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Guar gum as galactomannan source induces dysbiosis and reduces performance in broiler chickens and dietary β-mannanase restores the gut homeostasis

Marielen de Souza [*,b] Venessa Eeckhaut,[†] Evy Goossens [†] Richard Ducatelle,[†] Filip Van Nieuwerburgh [‡] Karl Poulsen,[§] Ana Angelita Sampaio Baptista,[‖] Ana Paula Frederico Rodrigues Loureiro Bracarense,[*] and Filip Van Immerseel[†,1]

[*]*Laboratory of Animal Pathology (LAP), Department of Preventive Veterinary Medicine, State University of Londrina, Londrina, Brazil;* [†]*Livestock Gut Health Team (LiGHT), Department of Pathobiology, Pharmacology and Zoological Medicine, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium;* [‡]*Ghent University Next Generation Sequencing Facility (NXTGNT), Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium;* [§]*ELANCO Animal Heath, Antwerpen, Belgium; and* [‖]*Laboratory of Avian Medicine (LAM), Department of Preventive Veterinary Medicine, State University of Londrina, Londrina, Brazil*

ABSTRACT Galactomannans are abundant nonstarch polysaccharides in broiler feed ingredients. In broilers, diets with high levels of galactomannans have been associated with innate immune response stimulation, poor zootechnical performance, nutrient and lipid absorption, and excessive digesta viscosity. However, data about its effects on the gut microbiome are scarce. β-Mannanases are enzymes that can hydrolyze β-mannans, resulting in better nutrient utilization. In the current study, we have evaluated the effect of guar gum, a source of galactomannans, supplemented to broiler diets, either with or without β-mannanase supplementation, on the microbiota composition, in an attempt to describe the potential role of the intestinal microbiota in β-mannanase-induced gut health and performance improvements. One-day-old broiler chickens (n = 756) were randomly divided into 3 treatments: control diet, guar gum-supplemented diet (1.7%), or guar gum-supplemented diet + β-mannanase (Hemicell 330 g/ton). The zootechnical performance, gut morphometry, ileal and cecal microbiome, and short-chain fatty acid concentrations were evaluated at different time points. The guar gum supplementation decreased the zootechnical performance, and the β-mannanase supplementation restored performance to control levels. The mannan-rich diet-induced dysbiosis, with marked effects on the cecal microbiota composition. The guar gum-supplemented diet increased the cecal abundance of the genera *Lactobacillus*, *Roseburia*, *Clostridium sensu stricto 1*, and *Escherichia-Shigella*, and decreased *Intestinimonas*, *Alistipes*, *Butyricicoccus*, and *Faecalibacterium*. In general, dietary β-mannanase supplementation restored the main microbial shifts induced by guar gum to levels of the control group. In addition, the β-mannanase supplementation reduced cecal isobutyric, isovaleric, valeric acid, and branched-chain fatty acid concentrations as compared to the guar gum-supplemented diet group, suggesting improved protein digestion and reduced cecal protein fermentation. In conclusion, a galactomannan-rich diet impairs zootechnical performance in broilers and results in a diet-induced dysbiosis. β-Mannanase supplementation restored the gut microbiota composition and zootechnical performance to control levels.

Key words: beta-mannanase, guar gum, microbiota, mannan, gut health

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INTRODUCTION

It is estimated that by 2030, poultry meat consumption will represent 41% of all meat protein sources worldwide, driven by the efficient production and the lack of cultural and religious hurdles (OECD and FAO, 2021). One of the major potential problems for the future is to keep the production cost at low levels. Currently, feed represents >60% of the final production cost (Noblet et al., 2022), so dietary additives that can improve feed utilization are of great value for the poultry industry. One of the most widely used dietary additives to enhance digestibility are carbohydrate-degrading enzymes, such as xylanases (Zhang et al., 2014). These

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[1]Corresponding author: filip.vanimmerseel@ugent.be

enzymes are targeting plant cell wall components, more specifically soluble nondigestible nonstarch polysaccharides (**NSP**), considered to be antinutritional factors (Jezierny et al., 2010; Amerah, 2015). NSP have β-glycosidic bonds that cannot be digested by monogastric animals, and data clearly show negative correlations between digestibility and dietary NSP concentrations (Jaworski et al., 2015). High concentrations of soluble NSP increase small intestinal viscosity, decrease passage rate, favor the expansion of harmful microbiota, such as *Escherichia coli* and *Clostridium perfringens*, reduce animal performance, and affect the intestinal microbiota composition (Hussain et al., 2012; Shojadoost et al., 2012; Latorre et al., 2015; Jha et al., 2019; Bushra et al., 2020).

While arabinoxylans are the most well-known NSP, there are a variety of other NSPs that can negatively affect animal performance (Căpriţă et al., 2010; Kermanshahi et al., 2018). As an example, β-mannans (galactomannans and glucomannans) are present in different concentrations in many feed ingredients. Dehulled soy bean meal (48% crude protein), one of the most important ingredients in poultry diets, has about 2.8 to 10 g/kg of β-mannans (Hove et al., 2018). Guar gum meal typically contains between 20 and 80 g/kg of galactomannans (mannose backbone with galactose side-chains), and is often used as β-mannan-rich source to evaluate the effect of β-mannans and β-mannan-degrading enzymes in experimental trials (Hussainet al., 2012; Saeed et al., 2019).

In humans, galactomannan ingestion has been related to glycemic index reduction in diabetics, alleviation of the irritable bowel syndrome symptoms, prebiotic effects, and improvement of short-chain fatty acid (**SCFA**) production (Singh et al., 2018; Rao and Quartarone, 2019; Miao et al., 2021). However, in broilers, diets with high concentrations of plant-derived galactomannans are associated with poor performance, reduced feed intake, nutrient and lipid absorption, innate immune response stimulation, and excessive digesta viscosity (Lee et al., 2003; Shirouchi et al., 2011; Shastak et al., 2015).

β-Mannanases are enzymes synthesized by strains of *Aspergillus niger, Paenibacillus lentus, Bacillus subtilis*, or *Trichoderma longibrachiatum* (Li et al., 2014; Saeed et al., 2019). These enzymes can hydrolyze β-1,4-glycosidic linkages in β-mannans resulting in a better nutrient utilization, improvement of innate immune responses, reduced intestinal viscosity and lower pathogen proliferation (Hussainet al., 2012). The effects of β-mannans and β-mannanase feed supplementation on performance and gut histology in chickens have been described previously (Maisonnier et al., 2003; Zou et al., 2006; Caldas et al., 2018; Latham et al., 2018). As NSP-degrading enzymes typically cause release of smaller oligosaccharides that can be used by the intestinal microbiota, it might be that shifts in microbial composition contribute to the observed health effects when β-mannanases are added to poultry diets (Hussainet al., 2012; Saeed et al., 2019). However, data on the effect of β-mannans and β-mannanase on the intestinal microbiome are lacking. In the current study, we evaluate the effect of guar gum (**GG**) supplementation to the diet of broilers, either or without β-mannanase, on the microbiota composition, in an attempt to describe the potential role of the intestinal microbiota in β-mannanase-induced gut health and performance improvements.

MATERIALS AND METHODS

Animal Trial

A total of 756 one-day-old Ross-308 broiler chicks were randomly divided in 3 treatments (12 pens per treatment with 21 animals each): control diet; GG diet; GG diet + β-mannanase (GG + E) (Hemicell 330 g/ton of feed), and housed on solid floors covered with wood shavings following European Union Directive 2007/43/EC (EU, 2007). Water, feed (Table 1), and heating were provided according to broiler guidelines (Aviagen, 2018). At 1, 14, 21, 28, and 35 d of age the animals and feed leftovers were weighed per pen to calculate the feed conversion ratio (**FCR**), body weight (**BW**), daily feed intake (**DFI**), and average daily gain (**ADG**). At 14 and 28 d of age, 1 animal per pen ($n = 12$ birds/treatment) was euthanized by an intravenous overdose of 20% sodium pentobarbital (Kela, Hoogstraten, Belgium), according to Annex I to the Council Regulation (**EC**) No 1099/2009 (EC, 2009), and content from ileum and cecum was collected and stored at −20°C, while part of the duodenum and ileum were fixed in 4% buffered formalin for 24 h.

Table 1. Composition of the experimental diets.

Ingredients (g/kg)	Control diet		GG diet	
	Starter	Grower	Starter	Grower
Maize	610.40	625.85	588.60	606.40
Guar gum	-	-	17.90	16.50
Soya bean meal	310	300	314	303
Full fat soya bean	2	-	2	-
Animal fat	25	25	25	25
Soybean oil	10	12.50	10	12.50
Premix	5	5	5	5
Lime fine (38% Ca)	15	13	15	13
Dicalcium phosphate	10.10	6.90	10.10	6.90
Sodium bicarbonate	2.70	2.20	2.70	2.20
L-lysine HCL	3.20	2.90	3.10	2.85
DL-methionine	3.25	2.90	3.25	2.90
L-threonine	1.25	1.10	1.25	1.10
Calculate composition (g/kg)				
ME (MJ/kg)	12.86	13.03	12.59	12.79
Crude protein	203.9	199.4	204	199.2
Crude fat	67.8	70.4	66.9	69.6
Starch	402.4	412.4	388.2	399.7
Sugars	43.7	42.9	43.7	42.8
Ca	9.0	7.8	9	7.8
Available P	4.8	4.2	4.8	4.2

The starter diet was provided from d 1 until d 14, followed by grower diet until d 35. ME, metabolizable energy. Hemicell 330 g/ton of feed was added only to the group that received the GG diet supplement with β-mannanase. Premix composition per kg of product: vitamin A 10,000 IU; vitamin D_3 2,500 IU; vitamin E 50 mg; vitamin K_3 1.5 mg; vitamin B_1 2.0 mg; vitamin B_2 7.5 mg; vitamin B_6 3.5 mg; vitamin B_{12} 20 μg; niacin 35 mg; D-pantothenic acid 12 mg; choline chloride 460 mg; folic acid 1.0 mg; biotin 0.2 mg; iron 80 mg; copper 12 mg; manganese 85 mg; zinc 60 mg; iodate 0.8 mg; selenium 0.15 mg. GG, guar gum.

Histological Analysis

The formalin-fixed tissue segments ($n = 12$/treatment/intestinal segment) were embedded in paraffin, 5 μm sections obtained, deparaffinized, and stained with hematoxylin and eosin (**H&E**). Duodenal and ileal villus length and crypt depth were assessed by random measurement of 15 villi and crypts using a PC-based image analysis system (Leica Application Suite V4.1, Leica, Diegem, Belgium). Afterward the villus to crypt ratio was calculated.

Molecular Analysis

DNA Extraction DNA was extracted from 100 mg cecal and ileal content of 1 bird per pen (12 birds/treatment), using the hexadecyltrimethylammonium bromide (**CTAB**) method according to Kowalchuk et al. (1998) with small modifications. Briefly the intestinal content was suspended in 0.5 mL CTAB (Sigma Aldrich, St. Louis, MO) buffer 5% (w/v), 0.35 M NaCl, 120 mM K_2HPO_4) and 0.5 mL phenol-chloroform-isoamyl alcohol (25:24:1). The mixture was homogenized by grinding with 0.5 g unwashed glass beads (Sigma-Aldrich) in a bead beater (2 × 3 min 30 Hz for ileal content and 2 × 2 min, 22.5 Hz for cecal content; TissueLyser II; Qiagen, Hilden, Germany) with a 30 s interval between shakings. Samples were centrifuged for 10 min at 8,000 rpm and 300 μL of the supernatant was transferred to a new tube. A re-extraction from the remaining content was performed by adding 0.25 mL CTAB buffer and homogenizing and centrifuging the sample as described above. An equal volume (0.6 mL) of chloroform-isoamyl alcohol (24:1) was added to the supernatant collected in order to remove the phenol from the samples. The mixture was further centrifuged at 16,000 × g for 10 s. Nucleic acids were precipitated with 1.2 mL of polyethyleenglycol-6000 solution (30% w/v; 1.6 M NaCl) for 2 h at room temperature. Samples were centrifuged (13,000 × g, 20 min, 4°C) and the pellet was washed twice with 1 mL of ice-cold ethanol (70% v/v). The obtained pellet was dried and resuspended in 100 μL deionized water (LiChrosolv Water, Merck, Darmstadt, Germany). The quality and the concentration of the DNA were examined spectrophotometrically using NanoDrop (Thermo Scientific, Waltham, MA). Only samples with a 260/280 purity value above 1.7 were selected.

16S rRNA Sequencing and Bioinformatics The extracted DNA was diluted to 20 ng/μL and the V3 to V4 hypervariable region of the 16S rRNA gene was amplified using the gene-specific primers (Table 2), as described by Aguirre et al. (2019). The final barcoded libraries were pooled at an equimolar concentration of 5 nM and sequenced with 30% PhiX spike-in using the Illumina MiSeq v3 technology (2 × 300 bp, paired-end) by the Ghent University next generation sequencing facility NXTGNT. After demultiplexing of the amplicon dataset and deletion of the barcodes, optimal trimming parameters were determined using the python-based application FIGARO (Weinstein et al., 2019). All further processing was performed in R (v4.1.2) (Bunn and Korpela, 2013). Raw sequence reads were trimmed, quality-filtered and dereplicated using the *DADA2* algorithm (v1.14.0) (Callahan et al., 2016). An initial amplicon sequence variant (**ASV**) table was constructed before chimeras were identified using the *removeBimeraDenovo* function. Finally, taxonomy was assigned using *DADA2*'s native naïve Bayesian classifier against the Silva database (v138) (Quast et al., 2013). To construct a phylogenetic tree, multiple sequence alignment was performed using the *DECIPHER* (v2.14.0) algorithm (Wright, 2015), after which a neighbor-joining tree was constructed using *PHANGORN* (v2.7.0) (Schliep, 2011). This neighbor-joining tree was used as the starting point to fit the final GTR + G + I (generalized time-reversible with gamma rate variation) maximum likelihood tree. The resulting phylogenetic tree and ASV table were loaded into *Phyloseq* (v1.28.0) (McMurdie and Holmes, 2013), after which potential contaminant chloroplastic and mitochondrial ASVs were removed from the dataset. Potential contaminant DNA reads originating from the DNA extraction or library preparation buffers were identified based on both the DNA concentration and prevalence of the ASVs in the negative control samples (DNA extraction controls) using *decontam* (v1.14.0) (Davis et al., 2018) and removed from the final dataset.

Quantitative PCR To confirm the main microbial shifts that were identified using 16S rRNA gene analysis, a *q*PCR for families *Enterobacteriaceae* and *Ruminococcaceae* and genus *Lactobacillus* was performed. Additionally, a *q*PCR quantifying the number of genes encoding the butyryl-CoA:acetate CoA-transferase was performed using the CFX384 BioRad detection system (BioRad, Nazareth-Eke, Belgium). Each reaction was done in triplicate in a 12 μL total reaction mixture using 1× SensiMix SYBR No-ROX mix (Bioline, Kampenhout, Belgium), 0.5 μM final primer concentration (2.5 μM for butyryl-CoA: acetate-CoA transferase enzyme), 2 μL of (20 ng/μL) DNA, and deionized water to complete the reaction volume. A standard curve was included in triplicate for each primerset. The amplification program consisted of 1 cycle at 95°C for 10 min, 40 cycles of 30 s at 95°C, followed by the annealing temperature, described in Table 2 for each primerset, for quantifying the number of gene copies encoding butyryl-CoA: acetate CoA-transferase a 3-steps protocol was used 1 cycle at 95°C for 10 min, 40 cycles of 30 s at 95°C, 30 s at 53°C, and 30 s at 72°C. The fluorescent products were detected at the last step of each cycle. A melting curve analysis was done after amplification and was obtained by slow heating from 60°C to 95°C at a rate of 0.5°C/5 s to confirm the specificity of the reaction. The primer sequences are described in Table 2.

Short-Chain Fatty Acid and Branched-Chain Fatty Acid Analysis

SCFA and branched-chain fatty acid (BCFA) were extracted from 200 mg of cecal content with diethyl ether and measured using a GC-2014 gas chromatograph

Table 2. Primer sequences and annealing temperatures used for quantification of the respective taxa and the butyryl-CoA CoA-transferase gene, in *q*PCR reactions.

Target	Primer	Sequence	Annealing temperature and time	Reference
V3−V4 region of the 16s rRNA gene	*S-D-Bact-0341-b-S-17* *S-D-Bact-0785-a-A-21*	Fw—5′ TCGTCGGCAGCGTCAGATG TGTATAAGAGACAGCCT ACGGG NGGCWGCAG 3′ Rv—3′ GTCTCGTGGGCTCGGAGAT GTGTATAAGAG ACAGGACTAC HVGGGTATCTAATCC 5′	55°C, 30”	Klindworth et al. (2013)
Enterobacteriaceae	*Eco1457F* *Eco1652R*	Fw—5′ CATTGACGTTACCCGCAGA AGAAGC 3′ Rv—3′ CTCTACGAGACTCAAGCTT GC 5′	55°C, 1’	Bartosch et al. (2004)
Lactobacillus spp.	*Lacto-16S-F* *Lacto-16S-R*	Fw—5′ GGA ATC TTC CAC AAT GGA CG 3′ Rv—3′ CGC TTT ACG CCC AAT AAA TCC GG 5′	60°C, 1’	Abdulamir et al. (2010)
Ruminococcaceae	*sg-Clept-F* *sg-Clept-R3*	Fw—5′ GCACAAGCAGTGGAGT 3′ Rv—3′ CTTCCTCCGTTTTGTCAA 5′	60°C, 1’	Matsuki et al. (2004)
Butyryl-CoA:acetate CoA-transferase	*BCoATscrF* *BCoATscrR*	Fw—5′ GCIGAICATTTCACITGGAAY WS ITGGCAYATG 3′ Rv—3′ CCTGCCTTTGCAATR TCIAC RAANGC 5′	53°C, 30”	Louis and Flint (2007)

Fw, forward; Rv, reverse.

(Shimadzu, ‘s-Hertogenbosch, the Netherlands) (Eaton et al., 1998; De Weirdt et al., 2010; Boesmans et al., 2018). The results are expressed as μmol of SCFA or BCFA per gram of cecal content.

Statistical Analysis

Statistical analysis of zootechnical performance parameters, intestinal morphology, *q*PCR, and SCFA data was performed using GraphPad Prism (version 7.04, San Diego, CA). Assumption of homoscedasticity (Bartlett’s test and Brown-Forsythe test) was evaluated and when this assumption was met ($P > 0.05$), ANOVA, at a 5% of significance level, followed by Tukey’s test, was performed. Due to the lack of homoscedasticity, the SCFA, BCFA, and *q*PCR values were log transformed and further subjected to ANOVA.

Statistical analysis of the gut microbiota results was performed using R (version 4.2.1). The microbial alpha diversity (number of observed ASVs and the Shannon diversity index) was calculated using *phyloseq* (v1.18.0). The effect of the dietary treatment on the microbial alpha diversity was assessed using a Kruskal-Wallis test, followed by a Dunn’s post hoc test. Prior to beta diversity analysis, the 16S sequencing data were transformed to portions. The Bray-Curtis distance was used as a measure for the microbial beta diversity. The dispersion (variance) in the beta diversity was calculated using the *betadisper* function in the *vegan* package (Dixon, 2003). ANOVA showed no difference in variances between the groups. Significant differences in the community composition between the groups were determined through a permutational multivariate analysis of variance using distance matrices (**PERMANOVA**), using the *adonis2* function in *vegan*. In case a significant effect of the diet was observed, pairwise comparison between the diets was performed using the function *pairwise.perm.manova* from the *RVAideMemoire* package and Bonferroni corrected *P* values were reported (Hervé, 2022). Differentially abundant taxa (phyla, families, or genera) in the ileal or cecal microbiome at the different sampling days were identified by applying DESeq2 on the nonrarefied community composition data (Love et al., 2014). Significant differences were obtained using a Wald test followed by a Benjamini-Hochberg multiple hypothesis correction.

RESULTS

Guar Gum Reduces Animal Performance and Dietary β-Mannanase Supplementation Restores Performance to Control Group Level

Through the experimental period GG supplementation impaired broiler performance (Table 3). The BW of the animals fed a GG-supplemented diet was significantly lower at all ages as compared to the animals fed the control diet, and β-mannanase supplementation restored the BW to control levels. Overall from 0 to 35 d, GG supplementation significantly reduced the ADG with 7.31 g/d and increased FCR with 0.14, relative to the control group ($P < 0.0001$), while the animals that received dietary β-mannanase as an additive to the GG diet, had an ADG and FCR that was not different from the animals fed the control diet (Table 3).

Effects of Guar Gum and β-Mannanase Supplementation on Gut Morphometry

The intestinal morphometry was evaluated at 14 and 28 d in both the duodenum and ileum segments (Table 4). At the duodenum level, no significant changes

Table 3. The body weight in grams (BW) at d 1, 14, 28, 35, and 42, and feed conversion ratio (FCR), daily feed intake (DFI), and daily weight gain (DWG) measured at 4 time intervals, for animals fed a control diet or a diet supplemented with guar gum, either with or without β-mannanase supplementation at 330 g/ton feed.

Parameter and period	Control	GG	GG + E	Control vs. GG	Control vs. GG + E	GG vs. GG + E
	Mean ± standard deviation			Adjusted *P* value		
BW 1 d	42.04 ± 1.10	42.31 ± 0.85	42.07 ± 0.35	0.8103	0.9978	0.8441
BW 14 d	490.80 ± 13.23	457.60 ± 15.09	497.90 ± 12.34	<0.0001	0.4186	<0.0001
BW 21 d	961.50 ± 27.68	901.20 ± 22.38	960.60 ± 29.12	<0.0001	0.9967	<0.0001
BW 28 d	1,551 ± 54.27	1,428 ± 51.13	1,544 ± 12.20	<0.0001	0.9364	<0.0001
BW 35 d	2,320 ± 138.30	2,038 ± 75.62	2,302 ± 112.30	<0.0001	0.9203	<0.0001
Period 1−14 d						
DFI (g/bird)	35.74 ± 0.96	34.66 ± 1.20	36.06 ± 1.51	0.0997	0.8081	0.0252
ADG (g/d/bird)	31.86 ± 0.89	29.35 ± 1.22	32.43 ± 0.88	<0.0001	0.3721	<0.0001
FCR	1.12 ± 0.02	1.18 ± 0.03	1.11 ± 0.03	0.0001	0.7025	<0.0001
Period 15−21 d						
DFI (g/bird)	86.97 ± 3.08	85.34 ± 3.07	86.09 ± 3.38	0.4319	0.7808	0.8323
ADG (g/d/bird)	67.16 ± 2.80	63.01 ± 2.17	65.46 ± 4.76	0.0150	0.4519	0.2028
FCR	1.29 ± 0.02	1.35 ± 0.03	1.32 ± 0.05	0.0020	0.3052	0.0782
Period 22−27 d						
DFI (g/bird)	123.80 ± 3.45	123.20 ± 7.33	124.80 ± 3.68	0.9616	0.8805	0.7368
ADG (g/d/bird)	84.13 ± 5.17	74.90 ± 4.48	81.86 ± 3.29	<0.0001	0.4237	0.0013
FCR	1.47 ± 0.06	1.64 ± 0.08	1.52 ± 0.05	<0.0001	0.1504	0.0003
Period 28−35 d						
DFI (g/bird)	165.90 ± 8.06	157.30 ± 5.32	168.90 ± 8.50	0.0202	0.5812	0.0015
ADG (g/d/bird)	108.10 ± 12.81	85.29 ± 9.53	106.10 ± 11.95	<0.0001	0.9115	0.0003
FCR	1.55 ± 0.15	1.86 ± 0.160	1.60 ± 0.14	<0.0001	0.6454	0.0006
Overall 0−35 d						
DFI (g/bird)	83.63 ± 2.46	81.24 ± 2.54	84.10 ± 2.69	0.0720	0.8960	0.0264
ADG (g/d/bird)	61.15 ± 3.35	53.84 ± 2.51	60.22 ± 3.01	<0.0001	0.7248	<0.0001
FCR	1.37 ± 0.04	1.51 ± 0.04	1.39 ± 0.03	<0.0001	0.2643	<0.0001

Values are the means for 12 pens of 21 chickens ± standard deviation of the mean. ANOVA, followed by Tukey multiple comparison test, was used to determine statistical differences among groups.

GG, guar gum; GG + E, guar gum + β-mannanase.

in villus height or crypt depth were observed between groups at both ages. In the ileum, GG ingestion significantly increased the villus height and the villus:crypt ratio as compared to the control group at 14 and 28 d, while both parameters did not differ between the enzyme-supplemented group and the control group.

Effects of Guar Gum and β-Mannanase Supplementation on the Ileal and Cecal Microbiota Composition

The composition of the ileal and cecal microbiome was evaluated at 14 and 28 d of age. The microbial richness

Table 4. Duodenal and ileal villus height, crypt depth and villus:crypt ratio, at 2 time points, for animals fed either a control diet or a diet supplemented with guar gum, either with or without β-mannanase supplementation at 330 g/ton feed.

Parameter and period	Control	GG	GG + E	Control vs. GG	Control vs. GG + E	GG vs. GG + E
	Mean ± standard deviation			Adjusted *P* value		
Duodenum 14 d						
Villus height, μm	1,470 ± 267.60	1,419 ± 143.20	1,484 ± 125.20	0.7848	0.9836	0.6817
Crypt depth, μm	184.80 ± 33.15	195.00 ± 24.59	179.90 ± 32.23	0.6871	0.9174	0.4464
Villi: crypt ratio	8.20 ± 2.01	7.34 ± 0.87	8.46 ± 1.47	0.3593	0.9089	0.1841
Duodenum 28 d						
Villus height, μm	1,874 ± 215.20	1,860 ± 219.60	1,720 ± 307.80	0.9898	0.3050	0.3723
Crypt depth, μm	180.60 ± 34.57	180.80 ± 22.57	176.10 ± 42.90	>0.9999	0.9455	0.9410
Villi: crypt ratio	10.71 ± 2.16	10.38 ± 1.39	10.12 ± 2.26	0.9138	0.7521	0.9461
Ileum 14 d						
Villus height, μm	389.20 ± 72.86	509.90 ± 117.40	416.70 ± 77.53	0.0076	0.7439	0.0455
Crypt depth, μm	119.70 ± 38.66	110.90 ± 29.47	122.50 ± 27.87	0.7848	0.9768	0.6613
Villi: crypt ratio	3.22 ± 0.70	4.69 ± 0.827	3.51 ± 0.860	0.0003	0.6553	0.0031
Ileum 28 d						
Villus height, μm	489 ± 124.40	640 ± 167	615.80 ± 130.50	0.0354	0.0880	0.9084
Crypt depth, μm	112.20 ± 36.18	114.60 ± 26.68	116.70 ± 28.57	0.9793	0.9323	0.9856
Villi: crypt ratio	4.51 ± 0.85	5.60 ± 0.76	5.42 ± 0.95	0.0103	0.0364	0.8611

Values are the means of 12 animals (1/pen/treatment) ± standard deviation. ANOVA, followed by Tukey multiple comparison test, was used to determine statistical differences among groups.

GG, guar gum; GG + E, guar gum + β-mannanase.

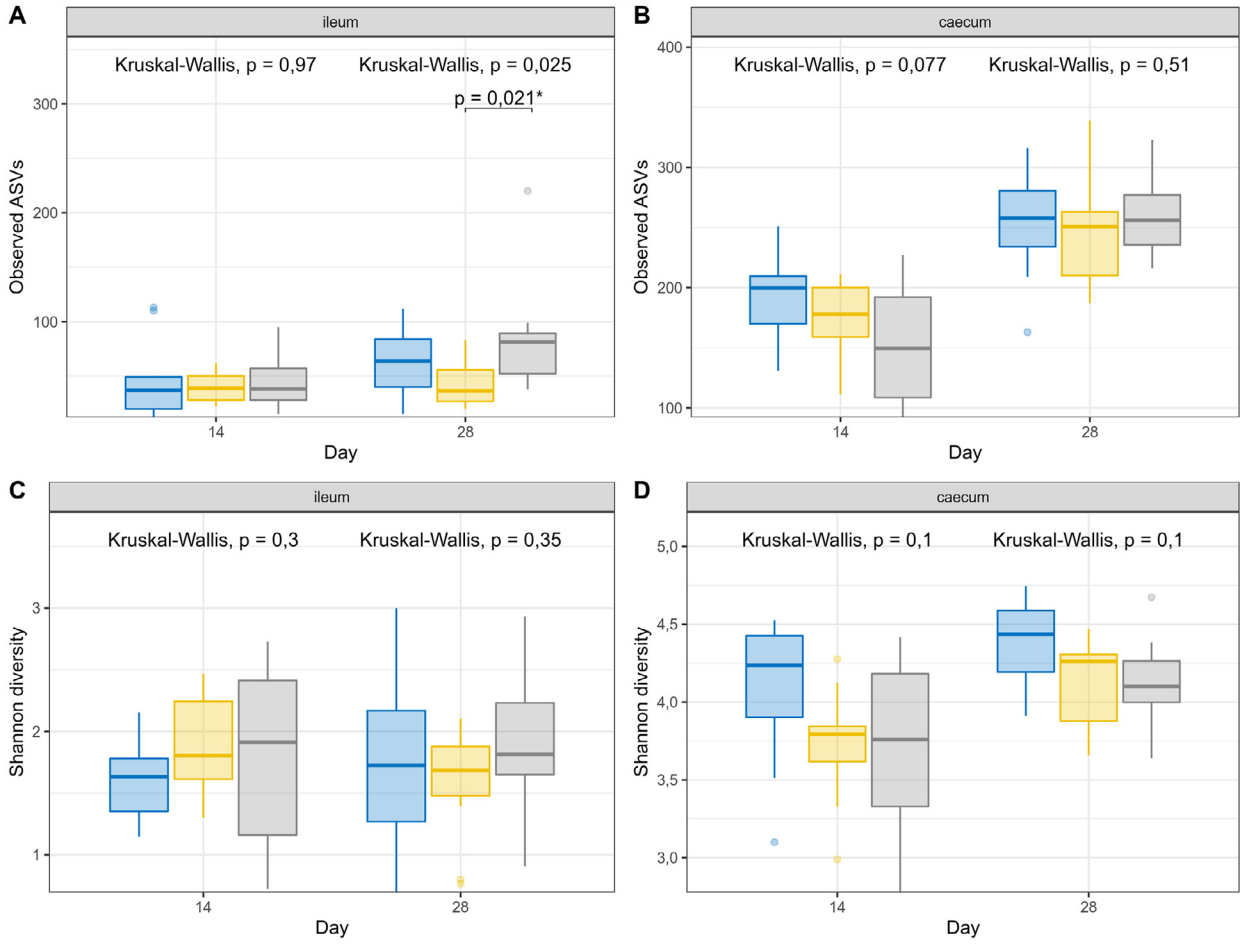

Figure 1. Ileal and cecal bacterial α-diversity measurements (A, B: observed ASVs; C, D: Shannon diversity) at 2 time points (14 or 28 d of age) among groups of animals fed a control diet or a diet supplemented with guar gum, either or not supplemented with β-mannanase at 330 g/ton feed. Values are the means of 12 animals (1/pen/treatment) ● Control; ● Guar gum; ● Guar gum + β-mannanase.

was evaluated through the number of observed ASVs and the estimated community diversity (Shannon index) in each sample (Figure 1). In the ileum, no significant changes were observed at 14 d. At 28 d GG ingestion reduced the microbial richness ($P = 0.021$) as compared to GG + E. No differences in α-diversity metrices were seen between groups in the ceca (Figure 1B and D).

Bray-Curtis dissimilarity metric was used to investigate β-diversity in the ileum and cecum between the treatment groups. In the ileum at 14 d a significant shift in the microbial communities was observed, in which 18% of the variation was due to the treatment. The β-diversity of the GG ($P = 0.0033$) and GG + E ($P = 0.0291$) groups were significantly different from the control group (Figure 2A). β-Mannanase supplementation of the GG diet did not affect the ileal microbial composition, as no difference between the GG and GG + E group could be observed ($P = 0.394$). No differences were observed in the ileum at 28 d. In the ceca, a significant difference in the microbial communities was observed at 14 d, in which 17% of the variation could be justified by the treatment, and all groups differed from each other (Figure 2B). At 28 d 22% of the difference in cecal microbial community composition could be attributed to the dietary treatment, and all groups differed from each other (Figure 2D).

The phylum *Firmicutes* was the most abundant phylum in both intestinal segments at both ages (>90%). In the ileum on 14 d, GG group had a significantly higher relative abundance of the phylum *Proteobacteria* as compared to the control group (6.2% vs. 0.08%, $P < 0.0001$). Adding β-mannanase to the GG group (GG + E) resulted in a significantly lower relative *Proteobacteria* abundance as compared to the GG group (1.7 vs. 6.2%, $P = 0.0264$) (Table 5). No significant differences were observed in the ileum at 28 d, at phylum level.

In the cecum at both 14 and 28 d, GG significantly increased the relative abundance of *Actinobacteriota* and *Proteobacteria* and reduced *Bacteroidota* as compared to the control group. β-Mannanase supplementation (GG + E) resulted in a significant decrease in *Actinobacteriota* and *Proteobacteria* and an increase in *Bacteroidota* relative abundance as compared to the GG group. No significant differences were observed between the control and GG + β-mannanase groups at phylum level.

At genus level in the ileum at 14 d, GG supplementation increased the relative abundance of *Bifidobacterium, Streptococcus, UCG-008, Tyzzerella, Escherichia-*

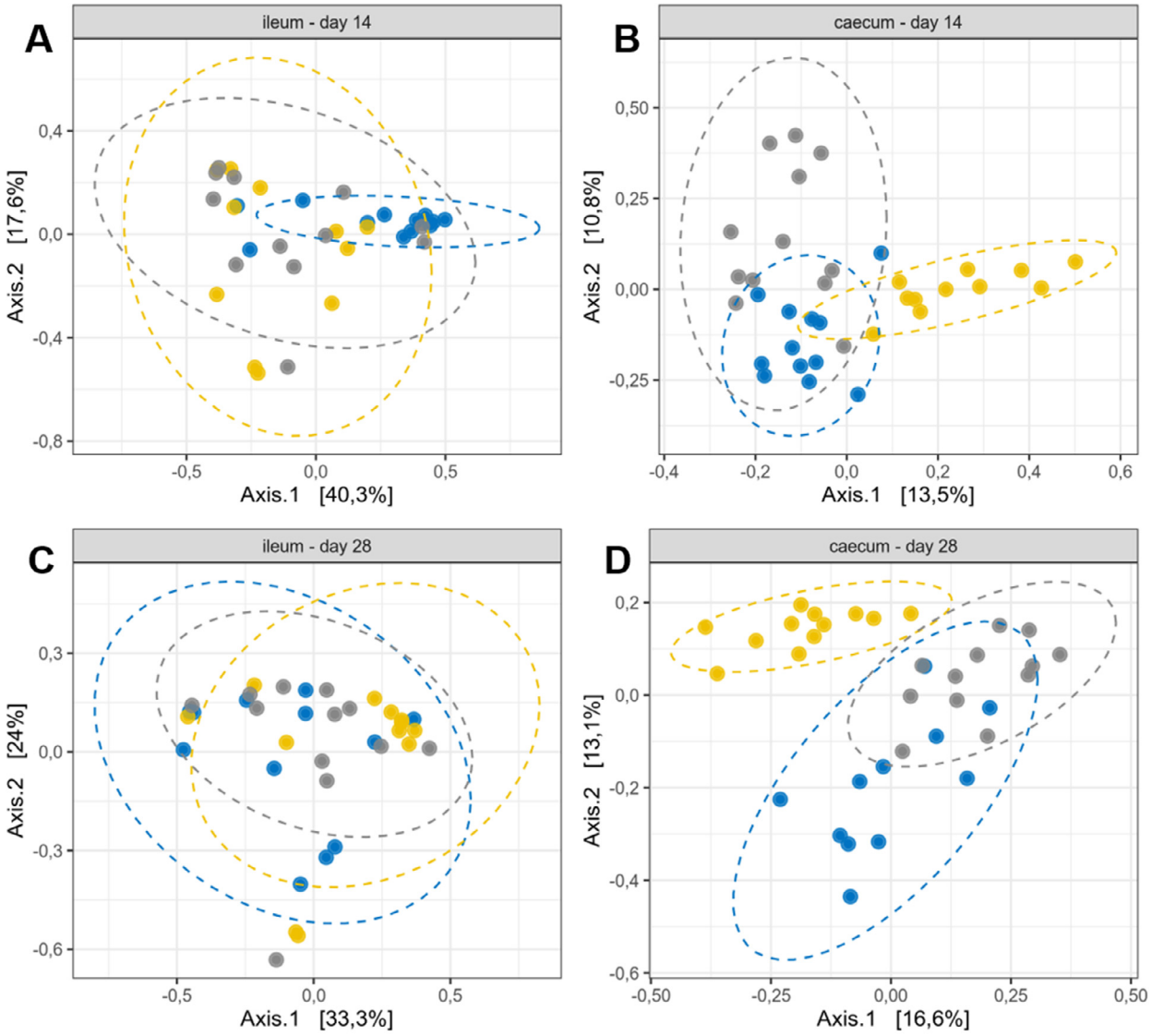

Figure 2. Principal coordinate analysis plot of bacterial β-diversity based on Bray-Curtis dissimilarities. Ileal and cecal bacterial β-diversity measurements at 2 time points among groups of animals fed a control diet or a diet supplemented with guar gum, either or not supplemented with β-mannanase at 330 g/ton feed. Values are the means of 12 animals (1/pen/treatment) ● Control; ● Guar gum; ● Guar gum + β-mannanase. Each dot represents an individual chicken microbiome. β-Diversity. Bray-Curtis dissimilarity metric. (A) Ileum 14 d: significant separation in the microbial communities ($P = 0.002$), 18% of the difference is attributed to the treatments, GG ($P = 0.0033$) and GG + E ($P = 0.0291$) differed from control. (B) Cecum 14 d: a significant difference of the microbial communities was observed ($P = 0.001$), 17% of the difference can be attributed to the treatments, all groups differ from each other (control vs. GG $P = 0.0003$; control vs. GG + E $P = 0.0006$; GG + E vs. GG $P = 0.0003$). (C) Ileum 28 d: there was no statically difference among the microbial communities ($P = 0.219$). (D) Cecum 28 d: a significant difference ($P = 0.001$) among the groups was observed. The treatments explain 22% of the variation between the samples, all groups differ from each other (control vs. GG $P = 0.0003$; control vs. GG + E $P = 0.0006$; GG + E vs. GG $P = 0.0003$).

Shigella, and reduced *Enterococcus, Family_ Peptostreptococcaceae*, and *Romboutsia*, as compared to the control group (Table 6). The β-mannanase supplementation was able to restore all these shifts to control levels. In the ileum at 28 d GG supplementation increased *Tyzzerella* abundance and decreased *Erysipelatoclostridium, Butyricicoccus, Faecalibacterium, Family_ Ruminococcaceae*, and *Romboutsia*, and again β-mannanase supplementation restored these changes to control levels (Table 6).

In the cecum, at 14 d of age, GG supplementation caused significant changes in 24 bacterial genera as compared to the control group, and dietary β-mannanase was effective to restore 20 (83.33%) of these to control levels (Table 7). The main changes induced by GG were an increase in genera belonging to the families *Lactobacillaceae* (*HT002, Lactobacillus*, and *Limasilobacillus*), *Streptococcacea* (*Streptococcus*), *Lachnospiraceae* (*Marvinbryantia* and *Roseburia*), and *Enterobacteriaceae* (*Escherichia-Shigella*) and a decrease in some genera from the families *Oscillospiraceae* (*Intestinimonas* and *Family_ oscillapiraceae*), *Ruminococcaceae* (*Anaerotruncus, Caproiciproducens, DTU089, Family_ Ruminococcaceae, Incertae Sedis*, and *Negativibacillus*) and *Peptostreptococcaceae* (Family_ *Peptostreptococcaceae*).

At 28 d, in the cecum, GG supplementation caused significant changes in 38 bacterial genera as compared to the

Table 5. Mean abundance of ileal and cecal phyla in the microbiota at 2 time points among groups of animals fed a control diet or a diet supplemented with guar gum, either or not supplemented with β-mannanase at 330 g/ton feed.

	CTR	GG	GG + E	CTR vs. GG	CTR vs. GG + E	GG vs. GG + E
Phylum	Mean abundance (%)			Adjusted *P* value		
Ileum 14 d						
Actinobacteriota	2.69	3.78	6.16	>0.05	>0.05	>0.05
Bacteroidota	0.01	0	0.02	>0.05	>0.05	>0.05
Firmicutes	97.21	90.01	92.07	>0.05	>0.05	>0.05
Proteobacteria	0.09	6.21	1.75	< 0.0001	0.0316	0.0264
Ileum 28 d						
Actinobacteriota	4.33	5.94	5.03	>0.05	>0.05	>0.05
Bacteroidota	0.04	0	0.16	>0.05	>0.05	>0.05
Firmicutes	95.10	92.30	92.87	>0.05	>0.05	>0.05
Proteobacteria	0.52	1.76	1.92	>0.05	>0.05	>0.05
Cecum 14 d						
Actinobacteriota	0.25	0.67	0.33	<0.0001	>0.05	<0.0001
Bacteroidota	6.04	1.00	4.65	<0.0001	>0.05	<0.0001
Firmicutes	91.73	92.29	92.06	>0.05	>0.05	>0.05
Proteobacteria	1.96	6.02	2.94	0.0163	>0.05	>0.05
Cecum 28 d						
Actinobacteriota	0.17	1.14	0.26	<0.0001	>0.05	0.0001
Bacteroidota	8.86	3.95	8.28	<0.0001	>0.05	0.0001
Firmicutes	90.25	92.30	90.51	<0.0001	>0.05	0.0001
Proteobacteria	0.71	2.59	0.94	0.0047	>0.05	0.0071

Values are the means of 12 animals (1/pen/treatment). DESeq2 analysis at 5% of significance level, was used to determine statistical differences among groups.

control group, and dietary β-mannanase restored 36 to control levels (Table 7). Relative to the control group, GG significantly increased the relative abundance of genera belonging to the families *Clostridiaceae* (*Clostridium sensu stricto 1*), *Lactobacillaceae* (*HT002* and *Lactobacillus*), *Lachnospiraceae* (*[Eubacterium] hallii group, Blautia, Family_Lachnospiraceae, Frisingicoccus, Lachnospira, Roseburia*, and *UC5-1-2E3*) and *Enterobacteriaceae* (*Escherichia-Shigella*), and reduced genera from the families *Bacillaceae* (*Bacillus* and *Family_Bacillaceae*), *Butyricicoccaceae* (*Butyricicoccus, UCG-008*, and *UCG-009*), *Oscillospiraceae* (*Family_Oscillospiraceae, Flavonifractor, Intestinimonas, Oscillospira*, and *UCG-005*), and *Ruminococcaceae* (*Anaerofilum, Anaerotruncus, Family_Ruminococcaceae, Fournierella*, and *Paludicola*).

In general, β-mannanase dietary supplementation was able to restore the main microbial shifts induced by GG and restore the gut microbiome to levels of the control group.

To confirm specific microbial changes induced by the diets, *q*PCR analysis was done to quantify the abundance of specific families and genera in cecal content. Regarding the family *Enterobacteriaceae* no significance was observed at both ages, however, at 14 d the GG group had a trend toward higher levels ($P = 0.0709$) (Figure 3). The family *Ruminococcaceae* was significantly reduced in the GG group at 14 d relative to the control. The abundance of the *Lactobacillus* genus was significantly higher in the GG group as compared to the control group at 14 and 28 d, which is in accordance with the 16SrRNA gene data.

The gene encoding butyryl-CoA: acetate CoA-transferase, which estimates the butyrate-producing ability of the microbiota (Louis and Flint, 2007), was also quantified. At 28 d a significant decrease was found in the GG group relative to the control group.

Guar Gum and β-Mannanase Supplementation Affect SCFA and BCFA Concentrations in the Cecal

SCFA and BCFA concentrations in cecal content were analyzed at 14 and 28 d of age (Table 8). At 14 d of age β-mannanase supplementation reduced propionate, valeric, isovaleric, isobutyric and BCFA levels, as compared to the GG group. At 28 d GG increased acetic and isocaproic acid levels relative to the control group, and again a reduction in propionate, caproic, valeric, isovaleric, isobutyric, isocaproic, and BCFA levels were induced by the enzyme, relative to the GG group levels.

DISCUSSION

Guar gum is produced from guar, a drought resistant legume. The byproduct guar meal may contain up to 45% protein and is used as a feed ingredient, but it contains a high concentration of β-mannans, considered to be an antinutritional factor, that leads to animal performance losses (Saeed et al., 2017). This was confirmed in our trial, and β-mannanase supplementation was able to restore the BW and FCR to control levels. The negative effects of GG on zootechnical performance was already observed in previous studies (Leeet al., 2003; Mishra et al., 2013; Rama Rao et al., 2014). The poor performance is attributed to the high intestinal viscosity induced by GG, that reduces the passage rate, increases the satiety, reduces the feed intake (also observed in this study), and also impairs the nutrient absorption (Maisonnier et al., 2003; Owusu-Asiedu et al., 2006). According to

Table 6. Differentially abundant genera in the ileal microbiota at 2 time points among groups of animals fed a control diet or a diet supplemented with guar gum, either or not supplemented with β-mannanase at 330 g/ton feed.

Phylum	Class	Family	Genus	CTR	GG	GG + E	CTR vs. GG	CTR vs. GG + E	GG vs. GG + E
				Mean abundance (%)			Adjusted *P* value		
ILEUM 14 d									
Actinobacteriota	*Actinobacteria*	*Bifidobacteriaceae*	*Bifidobacterium*	0.0004	0.0427	0.0086	0.0384	0.3273	1
Firmicutes	*Bacilli*	*Enterococcaceae*	*Enterococcus*	13.2080	0.9023	6.5246	0.0034	1	0.0073
Firmicutes	*Bacilli*	*Erysipelatoclostridiaceae*	*Erysipelatoclostridium*	0.0719	0.0104	0.0319	0.0571	1	0.6122
Firmicutes	*Bacilli*	*Lactobacillaceae*	*Lactobacillus*	66.8353	47.8278	49.2388	0.0571	1	0.4713
Firmicutes	*Bacilli*	*Lactobacillaceae*	*Weissella*	0	0.0082	0.0574	0.2450	0.0104	0.3590
Firmicutes	*Bacilli*	*Staphylococcaceae*	*Staphylococcus*	0.1599	0.0837	0.3014	0.4290	0.0104	0.1463
Firmicutes	*Bacilli*	*Streptococcaceae*	*Streptococcus*	2.8872	19.8697	7.4034	0.0226	0.3273	1
Firmicutes	*Clostridia*	*Butyricicoccaceae*	UCG-008	0	0.1665	0	<0.0001	1	<0.0001
Firmicutes	*Clostridia*	*Clostridiaceae*	*Candidatus Arthromitus*	2.5497	1.1646	9.3110	0.1829	1	0.0192
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Tyzzerella*	0.0028	1.3470	0.0176	0.0046	1	0.4713
Firmicutes	*Clostridia*	*Peptostreptococcaceae*	*Family_ Peptostreptococcaceae*	0.5989	0.0160	0.1894	0.0125	1	0.0046
Firmicutes	*Clostridia*	*Peptostreptococcaceae*	*Romboutsia*	0.2374	0	0.0415	<0.0001	1	<0.0001
Proteobacteria	*Gammaproteobacteria*	*Enterobacteriaceae*	*Escherichia-Shigella*	0.0879	6.2105	1.7457	0.0034	0.1327	0.9838
ILEUM 28 d									
Firmicutes	*Bacilli*	*Erysipelatoclostridiaceae*	*Erysipelatoclostridium*	0.0124	0.0003	0.1467	0.0210	1	0.0104
Firmicutes	*Clostridia*	*Butyricicoccaceae*	*Butyricicoccus*	0.0389	0	0.1264	0.0011	1	0.0104
Firmicutes	*Clostridia*	*Clostridiaceae*	*Clostridium sensu stricto 1*	4.0782	5.0610	0.0358	0.9294	0.2173	0.0104
Firmicutes	*Clostridia*	*Lachnospiraceae*	*[Ruminococcus] torques group*	0.2051	0.0134	0.6417	0.0987	1	0.0347
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Tyzzerella*	0	0.3303	0	<0.0001	1	<0.0001
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Faecalibacterium*	0.2367	0.0050	0.6200	0.0347	1	0.0347
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Family_ Ruminococcaceae*	0.0563	0.0025	0.2794	0.0443	1	0.0735
Firmicutes	*Clostridia*	*Peptostreptococcaceae*	*Romboutsia*	4.2001	0.1062	1.0038	0.0009	1	0.0735

Values are the means of 12 animals (1/pen/treatment). DESeq2 analysis at 5% of significance level was used to determine statistical differences among groups.
Only bacterial families with minimum relative abundance >0.05% in at least 1 group are reported. The taxonomic classification and relative abundance of each family are shown.

Table 7. Differentially abundant genera in the cecal microbiota at 2 time points among groups of animals fed a control diet or a diet supplemented with guar gum, either or not supplemented with β-mannanase at 330 g/ton feed.

Phylum	Class	Family	Genus	CTR	GG	GG + E	CTR vs. GG	CTR vs. GG + E	GG vs. GG + E
				Mean abundance (%)			Adjusted *P* value		
			Cecum 14 d						
Actinobacteriota	*Actinobacteria*	*Bifidobacteriaceae*	*Bifidobacterium*	0	0.2836	0.0008	<0.0001	<0.0001	0.0220
Actinobacteriota	*Actinobacteria*	*Corynebacteriaceae*	*Corynebacterium*	0.0075	0.0189	0	0.5852	NA	0.0220
Actinobacteriota	*Coriobacteriia*	*Eggerthellaceae*	*Eggerthella*	0.0520	0.1896	0.0235	0.0069	0.5446	0.0002
Firmicutes	*Bacteroidia*	*Bacteroidaceae*	*Bacteroides*	1.7703	0.3534	1.0694	0.4688	0.3784	0.0353
Bacteroidota	*Bacteroidia*	*Rikenellaceae*	*Alistipes*	4.2728	0.6545	3.5875	<0.0001	0.5750	<0.0001
Firmicutes	*Bacilli*	*Bacillaceae*	*Bacillus*	0.7770	0.0806	0.2986	0.0170	0.2633	0.4078
Firmicutes	*Bacilli*	*Lactobacillaceae*	*HT002*	1.0569	2.7934	0.7001	0.0069	0.5446	0.0002
Firmicutes	*Bacilli*	*Lactobacillaceae*	*Lactobacillus*	3.9770	11.3182	3.8157	<0.0001	0.7878	<0.0001
Firmicutes	*Bacilli*	*Lactobacillaceae*	*Limosilactobacillus*	0.0547	0.4844	0.0984	0.0006	0.7842	0.0220
Firmicutes	*Bacilli*	*Streptococcaceae*	*Streptococcus*	0.0830	7.7199	0.4476	<0.0001	0.3490	<0.0001
Firmicutes	*Clostridia*	*Defluviitaleaceae*	*Defluviitaleaceae UCG-011*	0.1487	0.4659	0.0962	0.1933	0.7045	0.0415
Firmicutes	*Clostridia*	*[Eubacterium] coprostanoligenes group*	*Family_[Eubacterium] coprostanoligenes group*	1.8682	5.6657	3.4489	0.0170	0.4980	0.1555
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Family_Lachnospiraceae*	2.6678	2.5480	2.2075	0.4118	0.4546	0.0460
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Frisingicoccus*	0.0296	0.2682	0	0.1802	0.0093	<0.0001
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Marvinbryantia*	0.4971	2.3199	0.4942	0.0245	0.6922	0.0566
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Roseburia*	0.0059	0.6579	0.0477	0.0418	0.4546	0.3246
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Tyzzerella*	3.1932	2.4835	1.0642	0.9897	0.0093	0.0087
Firmicutes	*Clostridia*	*Monoglobaceae*	*Monoglobus*	1.3950	3.5698	1.5500	0.0457	0.9020	0.0307
Firmicutes	*Clostridia*	*Oscillospiraceae*	*Family_Oscillospiraceae*	2.5308	0.7232	0.9058	0.0457	0.0086	0.5377
Firmicutes	*Clostridia*	*Oscillospiraceae*	*Flavonifractor*	0.4751	0.2950	1.4531	0.8243	0.0043	0.0003
Firmicutes	*Clostridia*	*Oscillospiraceae*	*Intestinimonas*	5.9153	0.4555	1.7173	<0.0001	0.0026	0.0566
Firmicutes	*Clostridia*	*Oscillospiraceae*	*Oscillibacter*	0.6241	0.3149	3.3616	0.3680	0.0577	0.0003
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Anaerotruncus*	2.8432	0.0803	0.5591	0.0009	0.3936	0.0410
Firmicutes	*Clostridia*	*Ruminococcaceae*	*CAG-352*	0.0016	0.3842	0.0591	0.0032	0.2258	0.2259
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Caproiciproducens*	0.0241	0	0.0941	0.0221	0.2939	0.0007
Firmicutes	*Clostridia*	*Ruminococcaceae*	*DTU089*	0.6647	0.1800	1.8231	0.0992	0.2633	0.0003
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Family_Ruminococcaceae*	6.0167	2.0304	5.1728	0.0244	0.3936	0.2973
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Fournierella*	0.0950	0.0411	0.0013	0.7692	0.0093	0.0410
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Incertae Sedis*	4.7382	1.7320	12.2317	0.0244	0.0382	<0.0001
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Negativibacillus*	0.9024	0.2068	0.8357	0.0049	0.5767	0.0401
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Subdoligranulum*	1.2818	5.5883	0.6227	0.0020	0.3784	<0.0001
Firmicutes	*Clostridia*		*Peptostreptococcaceae Family_Peptostreptococcaceae*	0.0419	0	0.0905	<0.0001	0.8898	0.0003
Proteobacteria	*Gammaproteobacteria*	*Enterobacteriaceae*	*Escherichia-Shigella*	1.9624	6.0210	2.8657	0.0049	0.8246	0.0120
			Cecum 28 d						
Actinobacteriota	*Actinobacteria*	*Bifidobacteriaceae*	*Bifidobacterium*	0	0.6695	0	<0.0001	1	<0.0001
Actinobacteriota	*Coriobacteriia*	*Eggerthellaceae*	*CHKCI002*	0.1540	0.3567	0.2271	0.0385	0.6846	0.2387
Bacteroidota	*Bacteroidia*	*Rikenellaceae*	*Alistipes*	4.9440	2.0440	3.4186	0.0039	0.6444	0.0665
Firmicutes	*Bacilli*	*Bacillaceae*	*Bacillus*	1.1595	0.2377	0.8679	0.0417	0.9439	0.1153
Firmicutes	*Bacilli*	*Bacillaceae*	*Family_Bacillaceae*	0.1304	0	0.0796	<0.0001	0.9687	<0.0001
Firmicutes	*Bacilli*	*Lactobacillaceae*	*HT002*	0.3678	1.9823	0.1494	0.0105	0.6651	0.0001
Firmicutes	*Bacilli*	*Lactobacillaceae*	*Lactobacillus*	0.9118	4.8281	0.8689	0.0003	1	0.0004
Firmicutes	*Bacilli*	*Streptococcaceae*	*Streptococcus*	7.9427	16.0682	6.0690	0.0982	0.9326	0.0186
Firmicutes	*Clostridia*	Butyricicoccaceae	*Butyricicoccus*	6.5845	2.0123	4.3069	0.0003	0.5928	0.0186

(*continued on next page*)

Table 7 (*Continued*)

Phylum	Class	Family	Genus	CTR	GG	GG + E	CTR vs. GG	CTR vs. GG + E	GG vs. GG + E
				Mean abundance (%)			Adjusted *P* value		
Firmicutes	*Clostridia*	Butyricicoccaceae	UCG-008	0.0397	0	0.0046	0.0288	0.6444	0.2325
Firmicutes	*Clostridia*	Butyricicoccaceae	UCG-009	0.1720	0.0328	0.1117	0.0026	0.7532	0.0253
Firmicutes	*Clostridia*	*Christensenellaceae*	*Christensenellaceae R-7 group*	4.2309	4.1057	2.3629	0.9887	0.1443	0.0349
Firmicutes	*Clostridia*	*Christensenellaceae*	*Family_ Christensenellaceae*	0.0459	0.0094	0.0363	0.0480	1	0.0665
Firmicutes	*Clostridia*	*Clostridiaceae*	*Clostridium sensu stricto 1*	0.0062	0.2236	0.0026	0.0367	0.7863	0.0023
Firmicutes	*Clostridia*	*Defluviitaleaceae*	*Defluviitaleaceae UCG-011*	0.3430	0.4338	0.1992	0.3254	0.5929	0.0186
Firmicutes	*Clostridia*	*[Eubacterium] coprostanoligenes group*	*Family_ [Eubacterium] coprostanoligenes group*	2.1012	4.6901	3.1611	0.0039	1	0.0031
Firmicutes	*Clostridia*		*Hydrogenoanaerobacterium Family_ Hydrogenoanaerobacterium*	0.0119	0.0020	0.0427	0.2526	0.6142	0.0167
Firmicutes	*Clostridia*	*Lachnospiraceae*	*[Eubacterium] hallii group*	0.1646	0.4483	0.2148	0.0155	0.9326	0.0599
Firmicutes	*Clostridia*	*Lachnospiraceae*	*[Eubacterium] ventriosum group*	0.0997	0.0135	0.0038	0.0340	0.0006	0.1633
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Blautia*	1.7790	4.3392	0.8162	0.0059	0.3236	<0.0001
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Dorea*	0.3769	0.2576	0.0072	0.6096	<0.0001	<0.0001
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Family_ Lachnospiraceae*	1.9547	2.6942	2.3210	0.0043	0.3236	0.2824
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Frisingicoccus*	0.1599	0.6978	0.0476	0.0518	0.5180	0.0004
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Lachnoclostridium*	1.4047	0.9023	1.5288	0.2635	0.7863	0.0498
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Lachnospira*	0.0110	0.3710	0.0267	<0.0001	0.4807	<0.0001
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Lachnospiraceae NK4A136 group*	0.6699	0.8944	0.1580	0.7390	0.1044	0.0047
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Marvinbryantia*	0.8277	1.5084	0.4420	0.1196	0.5928	0.0031
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Roseburia*	0.0045	0.2859	0.0218	0.0043	0.6142	0.0835
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Sellimonas*	1.0880	1.1354	0.4360	0.6137	0.0006	<0.0001
Firmicutes	*Clostridia*	*Lachnospiraceae*	*Tyzzerella*	2.9827	1.1992	1.5099	0.0315	0.3886	0.5791
Firmicutes	*Clostridia*	*Lachnospiraceae*	*UC5-1-2E3*	0.1178	0.4007	0.1730	0.0253	0.6611	0.1791
Firmicutes	*Clostridia*	*Monoglobaceae*	*Monoglobus*	0.7497	4.2514	0.5809	<0.0001	0.9439	<0.0001
Firmicutes	*Clostridia*	*Oscillospiraceae*	*Family_ Oscillospiraceae*	2.5731	1.0761	2.9011	0.0182	0.5787	0.0023
Firmicutes	*Clostridia*	*Oscillospiraceae*	*Flavonifractor*	0.6582	0.2162	0.6112	0.0203	1	0.0090
Firmicutes	*Clostridia*	*Oscillospiraceae*	*Intestinimonas*	2.8395	0.5787	2.7148	<0.0001	1	<0.0001
Firmicutes	*Clostridia*	*Oscillospiraceae*	*Oscillibacter*	0.5280	0.3254	0.7632	0.4564	0.3973	0.0186
Firmicutes	*Clostridia*	*Oscillospiraceae*	*Oscillospira*	0.2566	0.0622	0.1776	0.0315	0.9439	0.0319
Firmicutes	*Clostridia*	*Oscillospiraceae*	*Pseudoflavonifractor*	0.1228	0.0291	0.1126	0.0658	1	0.0399
Firmicutes	*Clostridia*	*Oscillospiraceae*	UCG-005	5.2923	2.4787	5.0788	0.0431	0.9687	0.0845
Firmicutes	*Clostridia*	Peptococcaceae	Family_ *Peptococcaceae*	0.4498	0.7504	0.5115	0.0431	0.9326	0.1516
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Anaerofilum*	0.2865	0.0282	0.0984	0.0272	0.6444	0.2236
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Anaerotruncus*	0.8918	0.0214	0.4581	<0.0001	0.6444	<0.0001
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Angelakisella*	0.0094	0.0013	0.0526	0.2987	0.6533	0.0348
Firmicutes	*Clostridia*	*Ruminococcaceae*	*CAG-352*	0	0.1907	0	<0.0001	1	<0.0001
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Caproiciproducens*	0.0172	0.0025	0.0372	0.0550	0.7296	0.0032
Firmicutes	*Clostridia*	*Ruminococcaceae*	*DTU089*	0.4591	0.3197	0.6368	0.5802	0.3973	0.0265
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Faecalibacterium*	12.9897	7.6660	21.6605	0.2828	0.2132	0.0007
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Family_ Ruminococcaceae*	5.0240	2.6720	5.4050	0.0059	0.9439	0.0006
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Fournierella*	0.9912	0.1160	0.1417	<0.0001	0.0006	0.7409
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Incertae Sedis*	1.7218	1.7541	4.2044	0.6137	0.0006	0.0017
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Paludicola*	0.4965	0.1426	0.3843	0.0368	0.9687	0.0665
Firmicutes	*Clostridia*	*Ruminococcaceae*	*Subdoligranulum*	3.0761	6.0777	1.3110	0.1316	0.3236	0.0004
Proteobacteria	*Gammaproteobacteria*	*Enterobacteriaceae*	*Escherichia-Shigella*	0.7102	2.5917	0.9393	0.0182	0.7865	0.0938

Values are the means of 12 animals (1/pen/treatment). DESeq2 analysis at 5% of significance level was used to determine statistical differences among groups.
Only bacterial families with minimum relative abundance >0.05% in at least 1 group are reported. The taxonomic classification and relative abundance of each family are shown.

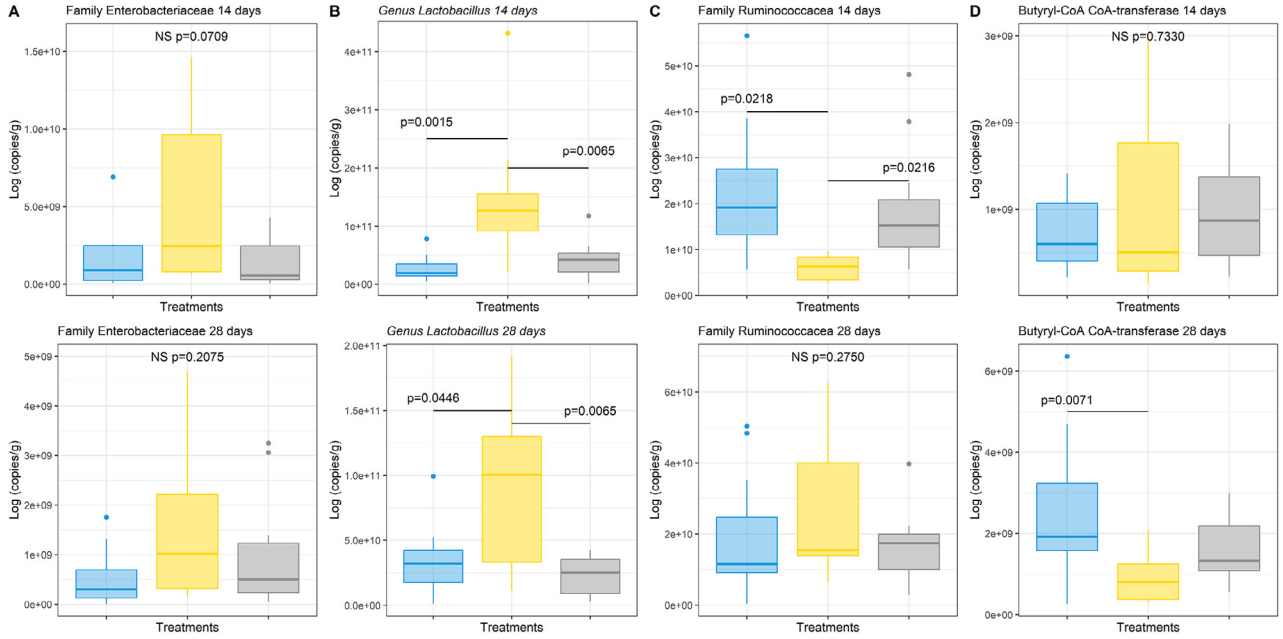

Figure 3. *q*PCR analysis from cecal content at d 14 (first line) and d 28 (second line) for animals fed a control diet or a diet supplemented with guar gum, either or not supplemented with β-mannanase at 330 g/ton feed. Values are the means of 12 animals (1/pen/treatment) ± standard deviation of the mean. ANOVA, followed by Tukey multiple comparison test, was used to determine statistical differences among groups *Y* axis—results are expressed as log of copies per gram of cecal content. Treatments: Control; Guar gum; Guar gum + β-mannanase. NS, nonsignificant.

previous studies the positive effects of dietary β-mannanase supplementation on broiler performance is a consequence of a higher ileal digestibility of carbohydrates and amino acids (Caldas et al., 2018; Gomez-Osorio et al., 2021; Latham et al., 2018; White et al., 2021).

Villus morphology was not negatively affected by GG supplementation to the diets. GG increased the ileal villus height as compared to the control group. Diets containing high levels of NSP significantly increase the length and weight of the gastrointestinal tract (Jorgensen et al.,

Table 8. SCFA concentrations in cecal content at 2 time points among groups of animals fed a control diet or a diet supplemented with guar gum, either or not supplemented with β-mannanase at 330 g/ton feed.

	CTR	GG	GG + E	CTR vs. GG	CTR vs. GG + E	GG vs. GG + E
SCFA	Mean ± SD (μmol/g)			Adjusted *P* value		
			Cecum 14 d			
Acetic acid	64.78 ± 22.60	66.12 ± 15.05	69.15 ± 21.53	0.9860	0.8560	0.9310
Butyric acid	10.37 ± 4.99	10.04 ± 4.67	10.65 ± 5.97	0.9871	0.9909	0.9579
Propionic acid	8.59 ± 5.22	7.68 ± 2.97	2.90 ± 0.29	0.9943	0.0001	0.0001
Caproic acid	0.31 ± 0.02	0.32 ± 0.02	0.31 ± 0.01	0.8284	0.9943	0.8669
Isobutyric acid	0.79 ± 0.19	0.92 ± 0.22	0.67 ± 0.14	0.2028	0.2943	0.0077
Isovaleric acid	0.81 ± 0.24	1.00 ± 0.22	0.64 ± 0.12	0.0726	0.1486	0.0006
Isocaproic acid	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.9655	0.9977	0.9774
Valeric acid	1.14 ± 0.31	1.22 ± 0.30	0.76 ± 0.25	0.8024	0.0073	0.0016
Total SCFA	90.33 ±31.53	87.66 ± 20.3	85.99 ± 27.36	0.9695	0.9180	0.9879
BCFA	1.91 ± 0.52	2.19 ± 0.42	1.57 ± 0.26	0.2713	0.1206	0.0033
			Cecum 28 d			
Acetic acid	58.25 ± 9.62	65.01 ± 17.51	41.41 ± 17.32	0.5426	0.0504	0.0040
Butyric acid	8.49 ± 2.81	14.62 ± 9.53	14.58 ± 12.36	0.2677	0.2723	>0.9999
Propionic acid	9.93 ± 2.73	11.94 ± 4.92	7.50 ± 4.17	0.4963	0.3656	0.0334
Caproic acid	0.30 ± 0.01	0.32 ± 0.01	0.30 ± 0.01	0.0579	0.9652	0.0292
Isobutyric acid	0.88 ± 0.17	0.93 ± 0.32	0.61 ± 0.11	0.9865	0.0064	0.0035
Isovaleric acid	0.91 ± 0.26	1.07 ± 0.49	0.67 ± 0.25	0.7524	0.1175	0.0211
Isocaproic acid	0.24 ± 0.01	0.26 ± 0.02	0.25 ± 0.01	0.0242	0.9679	0.0421
Valeric acid	1.06 ± 0.18	1.19 ± 0.16	0.87 ± 0.29	0.3485	0.1311	0.0052
Total SCFA	80.12 ± 10.74	95.55 ± 28.97	67.75 ± 37.17	0.3847	0.5659	0.0635
BCFA	2.04 ± 0.43	2.29 ± 0.78	1.48 ± 0.25	0.7004	0.0175	0.0019

Values are the means of 12 animals (1/pen/treatment) ± standard deviation. ANOVA, followed by Tukey multiple comparison test, was used to determine statistical differences among groups.

GG, guar gum; GG + E, guar gum + β-mannanase; SCFA, short-chain fatty acid; BCFA, branched-chain fatty acid. Quantification limit: acetic acid, 18.43 μmol/g; butyric acid, 1.45 μmol/g; propionic acid, 1.47 μmol/g; caproic acid, 0.29 μmol/g; isobutyric acid, 0.43 μmol/g; isovaleric acid, 0.42 μmol/g; isocaproic acid, 0.24 μmol/g; valeric acid, 0.43 μmol/g; total SCFA, 23.73 μmol/g; BCFA, 0.88 μmol/g.

1996). Maisonnier et al. (2003) also reported an increase in the villus height as a consequence of dietary GG supplementation and suggested that this finding is an adaptative process counteracting the negative effects of the diet, attempting to improve nutrient uptake.

Dysbiosis of the gut microbiome is a disruption in the balance, diversity, and function of intestinal microbial communities (Perez et al., 2019). In the current study guar gum ingestion caused changes in microbial diversity and composition in the cecal and ileal microbiome. Guar gum ingestion increased the relative abundance of the *Proteobacteria* phylum (ileum 14 d, cecum 14 and 28 d). A high abundance of phylum *Proteobacteria* is a hallmark of dysbiosis and poor chicken performance (Shin et al., 2015; Kollarcikova et al., 2019). The poor performance observed in the GG group in this study, might thus be associated with a diet-induced dysbiosis. The GG supplementation increased the relative abundance of bacterial families and genera known to contain opportunistic pathogens. *Escherichia-Shigella* (ileum 14 d, cecum 14 and 28 d) and *Clostridium sensu stricto* 1 (encompassing *C. perfringens*) (cecum 28 d) are the most well-known examples of bacterial members that were increased by dietary GG supplementation. The β-mannanase supplementation restored this shift to control levels. High levels of *Enterobacteriaceae* may increase intestinal lipopolysaccharide concentrations, often associated with increases in gut permeability, triggering inflammation (Bibbò et al., 2016). Guar gum supplementation also increased enterotoxigenic *Escherichia coli* proliferation in postweaning piglets (McDonald et al., 1999). In chickens, an increase of *Escherichia-Shigella* has been associated with a poor zootechnical performance (Rubio et al., 2015; Han et al., 2016). Similarly, in the present study GG supplementation impaired the zootechnical performance. Another important change was observed in the genus *Lactobacillus* that was increased in GG group as compared to the others, a finding that was confirmed by qPCR. β-Mannanase supplementation restored the levels to control values. *Lactobacillus* is a galactomannan fermenter and lactate producer (Ali et al., 2022). When high quantities of nondigestible carbohydrates reach the large intestine, they will be fermented and unusual high amounts of organic acids (lactic acid and SCFA) can be produced, resulting in a lower luminal pH, affecting the microbial composition (Petersen, 2005). While lactic acid bacteria are known for their prebiotic properties, intestinal overgrowth has been related to poor intestinal health in multiple studies (Park et al., 2021; Dey and Ray Chaudhuri, 2022; Slanzon et al., 2022).

Our data show that in the cecum, the mannan-rich diet increased the relative abundance of the genus *Bifidobacterium* and *Roseburia*, known by their capacity to ferment complex polysaccharides that have escaped proximal digestion (Jandhyala et al., 2015). This result supports our hypothesis of dysbiosis as consequence of poor ileal digestibility. The relative abundance of specific members of the *Ruminococcaceae* and *Lachnospiraceae* was reduced when GG was added to the broiler diets. These families contain butyrate-producing bacteria, of which a subset use lactate, and are shown to be of crucial importance to maintain gut health (Parada Venegas et al., 2019). Examples of beneficial bacterial genera from the *Ruminococcaceae* family that are negatively affected by GG supplementation (and restored to normal levels after supplementation with the β-mannanase) are *Butyricicoccus* and *Faecalibacterium*. These are known as butyrate-producing microorganisms that are highly anti-inflammatory, improve intestinal integrity because of effects on tight junction protein expression, and associated with optimal animal performance (Onrust et al., 2015; Bedford et al., 2017; Sikandar et al., 2017). As an example, *Butyricicoccus* supplementation to broiler diets has been shown to improve animal performance in a diet-induced dysbiosis models and was shown to reduce necrotic enteritis caused by *C. perfringens* (Eeckhaut et al., 2016). Also, it reduced the cecal abundance of *Escherichia*, an association seen in our study as well. While butyrate production has been hypothesized as the main driver for beneficial effects related to these bacterial genera, we did not observe effects on SCFA production, although a lower cecal (28 d) abundance of the gene encoding butyryl-CoA acetate CoA-transferase was observed in the GG-supplemented group.

Isobutyric, isovaleric, valeric acids, and BCFA are the result of protein fermentation (Fan et al., 2015). They were increased in the group that received the GG diet as compared to the group receiving the GG + β-mannanase. Probably the higher abundance of protein-derived BCFAs in the cecal content of the GG group is a consequence of poor protein absorption in the upper intestine.

In conclusion, guar gum diet supplementation impairs the broilers' zootechnical performance and for the first time a detailed effect of a mannan-rich diet on the broiler's gut microbiome has been described. GG causes a shift toward increases in opportunistic pathogens and reductions in beneficial microbiota and β-mannanase supplementation was able to restore the effects on the microbiota composition to control levels. Our results indicate that β-mannanase is a viable feed additive that can be used to counteract negative effects of mannan-rich feed ingredients on the microbiota composition.

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DISCLOSURES

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