or culling rate (data not shown).

3.2. Gut morphology

Gut morphology parameters (villous height, crypt depth and villous height/crypt depth) were assessed in the duodenum of chickens at day 21. Coccidiosis challenge reduced villous height ($P = 0.016$) and villous height/crypt depth ratio ($P = 0.017$) in the duodenum (Fig. 1). Interestingly, the supplementation of the MIX restored partially these two parameters. However, no significant difference can be observed between the IUC and IUC+MIX groups. Crypt depth was not significantly altered by treatments (Fig. 1).

Table 2
Effect of experimental treatments on performance from day 0 to day 10, day 10 to day 25, day 25 to day 35, day 0 to day 35.

	UUC	IUC	IUC+MIX	SEM	Treatment effect	Block effect
Day 0 to day 10						
BWG (g)	243	253	243	2.85	0.225	<0.05
FI (g)	240	249	237	2.71	0.145	<0.05
FCR (g/g)	0.988	0.984	0.975	0.004	0.415	<0.05
Day 10 to day 25						
BWG (g)	1163^{a}	1113^{b}	1150^{a}	6.50	0.001	<0.05
FI (g)	1473	1437	1447	7.08	0.096	<0.05
FCR (g/g)	1.267^{ab}	1.291^{b}	1.258^{a}	0.005	0.010	0.355
Day 25 to day 35						
BWG (g)	1163^{ab}	1108^{b}	1184^{a}	11.20	0.012	0.417
FI (g)	1728	1735	1779	10.20	0.065	0.288
FCR (g/g)	1.486^{a}	1.566^{b}	1.503^{a}	0.009	<0.001	<0.05
Day 0 to day 35						
BWG (g)	2569^{a}	2474^{b}	2576^{a}	18.94	0.003	0.428
FI (g)	3450	3431	3459	19.60	0.826	0.298
FCR (g/g)	1.343^{a}	1.387^{b}	1.343^{a}	0.007	0.006	<0.05
FBW (g)	2614^{a}	2519^{b}	2621^{a}	14.4	0.003	0.417

UUC: uninfected untreated control, IUC: infected untreated control, BWG: body weight gain, FI: feed intake, FCR: feed conversion ratio, FBW: final body weight. Values with different superscripts within a line differ significantly:.

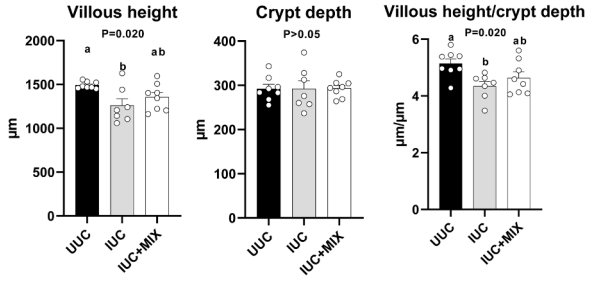

Fig. 1. Effect of the UUC, IUC and IUC+MIX treatments on duodenum morphology, UUC: uninfected untreated control, IUC: infected untreated control.

3.3. Lesion scores and oocyst count

Coccidiosis challenge increased the lesion scores of all Eimeria species as well as oocyst excretion in the feces ($P < 0.0001$, Table 3). Interestingly, the supplementation of the mixture reduced Eimeria acervulina lesion scores but did not alter the lesion scores for other species and total lesion scores as well as oocyst count in the feces compared to the IUC group (Table 3).

3.4. Protein and amino acid digestibility

Protein and amino acid digestibility were assessed on day 25 after a three-day trial performed on a subset of chickens from the UUC, IUC and IUC+MIX groups. The coccidiosis challenge reduced the digestibility of most amino acids except phenylalanine and proline for which the decrease is only numerical (Fig. 2). On average, the coccidiosis challenge reduced the AA digestibility by 5.8% points ($P < 0.0001$). The supplementation of the MIX partially restored the digestibility of cystine, valine and histidine and mitigated efficiently the negative effect

Table 3
Effect of the treatments on lesion scores and oocyst count at day 21.

	UUC	IUC	IUC+MIX	SEM	Treatment effect
Lesion scores					
Eimeria acervulina (duodenum)*	0.563^{a}	2.875^{b}	2.250^{c}	0.144	<0.0001
Eimeria maxima (ileum)	0.375^{a}	1.500^{b}	1.250^{b}	0.164	<0.0001
Eimeria tenella (cecum)	0.000^{a}	0.625^{b}	0.688^{b}	0.090	<0.0001
Total	0.938^{a}	5.000^{b}	4.188^{b}	0.318	<0.0001
Oocyst count					
Oocyst count (Log (oocyst)/g of feces)	3.635^{a}	4.834^{b}	4.831^{b}	0.118	<0.0001

*: lesion scores are noted from zero to four (zero for a normal appearance of the intestine, four for very severe damages), UUC: uninfected untreated control, IUC: infected untreated control.

of the coccidiosis challenge on the digestibility of lysine, threonine, glycine (Fig. 2). On average, the supplementation of the MIX increased digestibility of all amino acids relative to the IUC group by 2.4% points ($P = 0.017$).

4. Discussion

This study aimed at investigating to which extent the supplementation of an arginine, threonine, glutamine and grape extract polyphenols mix (MIX) could counteract the negative effect of a coccidiosis challenge on performance, gut morphology and nutrient digestibility.

Contrary to published literature that usually reports high doses of amino acid supplementation (e.g. >0.5% as fed basis) (Gottardo et al., 2016; Barekatain et al., 2021b), a more practical level of supplementation of the MIX was tested (0.1% as fed basis). The results showed that the addition of the MIX mitigated the effect of the challenge on performance and partially restored protein and amino acid digestibility but did not or poorly mitigated the effect of the challenge on gut morphology, lesion scores or oocyst excretion.

In accordance with the literature, our study showed that the challenge is associated with an impairment of gut morphology marked by decreased villous height, villous height/crypt depth ratio (Kettunen et al., 2001; Tan et al., 2014) and protein and amino acid digestibility (Rochell, 2015) which translated into lower performance. We can hypothesize that the lower digestibility could be related to the decreased expression of amino acid transporters. Indeed, it was shown that expression of the brush border membrane AA transporters EAAT3, bo+AT, CAT2 and B°A are downregulated in the duodenum of birds during an Eimeria acervulina infection (Paris and Wong, 2013; Rochell, 2015; Su et al., 2015). Gene expression measurements via qPCR or western-blot could help verifying this hypothesis.

Logically, the infection was associated with increased lesion scores in the different parts of the gut and higher oocyst excretion in the feces. Interestingly, the supplementation of the MIX partially restored the digestibility of protein and amino acids. The correlation between the loss of apparent digestibility due to the coccidiosis challenge and the recovery of this apparent digestibility in response to the supplementation of the MIX suggests that the solution can counteract the loss of digestibility of an amino acid all the more that its digestibility is negatively impacted by the challenge. This effect could be related to the stimulating effect of amino acids on nutrient transporter expression in the gut. Indeed, it has been reported that glutamine supplementation increased the expression of several amino acid transporters in piglet jejunum and ileum: ASCT2, b0+, y0LAT1 and EAAC1 (He et al., 2016). However, evidence of such effects in broilers are scarce (Barekatain et al., 2021b; Zhang et al., 2019). Therefore, to which extent amino acid supplementation can modulate nutrient transporter expression in broilers still needs further investigation. In addition, as we supplemented a mix, it is

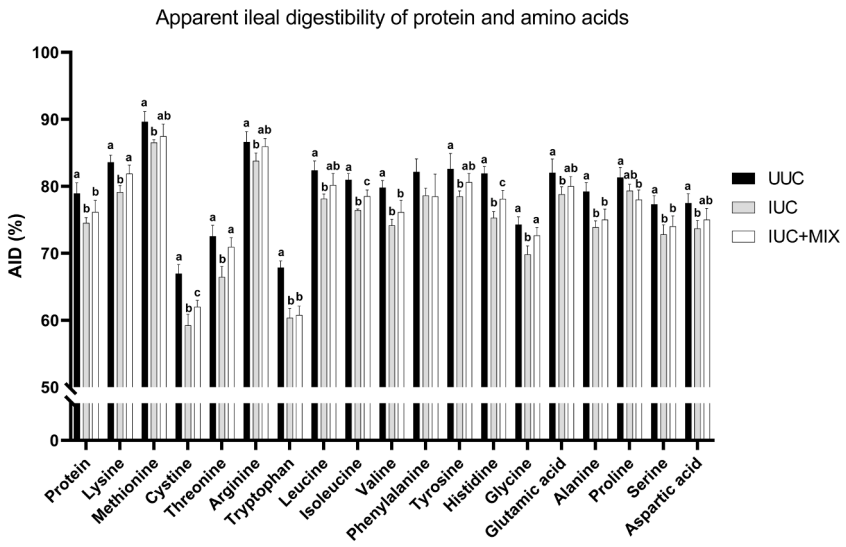

Fig. 2. Effect of the UUC, IUC and IUC+MIX treatments on apparent ileal digestibility of protein and amino acids.

difficult to conclude about which specific component is really driving the response. This aspect would require further research as well.

Logically, the infection was associated with increased lesion scores in the different parts of the gut and higher oocyst excretion in the feces. Some studies reported that supplementation of arginine can have a direct effect on the reduction of oocyst excretion (Allen, 1999). Indeed, it was shown that either single or dual daily doses of L-Arg (500 mg/kg of BW) reduced oocyst shedding of Eimeria tenella. In our study, the supplementation of the MIX did not affect this parameter which could be related to the different dose of supplementation and the method of administration used. Similarly, accumulating evidence show that L-Gln or L-Arg supplementation can improve gut morphology in a context of coccidiosis challenge suggesting trophic effect on intestinal epithelium of these amino acids (Laika and Jahanian, 2017; Luquetti et al., 2016). The supplementation of the MIX had only little effect on these parameters. Both the choices of the gut segment observed, and dose of supplementation used could explain these inconsistencies.

Even if digestibility and gut morphology were partially restored and oocyst count and lesion scores were not altered by the supplementation of the MIX, the performance of the animals was restored to the level of the uninfected birds. This suggests additional benefits of the supplementation that could not be grasped by the analysis performed in the trial. Published literature on the effect of the supplementation of single ingredient from the AA-based MIX on parameters of gut health can help us formulate hypothesis which would need to be verified in future studies. It is indeed possible that the supplementation of the MIX downregulated inflammation thus preventing the diversion of energy and amino acids from growth to immune system and in turn contributing to improved performance. In line with this hypothesis, it has been reported in two different studies that increased level of dietary threonine is associated with a downregulation of the expression of IL-1β, an interleukin controlling inflammation cascade (Wils-Plotz et al., 2013; Chen et al., 2017). In addition, as reported in the introduction, grape seed extract supplementation has been reported to downregulate oxidative stress which can in turn contribute to lower inflammation (Wang et al., 2008; Farahat et al., 2017) as these two parameters are tightly linked (Chen et al., 2008). Finally, grape seed extract supplementation decreased nitric oxide production (Wang et al., 2008), potentially increasing the availability of its precursor, arginine for growth. This lower inflammation in response to the supplementation of the MIX could rely on the improvement of several parameters related to gut health such as gut integrity and mucin synthesis. In line with this hypothesis, L-Thr supplementation has been associated with increased expression of MUC2 in the ileum (Chen et al., 2017), and L-Arg supplementation increased the density and number of goblet cells in the jejunum of coccidiosis- challenged birds (Tan et al., 2014). The effect of amino acids can also rely on their ability to support immune response and immunoglobulin synthesis. It has been reported that supplementing the diet with 1% L-Gln increased spleen relative weight, IgA, IgG, IgM concentrations, and anti-SRBC titers in broiler chicks (Bartell, 2006). In a rodent model, L-Arg supplementation was reported to activate innate immune system, including secretory IgA and mucins (Ren et al., 2014). In addition, L-Arg supplementation was associated with improved parameters related to microbiota balance such as reduced Firmicutes: Bacteroidetes ratio and increased abundance of *Lactobacillus* (Ren et al., 2014). The microbiota modulating effect of amino acids could therefore also account for the beneficial effect of the supplementation of the MIX. On this latter aspect, both the possibility of condensed polyphenols from grape extract to reach the lower part of intestine as reported in a rat model (Tsang et al., 2005) and to bind specifically to arginine through hydrogen bonds (Adamczyk et al., 2017) suggest that the supplementation of the combination of grape extract and arginine could also potentiate the effect of arginine in the large intestine. However, this hypothesis would need further investigation.

5. Conclusions

In summary, this work confirms the deleterious effect of coccidiosis challenge on performance, gut morphology and nutrient digestibility in broiler chickens. It also reveals that the supplementation of a combination of versatile and functional amino acids together with polyphenols supplemented at a low dose (0.1% as fed basis) can mitigate the effect of the challenge on performance by restoring nutrient digestibility and potentially modulating other parameters such as immune and oxidative stress status and microbiota balance. The understanding of the full mode of action of the mix warrants further research.

CRediT authorship contribution statement

Tristan Chalvon-Demersay: Conceptualization, Formal analysis, Writing – original draft. **Thanan Yamphet:** Investigation, Formal analysis, Writing – review & editing. **Saksit Srinongkote:** Investigation, Formal analysis. **Hiroyuki Ohara:** Funding acquisition, Writing – review & editing. **William Lambert:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The research was funded by Ajinomoto Co. Inc. The authors declare no direct conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.livsci.2021.104769.

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