Original Research Article

Potential of guar gum as a leaky gut model in broilers: Digestibility, performance, and microbiota responses

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ABSTRACT

Diet is a major modulator of animal resilience and its three pillars: host's immune response, gut microbiota, and intestinal barrier. In the present study, we endeavour to delineate a challenging condition aimed to degrade these pillars and elucidate its impact on broiler performance and nutrient digestibility. To attain this objective, we opted to use guar gum (GG) as a source of galactomannan. A series of three in vivo experiments were conducted employing conventional or semi-purified diets, supplemented with or without GG during the grower phase (14—28 d). Our findings demonstrate a substantial decline in animal performance metrics such as body weight (reduced by 29%, $P < 0.001$), feed intake (decreased by 12%, $P < 0.001$), and feed conversion ratio (up to 58% increase, $P < 0.001$) in the presence of GG at 2%. The supplementation of a semi-purified diet with incremental doses of GG resulted in a linear reduction ($P < 0.001$) in the apparent total tract digestibility of dry matter and apparent metabolisable energy. Additionally, a marked reduction in ileal endogenous losses, as well as apparent and standardised digestibility of all amino acids with varying proportions ($P < 0.05$), was observed. These alterations were accompanied by disrupted gut integrity assessed by fluorescein isothiocyanate-dextran (FITC-d) ($P < 0.001$) as well as an inflammatory status characterised by elevated levels of acute-phase proteins, namely orosomucoid and serum amyloid A in the sera ($P = 0.03$), and increased mRNA expression levels of *IL-1, IL-6, IL-8, Inos*, and *K203* genes in the ileum, along with a decrease in IgA levels in the gut lumen ($P < 0.05$). Microbial ecology and activity were characterised by reduced diversity and richness (Shannon index, $P = 0.005$) in the presence of GG. Consequently, our results revealed diminished levels of short-chain fatty acids ($P = 0.01$) and their producer genera, such as *Clostridium_XIVa* and *Blautia*, in the gut caeca, coupled with excessive accumulation of lactate (17-fold increase, $P < 0.01$) in the presence of GG at 2%. In addition to providing a more comprehensive characterisation of the GG supplementation as a leaky gut model, our results substantiate a thorough understanding of the intricate adjustments and interplay between the intestinal barrier, immune response, and microbiota. Furthermore, they underscore the significance of feed components in modulating these dynamics.

1. Introduction

Dietary components can influence animal growth, performance and intestinal health through their direct impact on the gut microbiota, intestinal barrier, and host immune balance (Alexander and Turnbaugh, 2020; Usuda et al., 2021). This triangular relationship is complex and variable but may represent an illustrative example of dynamic indicators of resilience (DIOR; van der Zande et al., 2020). The composition and activity of microbiota respond to dietary modulation (David et al., 2014). For instance, a high-fat diet reduces gut microbiota diversity (Kolodziejczyk et al., 2019; Wolters et al., 2019) which is reflected by a reduction of the relative

abundances of Bacteroidetes and increasing Firmicutes and Proteobacteria (Hildebrandt et al., 2009; de Meyer et al., 2019; Zhang et al., 2012). A high level in richness and diversity is pivotal for microbiota stability (Tap et al., 2015). It also ensures the finely regulated crosstalk between the host and the microbiota by modulating gut barrier functions and mucosal immune response, thereby influencing animal health and performances (Diaz Carrasco et al., 2019; Gilbert et al., 2018; Round and Mazmanian, 2010).

Numerous dietary (Osselaere et al., 2013; Tellez et al., 2015; Vicuña et al., 2015), environmental (Santos et al., 2019, 2014), and managemental (Baxter et al., 2019) stressors have been reported to induce a functional disturbance in tight junctions (TJ) causing increased intestinal permeability and inflammation. Nonetheless, the induced intestinal inflammation may be present in birds without clinical symptoms (Santos et al., 2021). Certain plant-based feed ingredients rich in non-starch polysaccharides (NSP) such as rye can increase viscosity resulting in digestibility and performance reduction due to limited access to endogenous enzyme, bacterial overgrowth, and intestinal inflammation (Vicuña et al., 2015). Several authors (Santos et al., 2021; Tellez et al., 2014; Vicuña et al., 2015) have used various levels of rye as a "leaky gut" model in birds. In this context, guar gum (GG) which contains β-galactomannan at 99% (Sharma et al., 2021), may also induce "leaky gut" and growth depression in broiler chickens (Sathe and Bose, 1962; Vohra and Kratzer, 1964). Several mechanism of β-galactomannan-induced poor growth performance can be involved but the most discussed mode of action is the poor nutrient absorption via high intestinal viscosity (Rainbird et al., 1984). Indeed, high intestinal viscosity has a wide-ranging influence on digestive enzyme activities and accessibility to nutrients, thereby drastically reducing the digestibility of all nutrients (Almirall et al., 1995; Smits et al., 1997). The negative repercussions of high intestinal viscosity are further aggravated by reduced glucose and water absorption up to 35% and 40%, respectively (Rainbird et al., 1984). Nunes and Malmlöf (1992) did not only validated the negative influence of β-galactomannan on glucose absorption but also the secretion of hormone involved in metabolism regulation including insulin, glucagon, and insulin-like growth factor 1. In addition, β-galactomannans can also bind and stimulate pattern recognition receptors (PRR) and activate innate cells which initiate inflammatory responses (Serrano-Gómez et al., 2004).

In chicken, most of the recent studies addressing the improvement of animal resilience focused primarily on zootechnical performances with very limited health and physiological parameters such as genetic background (Bedere et al., 2022), biodiversity (Fiorilla et al., 2022), pathogens and lesion scores (Santos et al., 2022; Wijnen et al., 2022) due to the complexity and dynamics of the numerous biological networks involved in animal health and resilience (Scheffer et al., 2018). Therefore, in the current study, we investigate whether GG supplementation degrades the broilers' performances by affecting gut integrity as well as other biological systems, such as, the immune response and the microbial ecology which can aggravate the impact on digestibility. We hypothesized, herein, that supplementation GG in broiler diets in higher amounts (2%) than those commonly found in raw material (approximately 0.3%; Hsiao et al., 2006) may trigger an exacerbated intestinal inflammation accompanied of an increase of the digesta viscosity which disrupts the caecal microbial ecology, therefore, culminating in a "leaky gut".

2. Materials and methods

2.1. Animal ethic statement

This study consisted of three independent in vivo experiments. They were realised at the Centre for Expertise and Research in Nutrition (CERN), Adisseo France S.A.S. All experimental procedures were performed according to legislation governing the animal welfare and research. They were approved by the animal welfare committee CEEA-002 under the project number 03488.01 and certified by the French government to conduct animal experiments under the authorisation G-03-159-4.

2.2. Experiment 1 − dry matter digestibility

A total of 72 one-day-old male Ross 308 chicks were obtained from a commercial hatchery and were fed a common starter diet from 1 to 13 d of age. On d 14, the broilers were randomly distributed into 4 dietary groups. Each group comprised 6 replicate cages containing 3 birds. From 14 to 24 d of age, the chickens from the dietary treatments received semi-purified diets based on wheat starch, corn, corn gluten meal, and β-casein (Table S1) containing either 0% (control), 2%, 4%, or 6% of GG as a purified source of β-galactomannan (Procol U special, Habgen Guargums Limited, Pakistan). Feed and water were provided ad libitum throughout the experiment. Body weight (BW) and feed intake were measured at d 13 and 24. From d 20 to 23, the excreta, pooled by cage, were collected, and stored at −20 °C to evaluate the apparent metabolisable energy (AME) and apparent total tract digestibility (ATTD) of dry matter. Lyophilised representative samples of each experimental diet were analysed for dry matter, crude protein, crude fat, ash, Ca, total P, and gross energy (GE, Table S2). Dry matter, crude protein, crude fat, and ash were determined using the method AOAC procedure (method 934.01; AOAC, 2006) and GE content was determined using the standardised methodology (methods 940.15 and 990.03; AOAC, 1990). Apparent metabolisable energy (AME), corresponding to the difference between the amount of ingested GE and the amount of excreta energy, was determined as previously described in Cozannet et al. (2019).

2.3. Experiment 2 − amino acids digestibility

A total of 150 one-day-old male Ross 308 broiler chicks were reared on a common starter diet from d 1 to 10. On d 11, the birds were divided into a control (CTRL) fed a standard diet, and a GG group fed a control diet supplemented with GG at 2% from 11 to 21 d. Both diets were isocaloric and iso-proteic and fed ad libitum. The composition and nutrient specifications of experimental diets are presented in Table S3. On d 21, all the birds from each group were weighed and further subdivided into 2 sub-groups, each containing 6 replicates of 4 birds. Birds from all the weight ranges were selected for each sub-group. All the birds were subjected to 12 h fasting period to ensure an empty gut before initiating the next dietary regimen. One sub-group within CTRL and GG groups received the same diet as before, while the other sub-groups were fed a nitrogen-free diet (Table S3). The re-feeding of the new dietary regime continued 1 h post fasting. After 4 h, all the birds were euthanised by intravenous injection of sodium pentobarbital (0.2 mL/kg of BW). The body cavity was immediately opened to collect ileal digesta from the ileum, determined as the section between Meckel's diverticulum and the caeca. Feed intake and BW were recorded during the experimental period to calculate BW gain and FCR. Diets and digesta samples were analysed for GE, amino acids, and TiO$_2$ contents. GE content of the experimental groups was determined using standardised methodology 930.15 and 990.03. Amino acid content in each group was measured by cation exchange chromatography after acid hydrolysis for 24 h (Directive 98/64/CE, 3/09/99, Norm NF EN ISO, 2005). Analysis of methionine was performed after initial oxidation of samples with performic acid while phenylalanine was analysed without oxidation. Dietary DL-Met and OH-Met were analysed using the methods previously described by Agostini et al. (2016). Briefly, feed samples were

grounded at 0.5 mm for added methionine sources extraction. OH-Met was extracted using water—methanol solution under stirring. The solution was treated under alkaline solution to hydrolyse oligomers and then neutralised before HPLC injection using a reverse phase column. The OH-Met peak was detected using UV detection at 214 nm. For DL-Met, extraction was done with 0.1 mol/L HCl solution containing thiodiglycol and adjusted to pH 2.2 by dilution in a citric/citrate buffer. DL-Met was separated using ion-exchange chromatography and determined after post column ninhydrin derivatisation with colorimetric detection at 570 nm. Titanium was determined by the procedure of Short et al. (1996). These values were used to determine the ileal endogenous amino acids (IEAA), apparent ileal amino acid digestibility (AIAAD), and standardised ileal amino acid digestibility (SIAAD) of amino acids by the method described by Adedokun et al. (2016).

2.4. Experiment 3 — gut health

A total of 176 male Ross 308 broilers at 14 d old were assigned to 2 experimental groups with 4 replicates for each group in floor pens containing 22 birds in each replicate pen. Broilers, previously fed a common starter diet (1—14 d) received a CTRL or a GG diet (2% GG) during the grower phase (14—28 d), as shown in Table S4. The experimental diets were iso-proteic and isoenergetic and were pelleted at a maximal temperature of 70 °C and cut into 2.5 mm pellets. The BW was measured individually on the 1st, 7th, 14th, and 28th d of age and the feed intake, the average daily weight gain, and the FCR were measured and calculated on weekly basis.

For caecal contents analysis, 3 birds of the average body weight were sampled from each replicate pen at d 28. Caecal contents were immediately preserved using sampling kits (BioFreeze, Alimetrics Diagnostics Ltd., Espoo, Finland) following the recommended protocol by the manufacturer to perform microbiota, ELISA and fermentation metabolites analysis as follows: short and branched chain fatty acids (SCFA and BFCA, respectively), ammonia, calprotectin, and immunoglobulin A (IgA). SCFA, lactic acid, BCFA were determined by gas chromatography using pivalic acid as an internal standard (Apajalahti et al., 2019). Ammonia was quantified using Weatherburn's colorimetric method (Weatherburn, 1967). Sequencing of 16S rRNA was performed on the Illumina platform (Alimetrics Diagnostics Ltd., Espoo, Finland) using primers for the V3-V4 16S hypervariable region (341F-785R) described by Thijs et al. (2017). We used Microbiome Analyst to calculate the alpha diversity indexes (Chao1, Shannon, and Simpson) and the beta diversity metrics (Bray—Curtis) as well as to graphically compare the gut microbial composition at taxonomic levels. Alpha 1 acid glycoprotein (α1GP), serum amyloid A protein (SAA), IL-6 and IL-10 cytokines were quantified in sera of broilers (at d 28) by ELISA kits from Abcam (#ab157690 kit, Abcam, Cambridge, UK) and G bioscience (#IT1361, #IT1317 and #IT1304 kits, St Louis, Missouri, US) according to the provider's instructions. Quantitative PCR of *Cd3, K203, Inos, IL-1, IL-6,* and *IL-8* genes was conducted on 12 ileal samples per experimental group at d 24 using SYBR Green GoTaq qPCR Master Mix (Promega, Madisson, Wisconsin, US). To measure gut integrity, we performed an identical independent experiment. After 24 h of fasting, fluorescein isothiocyanate dextran (FITC-d, Sigma—Aldrich, St Louis, Missouri, US) was administrated by gavage (8.32 mg/kg) to 60 broilers from CTRL and GG groups at d 21. Broilers' sera were collected 1 h after gavage and FITC-d concentrations were measure by fluorescence as described by Vuong et al. (2021).

2.5. Statistical analysis

In Exp. 1, the ATTD of dry matter was calculated according to the following equation:

$$ATTD \ (\%) = [(DM_i - DM_e)/DM_i] \times 100,$$

where DM_i = total dry matter intake (g) and DM_e = total excreta nutrient dry matter output (g), both corresponding to the period of faecal collection. All data from the Exp. 1 were analysed using GLM procedures of SAS software (version 9.1.3, SAS Institute, Inc., Cary, North Carolina, US) where cages or pens was used as the experimental unit. Linear and quadratic responses with increasing GG dose were analysed using orthogonal polynomials.

In the Exp. 2 and 3, the zootechnical performances were analysed by ANOVA. Significant means were separated by using Tukey's test and cytokines' expressions were analysed by two tailed *t*-test. Kaplan—Meyer test and presentation were used for survival results and Wilcoxon test was used to compare SCFA levels in gut contents. Beta diversity metrics based on the Bray—Curtis dissimilarities were visualised using principal coordinate analysis (PcoA) and analysed using multivariate permutational ANOVA (PERMANOVA). LEfSe algorithm was used to test the differential abundances among treatments using the Kruskal—Wallis rank sum. Correlations between BW and alpha diversities were evaluated by using Spearman's test. All statistical analyses were performed by using JMP statistical software (SAS Institute, Cary, North Carolina, USA). Statistical significance was set at $P < 0.05$.

3. Results

3.1. Guar gum reduces the ATTD of dry matter, AME, and broilers performances

To explore the effects of GG on broilers digestibility, we initially quantified the ATTD of dry matter and AME in broilers fed semi purified diets supplemented with incremental doses of GG from 0% to 6% (Exp. 1). The supplementation of GG in diet results in a linear decrease ($P < 0.001$) in ATTD of dry matter and AME (Fig. 1A—B). Dry matter digestibility and energy utilisation decreased by 1% and 60 kcal/kg DM, respectively, for each 1% of GG included in the diet. Additionally, both linear and quadratic ($P < 0.01$) decreases are also observed for feed intake and BW gain (BWG) with an increased dietary dose of GG (Fig. 1C—D). Due to the extremely low BWG (Fig. 1D) and animal welfare conditions in presence of GG at 4% and 6%, we limited the GG supplementation at 2% in the next experiment.

3.2. Guar gum reduces differentially the AID of amino acids

To further explore the effect of GG on broilers' performances, we performed the second in vivo experiment to assess the amino acids digestibility in presence of GG at 2% from d 10 to 21. The performance data show no impact of GG supplementation on feed intake during the experimental period. Nevertheless, BWG and FCR are negatively affected in the GG group compared with CTRL ($P < 0.05$, Table 1) with a decrease of 36% in BWG and an increase of 58% in FCR. A negative effect of GG supplementation on AIAAD is also noticed for all the indispensable and indispensable amino acids ($P < 0.05$, Table 2). The highest percent differences in AIAAD between GG and CTRL conditions are Met, within indispensable amino acids, and Cys, within dispensable amino acids ($P < 0.05$). Notably, similar differences are observed in SIAAD of both indispensable and dispensable amino acids where the difference in SIAAD for indispensable and dispensable amino acids is decreased in the GG diet compared with the CTRL diet (Table 3). The IEAA flow data showed that Leu, Lys, Thr, Val, and Arg are the most abundant in endogenous losses (Table 4). The quantitative difference for IEAA shows higher losses of amino acids in birds from GG group compared to the CTRL birds. For, Gln/Glu and Asp are more
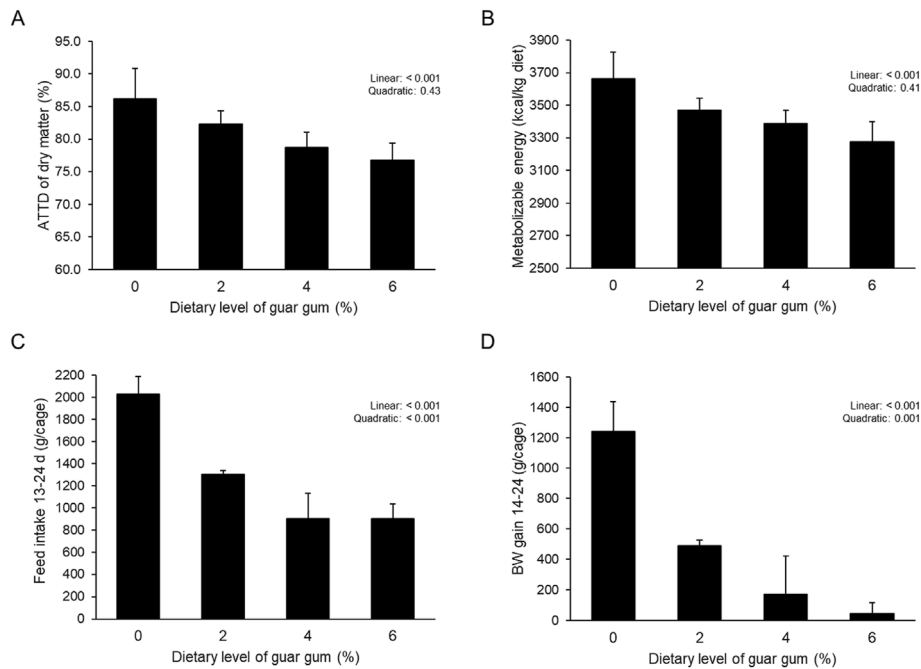
Fig. 1. Effects of dietary levels of guar gum on apparent total tract digestibility (ATDD) of dry matter, metabolisable energy, feed intake, and BW gain in broilers. Apparent total tract digestibility (A), metabolisable energy (B), feed intake (C), and BW gain (D) in broilers fed semi-purified diets from 13 to 24 d of age. Values are least square means ($n = 6$ cages per treatment), with their standard deviations represented by vertical bars. Linear and quadratic responses with increasing guar gum dose were analysed using orthogonal polynomials. Statistical significance was set at $P < 0.05$.

Table 1
Performance results of broilers fed control (CTRL) or guar gum (GG) supplemented diet (2%).

Item	CTRL	GG	SEM	*P*-value	Difference, %
10−17 d					
Feed intake, g	502	508	8.4	0.590	+1.23
BWG, g	401	293	8.3	0.001	−26.93
FCR	1.25	1.75	0.059	0.001	+40.00
17−21 d					
Feed intake, g	422	409	7.7	0.239	−3.08
BWG, g	301	156	6.1	0.001	−48.17
FCR	1.40	2.64	0.073	0.001	+88.57
10−21 d					
Feed intake, g	924	918	15.1	0.756	−0.64
BWG, g	703	450	12.7	0.001	−35.98
FCR	1.31	2.06	0.052	0.001	+57.25

BWG = body weight gain; FCR = feed conversion ratio.

Table 2
Apparent ileal amino acid digestibility (%) of broilers fed control (CTRL) or guar gum (GG) supplemented diet (2%).

Amino acids	CTRL	GG	SEM	*P*-value	Difference, %
Indispensable					
Arg	85.7	77.1	1.71	0.005	10.02
His	79.4	67.9	2.36	0.006	14.48
Ile	74.5	60.8	2.82	0.006	18.41
Leu	77.5	64.3	2.64	0.005	17.02
Lys	84.7	75.4	1.86	0.005	10.99
Met	79.4	63.8	2.94	0.003	19.74
Phe	78.5	66.0	2.50	0.005	15.98
Thr	76.3	65.7	2.21	0.006	13.94
Trp	75.3	61.3	2.58	0.003	18.58
Val	72.5	58.6	2.92	0.007	19.14
Average	78.4	66.1			15.83
Dispensable					
Ala	76.7	60.2	3.22	0.004	21.50
Asp	76.8	65.8	2.25	0.006	14.35
Cys	68.3	47.5	3.12	0.008	30.44
Glu/Gln	80.9	67.8	2.33	0.002	16.23
Gly	74.2	60.6	2.41	0.002	18.27
Pro	75.6	60.7	2.67	0.002	19.61
Ser	76.8	65.2	2.36	0.006	15.11
Tyr	79.1	69.2	2.37	0.014	12.48
Average	76.0	62.1			18.50

abundant in endogenous losses of dispensable amino acids in both treatment groups with quantitively higher losses in the GG group.

3.3. Effects of guar gum on broilers gut health

To better understand the effect of GG beyond digestibility and explain performance impairment, we explored the effects its supplementation on chicken gut health in a third trial. As in the previous experiments, the supplementation of 2% of GG drastically degraded the broilers' performances. Compared with the CTRL group, the individual BW of broilers from the GG group are significantly lower at 21 and 28 d (−26% and −29%, respectively, $P < 0.001$, Fig. 2A and Fig. S1B). The BWG and the average daily gain show similar differences (Fig. 2 B−C and Figs. S1C−D) during the grower period (14−28 d). Broilers from the GG group also present a significantly lower feed intake compared with the control birds

(2378.3 g vs. 2098.4 g, $P < 0.001$, Fig. 2D and Fig. S1E). Furthermore, FCR deteriorated with GG supplementation (1.807 vs. 1.267, $P < 0.001$, Fig. 2E and Fig. S1F).

3.4. Guar gum induce gut inflammation

Besides performances decrease, GG supplementation in diets highly disrupted gut integrity as shown with the increased concentrations of FITC-d broilers sera after oral administration

Table 3
Standardised ileal amino acid digestibility (%) of broilers fed control (CTRL) or guar gum (GG) supplemented diet (2%).

Amino acids	CTRL	GG	SEM	P-value	Difference, %
Indispensable					
Arg	86.6	78.6	1.74	0.008	9.31
His	80.5	69.7	2.45	0.01	13.41
Ile	76.3	63.5	2.86	0.01	16.83
Leu	78.5	65.9	2.67	0.007	15.95
Lys	85.6	76.9	1.92	0.009	10.10
Met	81.2	66.8	3.05	0.007	17.79
Phe	79.7	67.8	2.55	0.008	14.95
Thr	79.7	70.3	2.25	0.014	11.79
Trp	78.3	65.4	2.72	0.007	16.38
Val	74.9	62.1	2.96	0.012	17.04
Average	80.1	68.7			14.36
Dispensable					
Ala	78.2	62.6	3.27	0.007	19.98
Asp	78.4	68.2	2.30	0.013	13.10
Cys	72.5	52.6	3.20	0.001	27.43
Glu	81.9	69.3	2.37	0.003	15.37
Gly	76.4	63.9	2.47	0.005	16.40
Pro	77.4	63.4	2.69	0.004	18.07
Ser	79.3	68.7	2.39	0.01	13.40
Tyr	80.9	71.8	2.40	0.023	11.20
Average	78.1	65.1			16.87

Table 4
Ileal endogenous amino acid losses (mg/kg DMI) in 21-d-old broilers fed control (CTRL) or guar gum (GG) supplemented diet (2%) and diets are nitrogen free.

Amino acids	CTRL	GG	SEM	P-value
Indispensable				
Arg	150[a]	232[b]	16.5	0.005
His	71[a]	110[b]	9.4	0.014
Ile	173[a]	254[b]	17.3	0.007
Leu	196[a]	326[b]	27.2	0.007
Lys	140[a]	234[b]	16.6	0.002
Met	56[a]	92[b]	7.7	0.007
Phe	144[a]	215[b]	16.6	0.012
Thr	372[a]	506[b]	31.5	0.013
Trp	79[a]	107[b]	7.3	0.020
Val	244[a]	358[b]	21.3	0.003
Average	163	243		
Dispensable				
Asp/Asn	416[a]	597[b]	38.9	0.008
Ser	312[a]	424[b]	26.2	0.012
Glu/Gln	409[a]	621[b]	44.0	0.006
Pro	241[a]	336[b]	20.5	0.008
Cys	146	174	12.6	0.154
Gly	217[a]	314[b]	19.7	0.006
Ala	186[a]	277[b]	22.9	0.019
Tyr	150[a]	218[b]	12.7	0.003
Average	260	370		

[a], [b] Within a row, means without a common superscript differ at $P < 0.05$.

($P < 0.001$, Fig. 2F). We subsequently quantified mRNA expression levels of several immune markers to better understand the effects inclusion of GG (from 0% to 6%) on immune response in the broilers (Fig. 3). The expression levels of *Cd3* T cell marker do not reveal any notable change after GG supplementation (Fig. 3A). However, *K203* chemokine expression significantly increased with GG inclusion levels (Fig. 3B). As an important chemoattractant for macrophages (Lillehoj et al., 2007), the upregulation of *K203* expression was accompanied with an increased expression of their activation marker, *Inos* (Fig. 3C) and *IL-1*, *IL-6* and *IL-8* proinflammatory cytokines (Fig. 3D−E). Altogether, these data suggest an inflammatory status driven by innate immune response at ileal levels in presence of β-galactomannan starting from 2% in diets.

3.5. Guar gum induce systemic acute inflammatory markers

At systemic/sera level and compared to the control condition, broilers from GG group shows significantly higher levels of acute phase proteins inflammatory markers such as SAA (0.1 vs. 0.5 ng/mL, $P < 0.01$, Fig. 4C) and α1GP (orosomucoid, $P < 0.05$, Fig. 4B) known as an early inflammatory marker in the gastrointestinal tract (GIT; Chasser et al., 2021). However, we did not notice any changes in earlier secreted inflammatory cytokines (Fig. S2). Thus, GG supplementation induced significant decreases in IgA levels in gut lumen (2.9 vs. 4.5 mg/g of sample, $P = 0.03$, Fig. 4A).

3.6. Guar gum reduces caecal microbiota diversity

The impact of the GG supplementation on caecal microbiota composition was investigated by analysing 16S rRNA gene sequences from caecal samples collected on d 28. Paired-end sequencing generated 2,605,777 quality read counts with an average of 54,287 counts/sample representing a total of 2,216 amplicon sequence variants (ASV). Interestingly, and despite the common 223 ASVs, both experimental groups present unique microbial signatures with 123 and 60 exclusive ASVs in the CTRL and GG groups, respectively (Fig. 5A). The community structure of microbiota is significantly different in the two groups ($P < 0.001$) as shown by PCoA based on Bray−Curtis's dissimilarities. PERMANOVA tests explain 28.1% of these differences (Fig. 5B). In terms of alpha diversity, diets shows a significant reduction in microbiota diversity using Shannon ($P = 0.005$, Fig. 5C) or Simpson indexes ($P = 0.040$; Fig. 5D) in the GG group. However, no significant differences are observed when using Chao1 index ($P = 0.718$, Fig. 5E).

At the phylum level, the caecal microbiota of both groups is dominated by Firmicutes, followed by Bacteroidetes (Fig. 5F) which represent 90% of the microbial composition. The most abundant bacterial families are Lachnospiraceae, Bacteroidaceae, Lactobacillaceae and Ruminococcaceae in both groups (Fig. 5G). Interestingly, GG group broilers presents significantly higher abundances of Lactobacillaceae ($P = 0.003$) in detrimental of Erysipelotrichaceae ($P = 0.003$), Lachnospiraceae ($P = 0.007$), and Peptostreptococcaceae ($P < 0.001$, Fig. 5G). A total of 40 bacterial genera are assigned to the two groups, where more than 90% of microbiota is represented by *Ruminococcus, Bacteroides, Bifidobacterium, Lactobacillus, Clostridium_XlVa, Blautia, Streptococcus, Collinsella* and *Escherichia/Shigella*. However, their distribution changes in each diet group. *Ruminococcus*, followed by *Bacteroides*, are the most abundant in the control group while the GG group presents high abundances of *Lactobacillus* followed by *Bacteroides* and *Ruminococcus* (Figs. S4A−B). To further compare the abundance of all taxa, a Wilcoxon test was performed and plotted in the heat tree shown in Fig. S4C. Profound differences are observed between the two experimental conditions with 15 differential genera. Besides the increase of *Lactobacillus* in the GG group, we observe reductions, surpassing 2-fold decreases, in *Clostridium_IV, Clostridium_XIVa, Blautia* and *Anaerostipe* and (Fig. 5H, $P < 0.001$). These substantial ecological shifts in gut microbiota are likely to influence the activities and production of metabolites by gut microbiota.

3.7. Guar gum reduces caecal bacterial activity and metabolism

Based on the previous results, we quantified the microbial metabolites in chicken gut from the two groups. GG group presents a significantly higher concentration of lactic acid ($P < 0.01$) at the expense of acetic acid ($P = 0.01$, Fig. 6A−B). Acetic acid was the major fraction of SCFA (82.34 ± 25.83 mmol/kg) followed by butyric
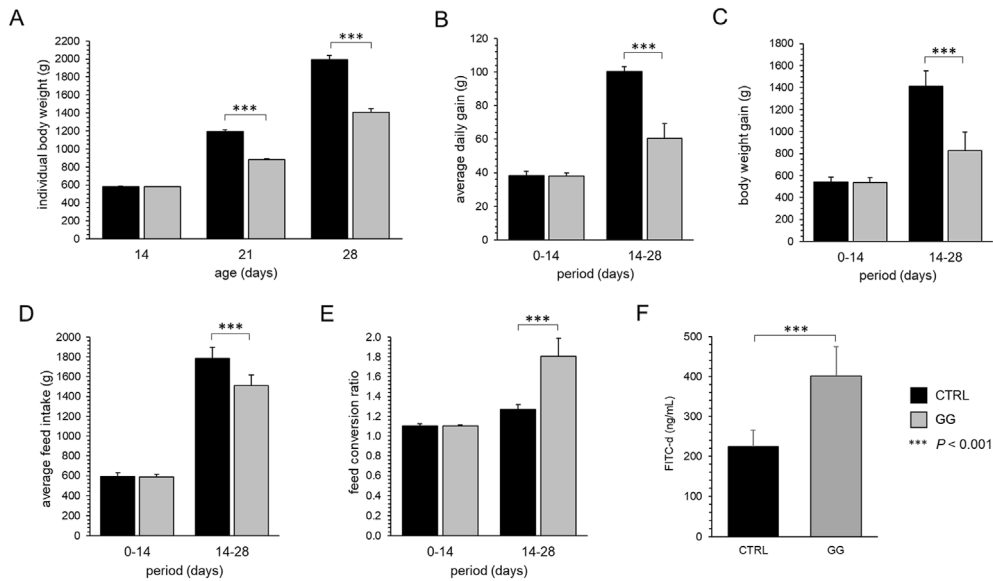
Fig. 2. Performances of broilers fed the common (0–14 d) and two experimental (14–28 d) diets. (A) Average body weights of chicken broilers from CTRL and GG diet groups at 14, 21, and 28 d of age. (B) Average daily weight gains (g) at 0 to 14, and 14 to 28 d of age. (C) Average body weight gains at 0 to 14, and 14 to 28 d of age. (D) Average feed intake (g) at 0 to 14, and 14 to 28 d of age. (E) Feed conversion ratio at 0 to 14, and 14 to 28 d of age. (F) Average FITC-d concentrations in broilers' sera at d 21. Data are represented as means ± SEM. Statistical differences were evaluated by impaired two tailed t-test. CTRL = control, GG = guar gum; FITC-d = fluorescein isothiocyanate dextran.
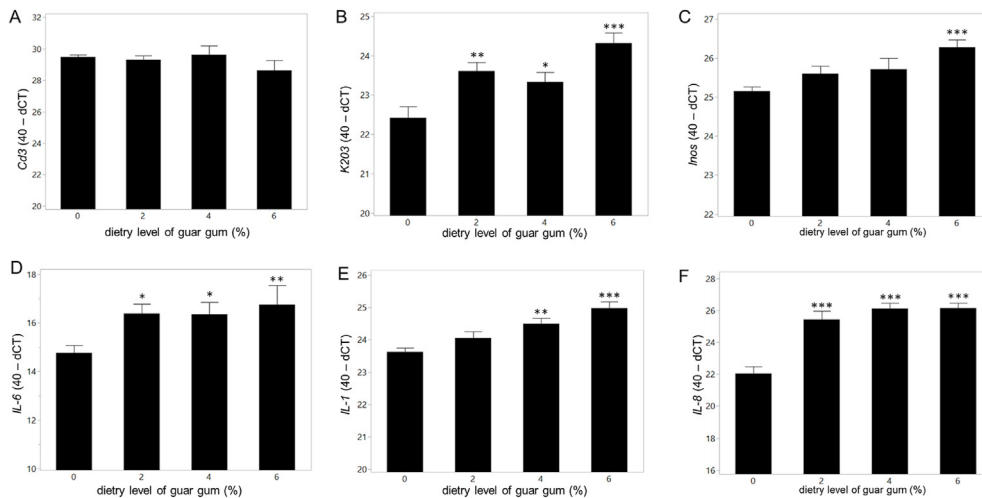
Fig. 3. Relative expression levels of immune markers in ileal tissues of broilers fed various levels of β-galactomannan. T lymphocytes marker *Cd3* (A), *K203* chemokine (B), induced NO synthase (*Inos*, C) and proinflammatory *IL-6*, *IL-1*, and *IL-8* cytokines (D, E and F, respectively) relative mRNA expression levels. Data represent the average 40 (qPCR cycles) − delta cycle threshold (dCT) values ± SEM. Statistical differences were evaluated by impaired two tailed t-test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
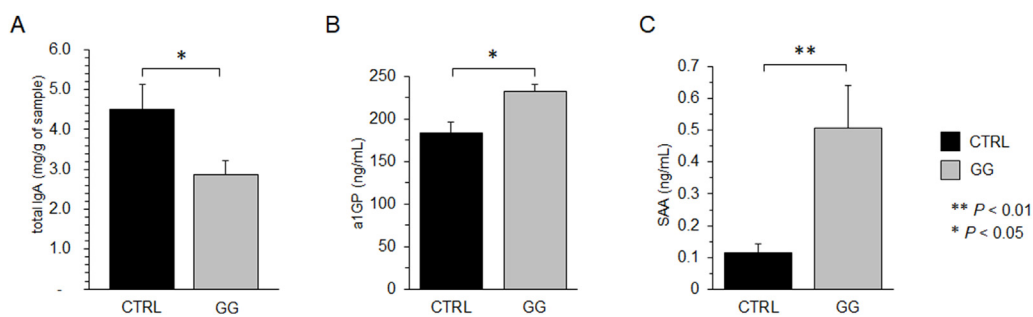

Fig. 4. ELISA quantification of immunoglobulin A (IgA) and acute phase proteins quantification in broilers' gut lumen and sera, respectively. (A) IgA average amounts (mg/g of sample) in broilers digesta from different diet groups. (B–C) Average concentrations (ng/mL) of α1GP (B) and SAA (C) inflammatory marker in sera of boilers fed the control and GG supplemented (2%) diets. Data are represented as means ± SEM. Statistical differences were evaluated by impaired two tailed t-test. CTRL = control; GG = guar gum; α1GP = alpha 1 acid glycoprotein; SAA = serum amyloid A protein.
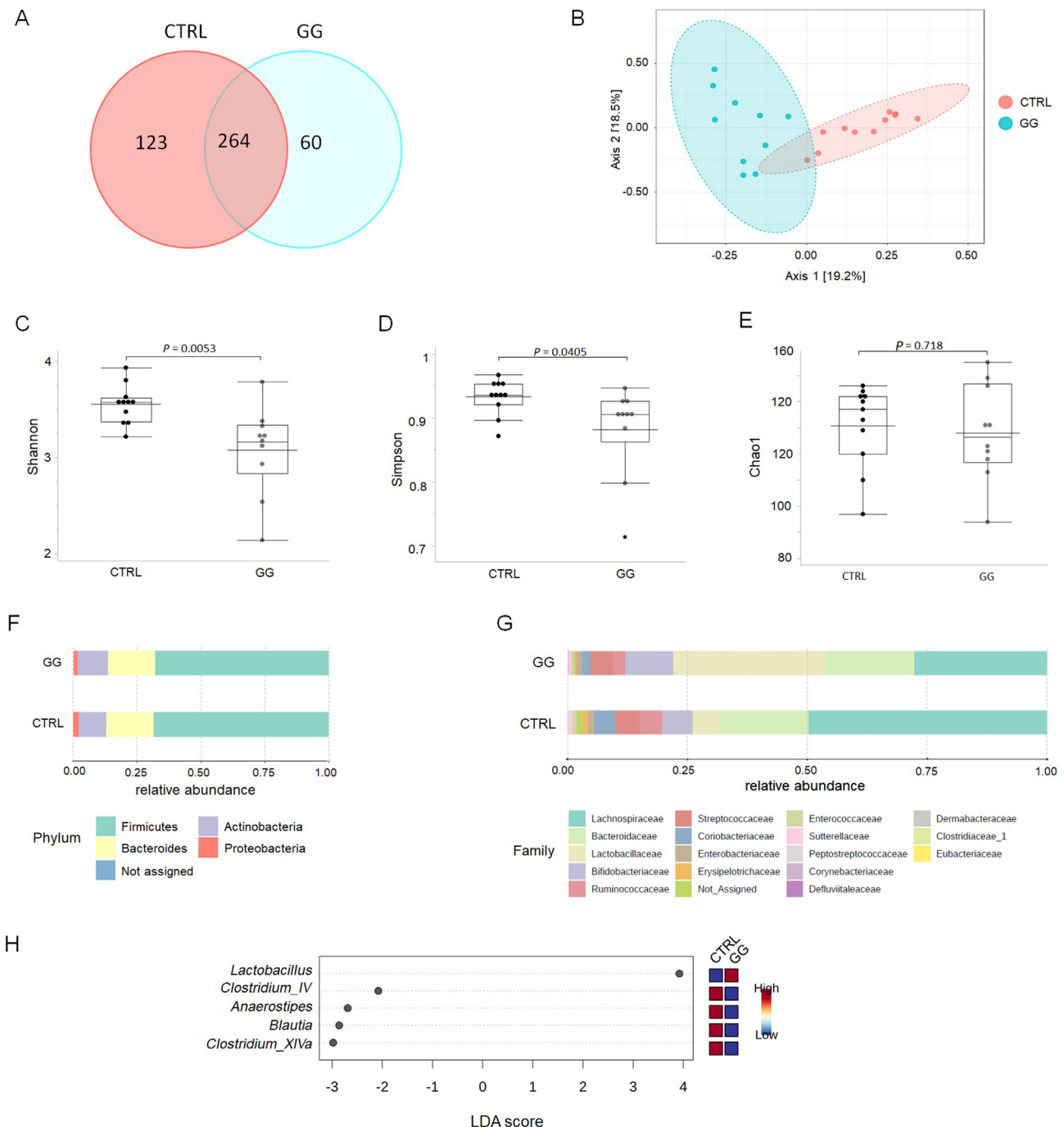
Fig. 5. Description of caecal microbial composition and structure in broilers fed the tested diets. (A) Venn's diagram representing the distribution of amplicon sequence variants between the 2 diets. (B) Beta diversity of microbiota by permutational multivariate ANOVA (PERMANOVA). (C-E) Alpha diversity indexes: Shannon (C) and Simpson (D) and Chao1 (E). (F-G) Relative abundance of bacterial phyla and families, respectively, in caecal content of broilers fed the two experimental diets. (H) Linear discriminant analysis (LDA) effect size (LEfSe) of bacterial genus abundances. CTRL = control; GG = guar gum (2%).

acid (14.56 ± 6.03 mmol/kg) and propionic acid (6.26 ± 2.32 mmol/kg, Fig. 6B). No significant differences were observed in propionic and butyric acids production (Fig. 6B) as well as protein metabolites (Fig. 6C−D).

4. Discussion

It is known that GG is high in galactomannan which is a cell wall component of legumes and is present in small quantities in soy hulls (<1%; Dierick, 1989; Hsiao et al., 2006; Ward, 1996; Whistler and Saarnio, 1957) and other raw materials. The linear decrease of ATTD of dry matter and AME in presence of GG at different concentrations, in the first experiment, are in accordance with Maisonnier et al. (2001) who reported that the addition of GG, in corn and soybean meal based diets, resulted in decreased nutrient digestibility and energy utilisation. Guar gum supplementation in broiler diets at various levels increases digesta viscosity (Latham et al., 2018) which impedes the digestion and utilisation of
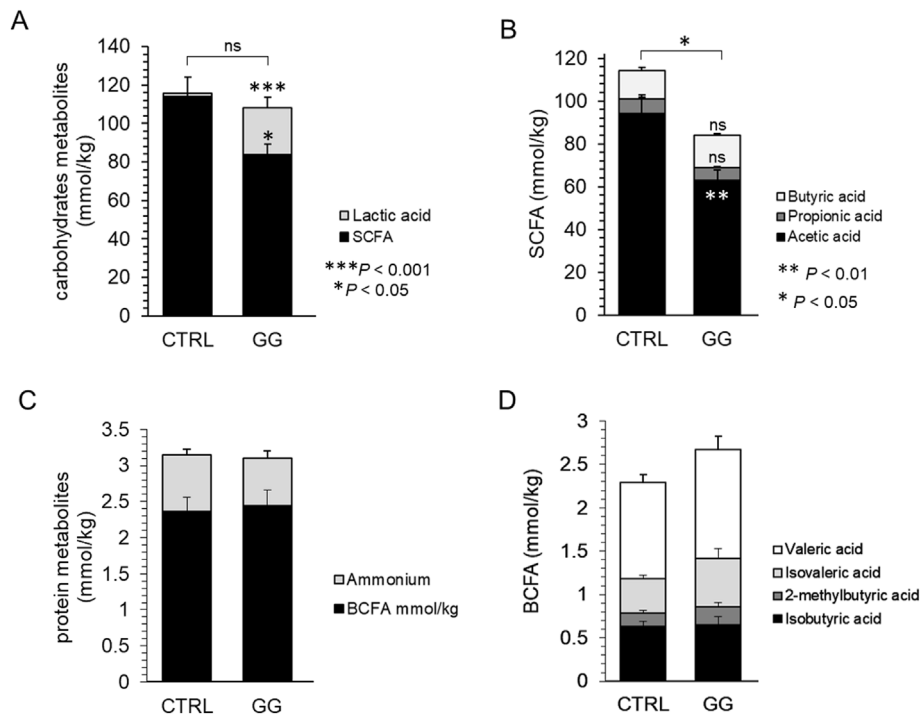
Fig. 6. Bacterial metabolite concentrations in broilers' caeca samples. (A) Average concentrations of carbohydrates metabolites: SCFA and lactic acids (mmol/kg) in caecal contents of broilers fed the CTRL or GG supplemented experimental diets. (B) Average concentrations of SCFA: butyric, propionic, and acetic acids (mmol/kg) in caecal contents of broilers fed the CTRL or GG experimental diets. (C) Average concentrations of ammonium and BCFA (mmol/kg) in caecal contents of broilers fed the CTRL or GG experimental diets. (D) Average concentrations of BCFA: valeric, isovaleric, 2-methylbutyric and isobutyric acids (mmol/kg) in caecal contents of broilers fed the CTRL or GG experimental diets. *P*-values displayed were obtained from Kruskal−Wallis's test. SCFA = short chain fatty acid; CTRL = control; GG = guar gum; BCFA = branched chain fatty acids.

nutrients in birds. Hence, high viscosity would slow down gastric emptying in conjunction with poor mixability of substrate with digestive enzymes. Collectively, this will reduce the nutrient contact with the absorptive epithelium (Read, 1986) resulting in a very low absorption of many important nutrients such as protein (Poksay and Schneeman, 1983), glucose (Blackburn and Johnson, 1981) and fat (Higham and Read, 1992) thereby deteriorating the growth performance. Thus, it can be assumed that the adverse effects of increasing doses of GG on dry matter digestibility, AME, feed intake, and BWG may have been induced by high digesta viscosity. Likewise, Choct and Annison (1992) and Latorre et al. (2015) also reported that soluble NSP including β-galactomannan, the main component of GG (99%; Sharma et al., 2021), can exert anti-nutritional effects in broilers especially through increasing viscosity in the intestinal environment, prolonging feed passage rate and increasing small intestinal fermentation which can reduce nutrient digestion and absorption resulting in impaired growth performance and intestinal health.

Unlike Exp. 1 and 3, we did not observe a difference in feed intake due to the supplementation of GG in Exp. 2. Souza et al. (2023) similarly reported no significant alterations in feed intake from 15 to 21 d with GG supplementation, yet they observed an overall reduction in global feed intake. This suggest a possibility that GG supplementation necessitate a longer duration to exert negative impact on feed intake, especially when the diets are adequately balanced for other essential nutrients. In line with our findings, which revealed a dramatic 36% decrease in BWG and a simultaneous increase of 58% in FCR, Daskiran et al. (2004) also documented severe depression in BW at a 2% inclusion rate of GG, consistent with several other studies (Ray et al., 1982; Vohra and Kratzer, 1964). On average, AIAAD for all the indispensable amino acids saw a noteworthy average reduction of 15.8% in the GG group.

Similarly, the AIAAD of dispensable amino acids was notably impaired by GG treatment, exhibiting an average decrease in digestibility of approximatively 18% when compared to the CTRL diet. Likewise, the SIAAD of indispensable and dispensable amino acids was decreased by 14% and 16%, respectively. The quantification of IEAA losses (Table 4) allowed us to indirectly calculate the fraction of indigestible amino acids at the terminal ileum in both CTRL and GG groups. The data indicated a substantial increase in the indigestible amino acids in the GG group, despite the increase in IEAA, underscored that the reduced digestibility of the amino acids at terminal ileum might elucidate the diminished performance observed in chickens.

The research conducted by Siegert and Rodehutscord (2020) focused on measuring the extract viscosity of guar meal, revealing that the viscosity decreases with increasing shear rates, similar to other grains such as barley, triticale, wheat, oats, and maize (Rodehutscord et al., 2016; Siegert et al., 2017). The high viscosity at low shear rate is attributed to soluble components like galactomannan. Although the exact impact of shear rates on digesta in broilers remain uncertain (Siegert and Rodehutscord, 2020), studies on possum (Lentle et al., 2005) and rats (de Loubens et al., 2013) suggest a shear rates below 1/s in the small intestine. Interestingly, the extract viscosity of guar meal was approximately 4.9 times higher compared to barley shear rate up to 1/s (Siegert and Rodehutscord, 2020). Increased digesta viscosity, combined with the slow distribution of digesta in the gut, typically leads to reduced feed intake and nutrient digestibility (Abdollahi et al., 2013). Although, no difference in feed intake was observed in this experiment, the diminished amino acid digestibility in GG supplemented group likely resulted from high digesta viscosity.

Previous reports have highlighted that the acute inflammation and adaptive immune responses significantly affect the amino acid

digestibility and retention (Iseri et Klasing, 2013; Klasing, 2017). During inflammation, Cys and Met become particularly limiting due to their involvement in acute phase proteins and antibodies secretion (Takahashi et al., 1997). Corresponding to these findings, our results indicated relatively lower digestibility and retention for Cys and Met (Tables 2 and 3) compared to the other amino acids. Furthermore, our study identified a potential macrophage-driven acute inflammation, evidenced by the increased levels of *k203* macrophage chemokine, *inos* activation marker, *IL-1, IL-6, IL-8* in gut tissues of broilers fed GG diets. The expression of inflammatory cytokines (*IL-1, IL-6, IL-8*) corresponded to elevated levels of acute phase proteins (APPs) such as α1GP and SAA (Li and Liao, 1991) in the broilers' sera from the GG group. This inflammatory status also affected adaptive humoral immune response, resulting in a significant decrease in IgA secretion in the gut lumen (Fig. 4A), indicating an imbalanced class switch favouring IgY over IgA secretion in the GIT (Castro-Dopico et al., 2019). Thus, for the first time to our knowledge, we demonstrate that GG supplementation induced disruption of gut integrity leading to a leaky gut syndrome (Fig. 2F) which initiate and/or exacerbated gut inflammation. Collectively, these results reveal a detrimental circle induced by GG supplementation, causing decreased amino acid and dry matter digestion not only due to increased viscosity but also through degradation of gut health via disruption of gut integrity and inflammation. Additionally, these alterations may also impact microbial homeostasis and ecology in the gut (Fadlallah et al., 2019).

Caecal microbiota of broilers in the CTRL group exhibited significantly higher microbiota diversity compared to the GG group. Notably, these diversity metrics displayed a positive correlation with the animal's body weights at 28 d, with the Shannon index showing the strongest correlation ($\rho = 0.52$, $P = 0.008$). The inclusion of GG in the feed led to a substantial decrease in alpha diversity and alterations in beta diversity alongside changes in animal performances. These shifts in diversity and microbial structure might be attributed to the increased presence of *Lactobacillus*, resulting in a shift in metabolites production from carbohydrate fermentation. This shift led to an increased lactic acid production, consequently reducing acetate levels. Our hypothesis revolves on around the potential of *Lactobacillus* to degrade galactomannan, producing a high concentration of lactate, which, in turn, could affect animal performances. Indeed, the accumulation of high levels of lactate in the caeca has been shown to have toxic effects leading to detrimental consequences for the animal (Ewaschuk et al., 2005; Wang et al., 2020). Surprisingly, while *Bacteroides,* known for their galactomannan metabolising capabilities due to their glycoside hydrolases (Bågenholm et al., 2017), were not significantly over-represented in the GG diet, SCFA producers like *Clostridium*_XIVa and *Blautia* decreased. These genera play an important role in gut homeostasis and animal health (Lopetuso et al., 2013; Parada et al., 2019). In humans, the deficiency of SCFAs has been associated with metabolic syndromes, appetite control alteration, and neurological diseases (Deleu et al., 2021). Chickens from the GG group displayed the lowest level of SCFAs likely due to the restrictions caused by the feed intake decrease (Metzler-Zebeli et al., 2019). When bacterial families are differentially compared, families harbouring SCFA producers, highly efficient at cellulose and NSP degradation (Biddle et al., 2013), were more abundant in the control group. The released SCFAs play several beneficial roles in the host mainly by enhancing metabolic pathways. Butyric acid provides energy for colonocytes, improves cell differentiation, strengthens the epithelial barrier, and reduces intestinal inflammation while, in the liver, propionate favours the gluconeogenesis and acetate contributes to lipogenesis (Deleu et al., 2021; Hamer et al., 2008; Louis et al., 2014). Conversely, the lack of SCFA production might amplify the inflammatory status in the model. Overall, these results strongly indicate that the GG challenge has profoundly detrimental effects on multiple levels, including acute inflammation markers, performance, microbial composition, and bacterial metabolites, leading to decreased microbial diversity, increased lactic acid concentrations, and elevated proinflammatory markers in the host.

5. Conclusions

A holistic view of this study showed that the GG supplementation, as expected, explicitly reduced growth performance in broilers. The severe degraded performances and the decrease of dry matter and amino acid digestibility are not only due to β-galactomannan-induced viscosity but also to gut integrity disruption, gut inflammation as well as altered caecal microbiota ecology and activity. These results validate the GG supplementation in diet as an accurate model for "leaky gut" in broilers as well as for DIOR (van der Zande et al., 2020). Indeed, the dynamic, and intricate crosstalk between these networks makes it difficult to single out the impact magnitude of one over the other. Future studies should also encompass the hierarchy of contributing factor(s) in the "leaky gut model" for broiler chickens.

Author contributions

Amine Mellouk: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing − original draft. **Tahir Mahmood:** Conceptualization, Formal analysis, Investigation, Methodology, Writing − original draft. **Maamer Jlali:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Writing − original draft. **Nuria Vieco-Saiz:** Methodology, Formal analysis, Investigation, Writing − review & editing. **Virginie Michel:** Methodology, Formal analysis, Project administration. **Pierre Cozannet:** Methodology, Investigation, Writing − review & editing. **Sarper Ozbek:** Funding acquisition, Resources. **Yves Mercier:** Funding acquisition, Project administration, Resources, Writing − review & editing. **Estelle Devillard:** Funding acquisition, Project administration, Resources, Writing − review & editing. **Jessika Consuegra:** Conceptualization, Funding acquisition, Project administration, Resources, Writing − review & editing, Supervision.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Appendix Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2024.01.005.

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