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Effects of Glucomannan Feed Additives on Large White Turkey Female Performance, Gastrointestinal Microbial Population, and Blood Corticosterone Levels in a Simulated Transport Challenge

KR Flores[1], AA Gernat[1], A Fahrenholz[1], JE de Oliveira[2], G Hosotani[2], and JL Grimes[1,*]

[1]Prestage Department of Poultry Science, North Carolina State University, Raleigh, NC, USA
[2]Cargill R&D Center Europe, Vilvoorde, Belgium

***Corresponding author:** JL Grimes, Prestage Department of Poultry Science, NC State University, Campus Box 7608 Raleigh, NC, USA, Phone: 919-515-5406; Email: jgrimes@ncsu.edu

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Abstract

Turkeys may experience stress due to conditions of transport, heat, and in some cases, feed and water restrictions. This may include experiencing conditions present when female turkeys are transported from the brooder house to the grow-out house, typically at 4 to 5 wk of age. This study's objective was to determine the effect of glucomannan feed additives on performance and corticosterone response in Large White commercial females after an application of simulated conditions which birds might experience during transportation at 4 to 5 wk of age. The transport simulation consisted of reducing the space per bird from 0.198 m^2/bird to 0.028m^2/bird, with no food and water, and exposed to heat lamps, for no more than 18 h. Fourteen hundred and forty Large White female turkeys were randomly assigned to 48 concrete, pine shavings covered floor pens. The experimental design was a completely randomized block design with a one-factor arrangement of 2 sources of glucomannans (medium-chain and long-chain) with two inclusion levels of each (0.02 and 0.20% of the diet). These treatments were compared to two control treatments (no glucomannans), however, one with no simulated transport conditions and one exposed to simulated transport conditions. Bird's performance, bursa, spleen, and blood corticosterone levels were analyzed in SAS 9.4 in a mixed model. No effects on growth performance of birds fed 0.02% glucomannans were observed. Birds fed glucomannans at 0.20% in the diet had a higher corticosterone level after the transport simulation was applied when compared to other treatments. However, birds fed medium-chain glucomannans at 0.20% had an improved performance after the transport simulation compared to birds fed the other glucomannan treatments. All birds recuperated their performance by d 45. No differences were found in the spleen and bursa weights at 45 d of age. Both glucomannans reduced the stress impact on intestinal bacteria by controlling the overgrowth of *Lactobacillus* and *Fusobacteria* while maintaining *Bacteroidetes*. In conclusion, glucomannans can be used in diets with no change in performance or health at 45 d of age while preserving intestinal microbiota from the negative effects of stress. At 45d, all birds recuperated from the simulated transport conditions applied at 29 d. Thus, the effects of glucomannans on turkey performance and health should be further investigated.

Introduction

During turkey production in the USA, young turkey females may be transported from the brooder house to the grow-out house, creating a potentially stressful situation for the birds due to variations in environmental temperature and reduced bird floor space during transport. Some of the stress to which birds may be exposed include but are not limited to, increased environmental temperature, increased animal density per area, and feed and water restriction. Therefore, bird transportation is also considered an animal welfare issue [1]. It has been established that an increase in density [2] and heat stress can be detrimental while rearing turkeys [3]. Stress can reduce feed intake [4], affect bird performance and even meat quality [5], which is crucial because it is the income variable in the long-term sustainability of the poultry industry. Birds exposed to stressful conditions, diseases, and harsh environments may result in severe economic losses [6]. Transferring birds between production houses is a challenge and it has the potential to create a stress response. The effect of stress on production costs should not be underestimated. St-Pierre, et al. [7] reported that the US animal industry, including turkeys, had a total loss of 2.4 billion dollars because of heat stress alone. It can only be speculated that this figure has increased over the years because of an increased number of animals reared, increased environmental temperatures, increases in animal genetic potential for growth, and the reduced use of antibiotics as growth promoters. It can also affect costs due to changes in housing systems, especially changes in ventilation systems. A challenge model for simulated transport challenge (STC) has been created to simulate transport stress, cold, and heat stress, so it can be used to find ways to reduce its impact on commercial turkey operations [8].

Nutrient and non-nutrient components in the diet can impact the immune system's maintenance, development, and response [9]. Among feed additives, prebiotics have been proposed as a feeding intervention strategy to overcome stress situations, mainly due to the positive effects on intestinal health [10,11]. However, most controlled studies have included a single stressor (e.g. heat stress, vaccination) [12], while the challenge model in the current study included heat, food and water deprivation, and high stocking density. Glucommanans is considered a feed additive with prebiotic effects that can be produced by combining cereal-derived mannose units to create different types of water soluble fibers. Previous studies with poultry have shown that glucomannans may have a positive impact on growth through modulation of the immune system, can act as prebiotics impacting gut microbiota, and by interacting with specific binding sites of the intestinal mucosa trigger physiological state [10,13,14].

Thus, the objective of this study was to determine if feeding turkey females two glucomannans with medium- or long-chain lengths would overcome the negative effects of a simulation of transport challenges, and the effects on growth performance, gastrointestinal microbial population, and blood corticosterone levels.

Materials and Methods

Treatments and experimental design

The experimental design was a completely randomized block design with a one-factor arrangement of dietary glucomannan levels. Birds were fed either a control diet or diets with two different glucomannans at 0.02% or 0.20% inclusion levels. The inclusion levels of glucomannans were chosen based on the results from *in ovo* and *in vivo* broiler trials [13,14] and from previous studies with the same glucomannans on turkeys (non-published data). Glucomannans were labeled medium-chain glucomannan (MG) and long-chain glucomannan (LG) (Cargill Incorporated, Minneapolis, MN). Thus, there were six treatments (Table 1): birds fed a control diet without challenge (NC), birds fed a control diet and subjected to simulated transport challenge (PC), and birds fed MG at 0.02% (MG low), MG at 0.20% (MG high), LG at 0.02% (LG low), and LG at 0.20% (LG high).

Feed manufacturing

A common basal diet was prepared and shared with all treatments to achieve the same nutritional values for starter 1 and starter 2 diets. Corn was ground in a hammer mill (Model 1522, Roskamp Champion, Waterloo, IA). The hammer mill was fitted with 6 and 8-mm screen sizes. All the main ingredients were batched into a basal and mixed in a 2-ton counterpoise ribbon mixer (Model TRDB126060, Hayes & Stolz, Fort Worth, TX) for 3 minutes of a dry mix followed by 90 seconds of a wet mix. The glucomannan treatments and a portion of the liquid poultry fat were added to the basal diets and then mixed for five minutes. All treatments were then conditioned for 30 seconds at 80˚C (176˚F) in a single pass conditioner (Model C18LL4/F6, California Pellet Mill, Crawfordsville, IN). Feeds were then pelleted by a 30 HP pellet mill (Model PM1112-2, California Pellet Mill, Crawfordsville, IN), using an 11/64" × 1 3/8" pellet mill die. Each treatment's pellets were cooled in a counter flow cooler (Model VK09X09KL, Geelen Counter flow USA, INC, Orlando, FL). Starter 1 diets were then crumbled (Model 624s, Roskamp Champion, Waterloo, IA). Starter 2 diets were fed as small pellets. Protein analysis for each treatment diet was outsourced (Carolina Analytical Services, Bear Creek, NC).

Table 1: Dietary treatments and simulated transport challenges applied to turkey females.

Treatment Label	Glucomannan Level	Simulated Transport Challenge
Negative Control	none	none
Positive Control	none	Yes
MG[1] Low[3]	0.02%	Yes
MG High[4]	0.20%	Yes
LG[2] Low	0.02%	Yes
LG High	0.20%	Yes

[1]MG: medium-chain glucomannan
[2]LG: long-chain glucomannan
[3]Low inclusion rate (0.02% of the diet)
[4]High inclusion rate (0.20% of the diet)

Feeding program

Birds were fed 2 dietary phases (starter 1, and starter 2) [15] on a feed weight per bird feeding program (Tables 2 and 3). Feed weight was recorded when added to feeders. At d 29, 31, and 45, the remaining feed at the feeders was weighed to calculate feed disappearance as intake (FI) which was used to determine feed conversion ratio (FCR).

Housing and management

This study was conducted in a curtain-sided house with a concrete floor. There were 48 total pens, each with 5.95 m^2 of space. Each treatment was assigned to eight pens of female birds (30 birds/pen).

Table 2: Turkey females basal dietary ingredient composition.

Ingredients (%)	Starter 1	Starter 2
Corn	18.6	22
Wheat	20	20
Soybean meal	38	35
Poultry meal	10	10
Poultry fat	7.03	7.03
Calcium carbonate	1.8	1.65
Monocalcium phosphate	2.55	2.35
Lysine[1]	0.45	0.44
Methionine[2]	0.45	0.43
Threonine	0.15	0.13
Salt	0.2	0.2
Sodium bicarbonate	0.13	0.13
Mineral premix inorganic[3]	0.2	0.2
Vitamin mix[4]	0.2	0.2
Choline chloride	0.2	0.2
Selenium mix	0.05	0.05
Ingredient Total:	100.00	100.00

[1]Donated by Evonik North America
[2]Donated by Ajinomoto North America
[3]The mineral premix provided the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg.
[4]Donated by DSM Nutritional Products; vitamin premix provided the following per kg of diet: vitamin A, 26455 IU; vitamin D3, 7936 IU; vitamin E, 132 IU; vitamin B12, 0.08 mg; biotin, 0.51 mg; menadione, 8 mg; thiamine, 8 mg; riboflavin, 26.67 mg; pantothenic acid, 44 mg; vitamin B6, 16 mg; niacin, 220 mg; folic acid, 4 mg.

Table 3: Calculated nutrient contents of dietary treatment in each feeding phase.

Nutrient (%)	Starter 1	Starter 2
Crude protein	30.70	29.50
Crude protein (Analyzed)[1]	27.90	25.60
ME (kcal/Kg)	3,087	3,126
Crude Fat	9.80	9.80
Lysine	1.89	1.80
Methionine[2]	0.84	0.81
Methionine+Cysteine[2]	1.23	1.18
Tryptophan[2]	0.33	0.31
Threonine[2]	1.19	1.12
Arginine[2]	1.89	1.8
Valine[2]	1.31	1.25
Calcium	1.50	1.41
Available Phosphorus	0.75	0.71
Sodium	0.19	0.19
Chloride	0.18	0.18

[1]Feed analysis was performed by Carolina Analytical Services (17570 NC Highway 902, Bear Creek, NC 27207).
[2]Calculated as digestible amino acids.

There was an initial density of 0.198 m^2/bird. All pens were bedded with fresh pine shavings. Large White female turkey females (Nicholas Select, Aviagen Turkeys, Lewisburg, WV, n=1,440) were placed on the day of hatching. Each pen of turkey females was weighed at placement. Birds were weighed individually at 29 and 31 d, before and after applying the STC procedures, respectively, and then at 45d. The weight of each pen of birds plus culls and mortalities was used to determine the FCR. Feed and water were offered *ad libitum* throughout the study except during the application of STC. All animal handling procedures were approved by the NCSU Institutional Animal Care and Use Committee.

Transport stress simulation and tissue samples

Birds in all treatments, except those in the negative control, were subjected to procedures of STC. These conditions were designed to simulate potential transportation conditions that may exist when birds are moved from the brooder house to a grow-out house during a typical North Carolina summer day as described by Bartz BM, et al. [8]. In STC, panels were applied to reduce the space per bird in each pen from 0.198m^2 to 0.028m^2/bird. This density is similar to the conditions that 4 to 5 wk old turkey females might experience during commercial transportation in a poult trailer with cages. Space was reduced overnight with no bird access to food or water for 18 h. Heat lamps were placed over each group of birds for 6 h to increase the environmental temperature. Bodyweight (BW) was measured before and after the simulated transport process. After the 18 h simulation, blood samples were collected from the bracheil vein of 3 randomly chosen birds per pen for corticosterone analysis. Levels of corticosterone were measured with an ELISA methodology kit (Item No. 501320, Cayman Chemical, Ann Arbor, MI). At 45 d of age, one bird per pen was euthanized and sampled for bursa and spleen weights.

Intestinal microbial analysis

Microbial sample collection and analysis were performed according to the procedure previously described [13]. In short, cloaca swabs were used to collect digesta material from the same 3 birds per pen at 29, 31, and 45 d of age. Samples were collected only from birds with dietary treatments NC, PC, MG at 0.20%, and LG at 0.20%. Following swab collection, samples were immediately stored at -80˚C until DNA extraction. The DNA from cloaca swabs was extracted and labeled with a cy-5 fluorescent-labeled nucleotide in order to assess the relative abundance of a previously defined list of bacteria biomarkers by microarray analysis (Cargill Inc., proprietary). Fluorescence intensity values for each probe were used to compare the relative abundance of microbiota between feed groups according to the experimental design [13].

Statistical analysis

The experiment had a completely randomized block design. All performance parameters measured were analyzed using the PROC MIXED procedure from SAS 9.4. (SAS Institute Inc., Cary, NC). The blocks were 4 spatial separation of 12 pens each and were considered a random variable in all analyses. Significant differences in main effects were separated using the Tukey HSD test. A value of $P \leq 0.05$ was used to set a significant difference between the main and interaction effects of the parameters analyzed. For microbial composition, the dataset was submitted for data distribution analysis, followed by data standardization. The resulting dataset was submitted to ANOVA with array and block as random effects while bird age, diets, and STC were analyzed as fixed effects in a factorial arrangement in JMP Genomics 9 (SAS Institute Inc., Cary, NC). A correction for multiple testing was applied so comparisons were considered significant when FDR<0.05. Microbiota annotation used in the analysis was at the phylum level and, when available, to species. LS Means were submitted to cluster analysis using the Fast Ward method as well as to principal component analysis. Volcano plots were created to show significant differences from pair wise comparisons.

Results

Performance parameters

Growth performance results are shown in table 4. Before birds were submitted to the STC, at 29 d of age, the only difference observed among all parameters measured was the greater feed intake from birds in the MG High group compared to the NC group. Following exposure to the STC, from 29 to 31 d of age, birds from the NC group had the greatest BWG with only birds from MG High reaching a similar BWG. At 31 d of age, the cumulative FI was the greatest for birds from the MG High group, with birds from NC and LG Low groups reaching similar cumulative FI. Two weeks after STC, at 45 d of age, there were no statistical differences in BW, BWG, or FCR due to treatment. However, birds from the PC group had the lowest FI, birds from MG High had the greatest FI, and FI from birds within other treatment groups were in between. Also birds fed MG at 0.20% of the diet had a greater FI than the birds fed the control diet and were exposed to the STC.

Corticosterone

Results on blood corticosterone levels are shown in table 5. At 31 d of age and after the application of STC, birds fed either MG or LG at 0.20% had the highest corticosterone levels. Birds fed either MG or LG at 0.02% had similar stress levels as birds fed the control diet and subjected to STC. Among treatments fed the control diet, birds

Table 4: Effects of glucomannans and simulated transport challenge (STC) on female turkey performance.

Diet	0 d (g)	29 d, before STC	31 d, after STC	45 d
(Body Weight, kg/bird)				
NC	61.7	1.16	1.29[a]	2.59
PC	61.5	1.14	1.19[b]	2.5
MG[1] Low[3]	61.4	1.15	1.21[ab]	2.52
MG High[4]	61.1	1.18	1.25[ab]	2.57
LG[2] Low	61.8	1.14	1.21[ab]	2.5
LG High	61.9	1.17	1.19[b]	2.56
SEM[5]	0.3	0.02	0.02	0.03
P-value	0.23	0.59	0.02	0.25
(Body weight Gain, kg/bird)				
NC	NA[6]	1.1	0.13[a]	1.31
PC	NA	1.07	0.06[b]	1.3
MG Low	NA	1.09	0.05[b]	1.31
MG High	NA	1.11	0.07[ab]	1.33
LG Low	NA	1.08	0.05[b]	1.33
LG High	NA	1.11	0.03[b]	1.36
SEM	NA	0.02	0.01	0.02
P-value	NA	0.59	0.002	0.34
(Feed Intake, kg/bird)				
NC	NA	1.57[b]	1.71[ab]	3.51[ab]
PC	NA	1.60[ab]	1.67[b]	3.38[b]
MG Low	NA	1.58[ab]	1.65[b]	3.44[ab]
MG High	NA	1.65[a]	1.74[a]	3.57[a]
LG Low	NA	1.60[ab]	1.69[ab]	3.40[ab]
LG High	NA	1.59[ab]	1.67[b]	3.45[ab]
SEM	NA	0.02	0.02	0.04
P-value	NA	0.05	0.01	0.04
(Feed Conversion Ratio, F:G)				
NC	NA	1.36	1.335	1.325
PC	NA	1.399	1.385	1.328
MG Low	NA	1.349	1.351	1.312
MG High	NA	1.386	1.38	1.352
LG Low	NA	1.38	1.385	1.319
LG High	NA	1.36	1.39	1.328
SEM[3]	NA	0.014	0.016	0.012
P-value	NA	0.302	0.135	0.293

[a,b]Means within a column lacking a common superscript differ (P ≤ 0.05).
[1]Medium-chain glucomannan
[2]Long-chain glucomannan
[3]Low inclusion rate (0.02% of the diet)
[4]High inclusion rate (0.20% of the diet)
[5]The standard error of the mean (SEM) n=8 pens per main effect with 30 birds per pen at placement.
[6]Non-Applicable

Table 5: Corticosterone levels following simulated transport challenge at 31 days of age.[1]

Diet	Corticosterone ng/ml
PC	2.91[c]
NC	21.75[b]
MG[2] Low[4]	20.46[b]
MG High[5]	29.07[a]
LG[3] Low	20.54[b]
LG High	27.71[a]
SEM[6]	1.57
P-value	<0.0001
BW (covariate) (P-value)	0.02

[1]Multiple comparison done with student's T test.
[2]Medium-chain glucomannan
[3]Long-chain glucomannan
[4]Low inclusion rate (0.02% of the diet)
[5]High inclusion rate (0.20% of the diet)
[6]The standard error of the mean (SEM) n=8 pens per main effect, each with 3 subsamples.

not exposed to STC had lower blood corticosterone levels than birds exposed to the STC.

Bursa and spleen weights

Results on organ weights are shown in table 6. No statistical differences due to treatments were observed for spleen weights, bursa weights, or relative organ weights (bursa or spleen percentage of BW) at 45 d of age.

Cloacal Microbial analysis

Principal components analysis (Figure 1): At 29 d of age, before stress, samples were grouped according to the diet composition (with or without glucomannan included at 0.20% of the diet). Following the applied STC at 31 and until 45 d of age, the microbial composition was grouped as PC with MG high or NC with LG high.

Clustering analysis-bacteria phylum (Figure 2): At the phylum level, a distinction between microbiota population before STC at 29 d of age and after STC at 31 d of age combined with diet phase change was observed, characterized by increased abundance of *Firmicutes, Fusobacteria* and an unclassified bacteria in exchange of *Bacteroidetes* and *Proteobacteria*. These changes were less pronounced in the microbiota of turkey females from NC and LG High groups than in turkey females from PC and MG High groups. At 45 d of age, the microbiota from turkey females from NC and LG High groups had a more pronounced reduction in the abundance of *Firmicutes, Fusobacteria*, and unclassified bacteria, while in turkey females from PC and MG High groups a minor reduction in the abundance of similar bacteria was observed (Table 7).

Microbial taxa relative abundance among treatment groups: The microbial population at 29 d of age: Microbial population differences within control groups (NC and PC) at d 29 are shown in figure 3. Prior to the exposure to STC, there were five different bacteria between NC and PC, with a greater abundance of *Serratia marcescens* and *Leuconosoc 2* in NC and a greater abundance of *Incertae Sedis XI Gallicola*, *Bacteroides uncult*, and *Porphyromonadaceae unclassified* in PC.

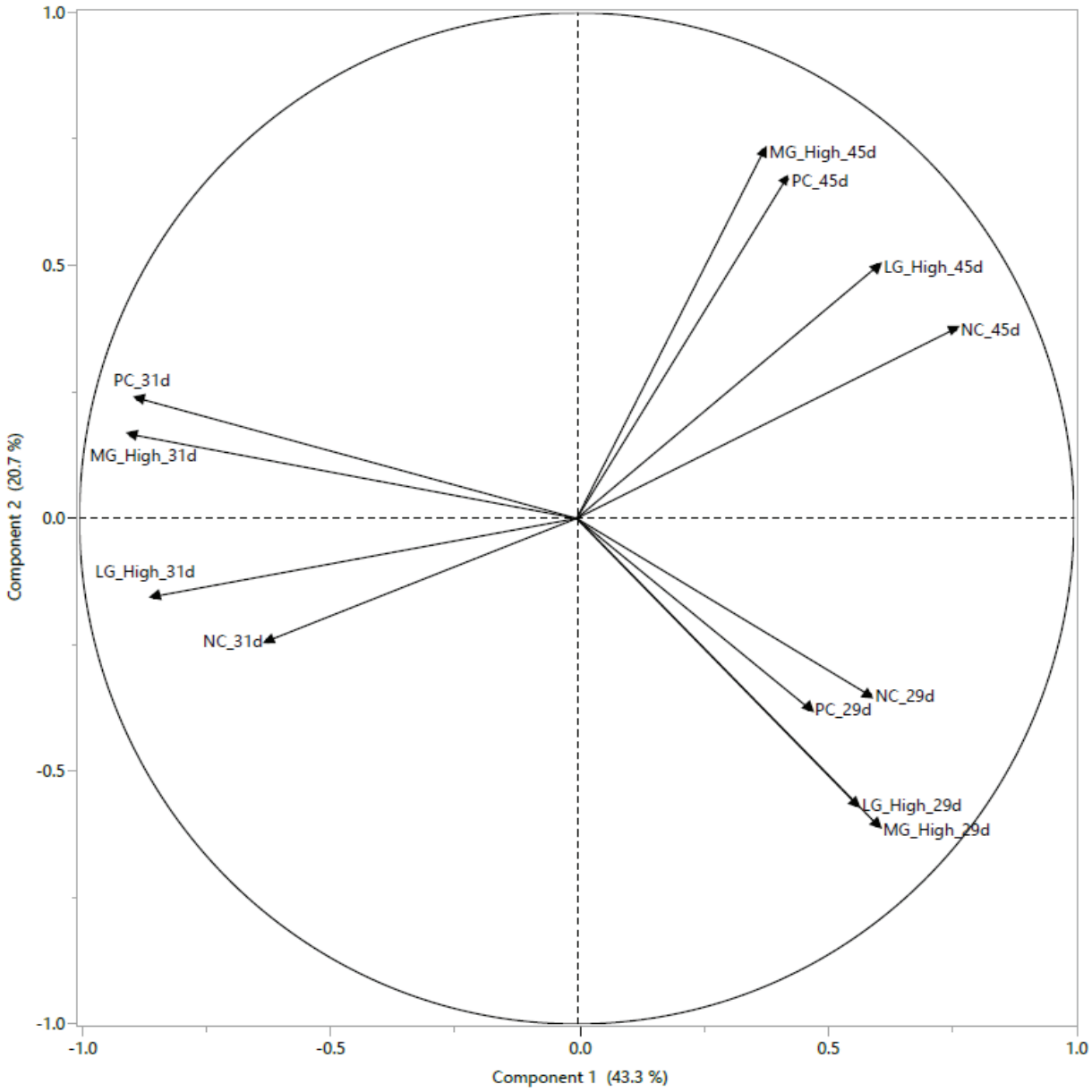

Figure 1: Principal components analysis of microbial population from cloacal samples at 29 days of age (before stress), 31 days of age (after stress), and 45 days of age of turkeys fed Negative Control diet (NC), Positive Control diet (PC), diet containing medium-chain glucomannan (MG) at 0.20% inclusion level, diet containing long-chain glucomannan (LG) at 0.20% inclusion level.

Microbial population from 29 to 31 d of age: The normal development of the microbiota population in turkey females from 29 d to 31 d of age with no exposure to STC is shown in figure 4. Turkey females at 31 d of age had a greater abundance of *Lactobacillus* species (*Lactobacillus species*, *Lactobacillus crispatus 1*, *Lactobacillus crispatus 3*, *Lactobacillus gasseri 2*) and a lower abundance of *Brenneria*, *Bacillus pumilus, Leuconostoc 2, Lachnospiraceae Incertae Sedis 1, Clostridium botulinum,* and *Lachnospiraceae unclassified.*

The microbial population at 31 d of age: Following the exposure to STC, at d 31 (Figure 5), the stress challenge affected the microbial population by increasing the abundance in PC of *Lactobacillus* species (*Lactobacillus* species, *Lactobacillus 1*, *Lactobacillus 4*, *Lactobacillus gasseri 1*, *Lactobacillus gasseri 2*) and reducing the abundance of *Holdemania* in NC. The effects of diet supplementation with MG at 0.20% of the diet on the microbial population at 31 d of age compared to PC are shown in figure 6. There was a difference in abundance of one bacterium, *Lactobacillus panis*, which was increased in turkey females fed MG. In comparison with turkey females not exposed to STC (NC), the effects of feeding MG at 0.20% of the diet at 31 d of age are shown in figure 7. Dietary supplementation with MG at 0.20% of the diet increased the abundance of *Lactobacillus* species (*Lactobacillus species, Lactobacillus crispatus 1, Lactobacillus crispatus 2, Lactobacillus crispatus 3, Lactobacillus gasseri 1, Lactobacillus gasseri 2, Lactobacillus panis*), and reduced the abundance of *Holdemania, Lachnospiraceae*

Table 6: Turkey bursa and spleen weights at 45 days of age[1].

Diet	Bursa (g)	Spleen (g)	Bursa % of BW	Spleen % of BW
PC	3.24	2.75	0.125	0.106
NC	3.31	2.98	0.123	0.111
MG[2] Low[4]	3.59	3	0.137	0.113
MG High[5]	3.26	2.93	0.125	0.114
LG[3] Low	3.54	3.14	0.136	0.124
LG High	3.54	3.04	0.131	0.113
SEM	0.12	0.11	0.005	0.005
P-value	0.14	0.26	0.11	0.41

[1]These values represent the weights of the organs and their percentage relative to the bird's bodyweight.
[2]Medium-chain glucomannan
[3]Long-chain glucomannan
[4]Low inclusion rate (0.02% of the diet)
[5]High inclusion rate (0.20% of the diet)

Table 7: Cluster analysis of cloaca microbial population between gram positive and gram-negative bacteria.[1,2]

Diet	29 days (before stress)	31 days (after stress)	45 days
PC	Aa	Ba	Ba
NC	Aa	Ba	ABa
MG[3] High[5]	Aa	Ba	Aa
LG[4] High[6]	Aa	Ba	Aa

[1]Same lower-case letters in a row were not significantly different (P>0.05).
[2]Same upper-case letters on a column were not significantly different (P>0.05).
[3]Medium-chain glucomannan
[4]Long-chain glucomannan
[5]Low inclusion rate (0.02% of the diet)
[6]High inclusion rate (0.20% of the diet)

Incertae Sedis tyrobutyricum, Lachnospiraceae Incertae Sedis 10, Lachnospiraceae Incertae Sedis ramulus, Lachnospiraceae Incertae Sedis torques,Lachnospiraceae Incertae Sedis XIII unclassified, Brenneria, Eubacterium, Streptococcus group, Bacillus pumulus, Pseudomonas group, and Salmonella 1. The effects of diet supplementation with LG at 0.20% of the diet on the microbial population at 31 d of age compared to PC are shown in figure 8. Dietary inclusion of LG at 0.20% of the diet reduced the abundance of *Lactobacillus 1, Lactobacillus 4,* and *Lactobacillus gasseri 1*.

At 31 d of age, compared with turkey females not exposed to STC, the inclusion of LG at 0.20% of the diet increased the abundance of *Lactobacillus crispatus 1, Lactobacillus crispatus 2, Lactobacillus crispatus 3, Lactobacillus species,* and reduced the abundance of *Holdemania, Facklamia, Brenneria,*and *Lachnospiraceae Incertae Sedis tyrobutyricum* (Figure 9).

A direct comparison between microbial populations from groups supplemented with either MG or LG at 0.20% of the diet is shown in figure 10. The inclusion of MG increased the abundance of *Lactobacillus 1, Lactobacillus 4, Lactobacillus gasseri 1,* while the inclusion of LG increased the abundance of *Bacteroidales unclassified.*

Microbial population from 31 to 45 d of age: The normal development of the microbiota population in turkey females from 31 d to 45 d of age with no exposure to STC is shown in figure 11. At 45 d of age, there was an increase in the abundance of *Lactobacillus 2, Bifidobacterium gallinarum, Rikenellaceae Alistipes 2, Facklamia, Lachnospiraceae unclassified, Campylobacter, Bacillus pumilus, Clostridium botulinum,* and a reduction of *Lactobacillus 1, Lactobacillus 4, Lactobacillus gasseri 1,*and *Citrobacter*. The development of the microbiota population from 31 to 45 d of age in turkey females exposed to STC in the PC group is shown in figure 12. At 45 d of age, there was an increase in the abundance of *Bifidobacterium gallinarum, Bifidobacterium pullorum, Campylobacter, Lachnospiraceae incertae Sedis ramulus, Leuconostoc 1, Faecalibacterium prausnitzii*, and a reduction in the abundance of *Lactobacillus 1, Lactobacillus 4, Lactobacillus crispatus 1, Lactobacillus crispatus 2, Lactobacillus crispatus 3, Lactobacillus gasseri 2,* and *Lactobacillus species.*

The microbial population at 45 d of age: At 45 d of age, the difference in the microbial population of turkey females from NC and PC groups was restricted to two bacteria (Figure 13). In turkey females subjected to STC, there was an increase in the abundance of *Lactobacillus 2* and a reduction in the abundance of *Rikenellaceae Alistipes.* In comparison with PC, the supplementation with MG at 0.20% of the diet resulted in changes in two bacteria (Figure 14), an increase in abundance of *Campylobacter,* and a reduction in *Lactobacillus 2*. Similarly, in comparison with NC, the supplementation with MG at 0.20% of the diet resulted in changes intwo bacteria (Figure 15), with an increase in *Clostridium bartlettii 1* and *Clostridium bartlettii 2.*

Compared with PC, diet supplementation with LG at 0.20% of the diet resulted in an increased abundance of *Bifidobacterium 2, Campylobacter,*and a reduction in the abundance of *Lactobacillus 2* (Figure 16). Compared with NC, diet supplementation with LG at 0.20% of the diet increased *Bifidobacterium 2* (Figure 17). A direct comparison between the effects of diet supplementation with either MG or LG at 0.20% of the diet resulted in an increased abundance of *Bifidobacterium 2* with the inclusion of LG, and an increased abundance of *Clostridium bartlettii 1* with the inclusion of MG (Figure 18).

Measurement of Campylobacter and Salmonella: The measurements of specific bacteria species relevant to poultry were performed (n=95). However, there were no differences among treatments throughout the study for most of the bacteria species. Data are shown only for *Campylobacter* and *Salmonella* due to the significant differences and relevance (Figures 19 and 20, respectively).

There were no differences in the abundance of *Campylobacter* within 29 and 31 d of age among treatment groups. A reduction in the PC group was observed compared to MG high and LG high however, all treatments exposed to STC having *Campylobacter* were not different from than NC group. No differences were observed in *Salmonella* between 29 and 45 d of age among different treatment groups. At 31 d of age, there was a reduction of *Salmonella* in turkey females from MG high group compared to NC group, with PC and LG high in between.

Discussion and Conclusion

The STC applied at 29 d of age had a negative impact on growth performance parameters with a lower BW and BWG of birds from PC compared to the NC group. This can be partially explained by one of the STC restrictions, the FI difference from d 29 to 31, with an intake of 70g and 140g per bird from PC and NC, respectively. The growth

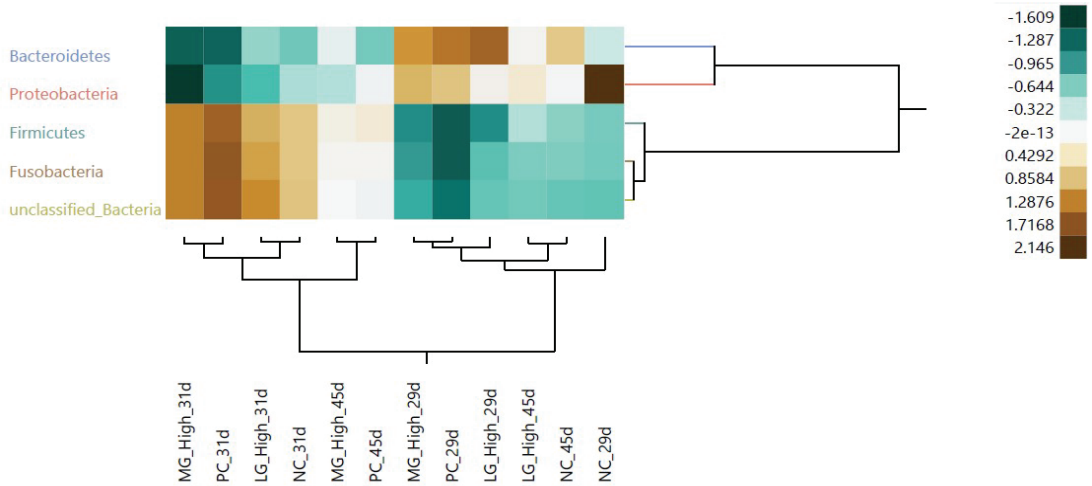

Figure 2: Hierarchical cluster analysis of relative abundance of microbial taxa (phylum) targeted by microarray in the cloacal samples at 29 days of age (before stress), 31 days of age (after stress), and 45 days of age of turkeys fed negative control diet (NC), positive control diet (PC), diet containing medium-chain glucomannan (MG) at 0.20% inclusion level, diet containing long-chain glucomannan (LG) at 0.20% inclusion level.

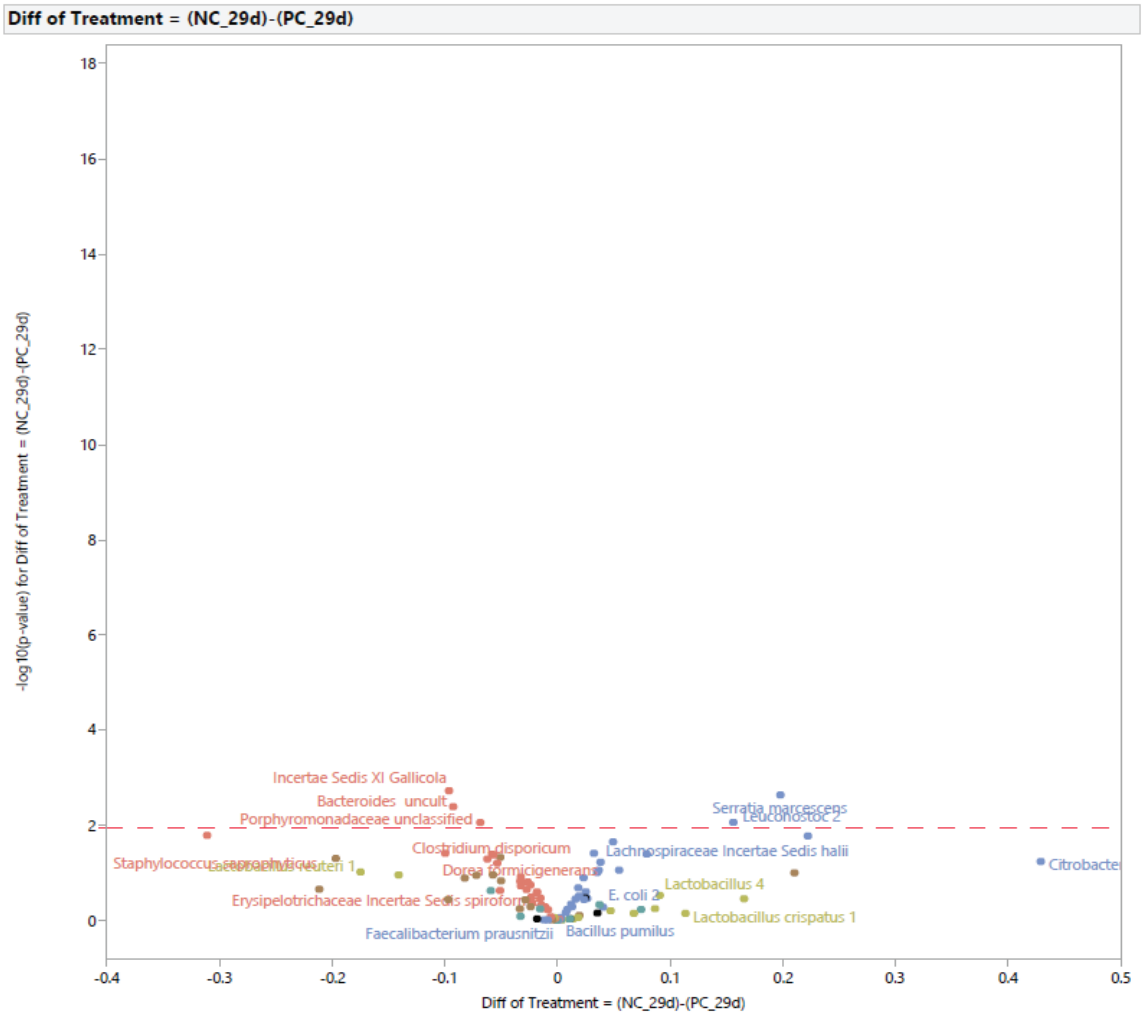

Figure 3: Effects of dietary treatments between negative control (NC) and positive control (PC) on intestinal microbiota of turkey females at 29 days of age (before stress).

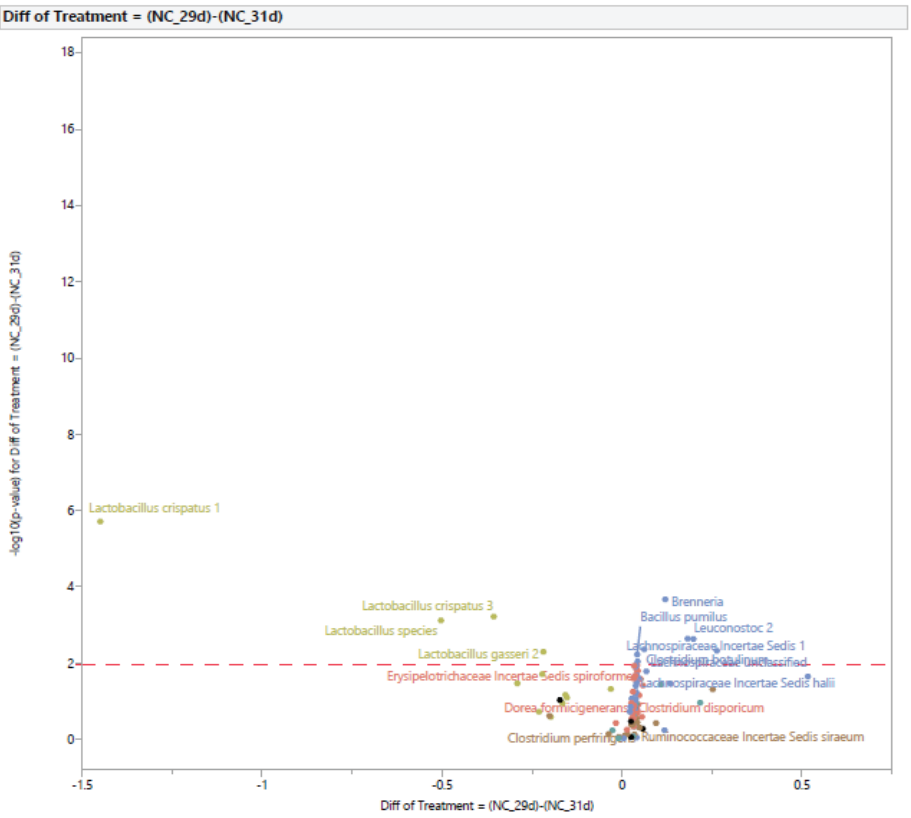

Figure 4: Age effect on the development of intestinal microbial population of turkey females within negative control treatment (NC) from 29 to 31 days of age.

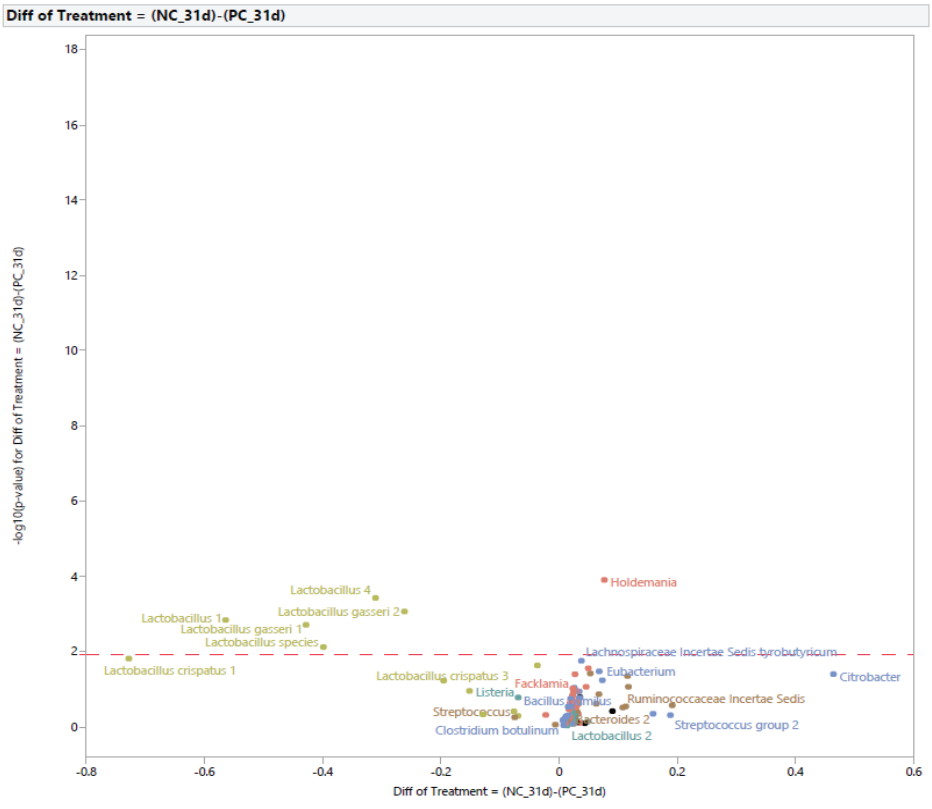

Figure 5: Effects of simulated transportation challenge on intestinal microbiota of turkey females at 31 days of age (after stress).

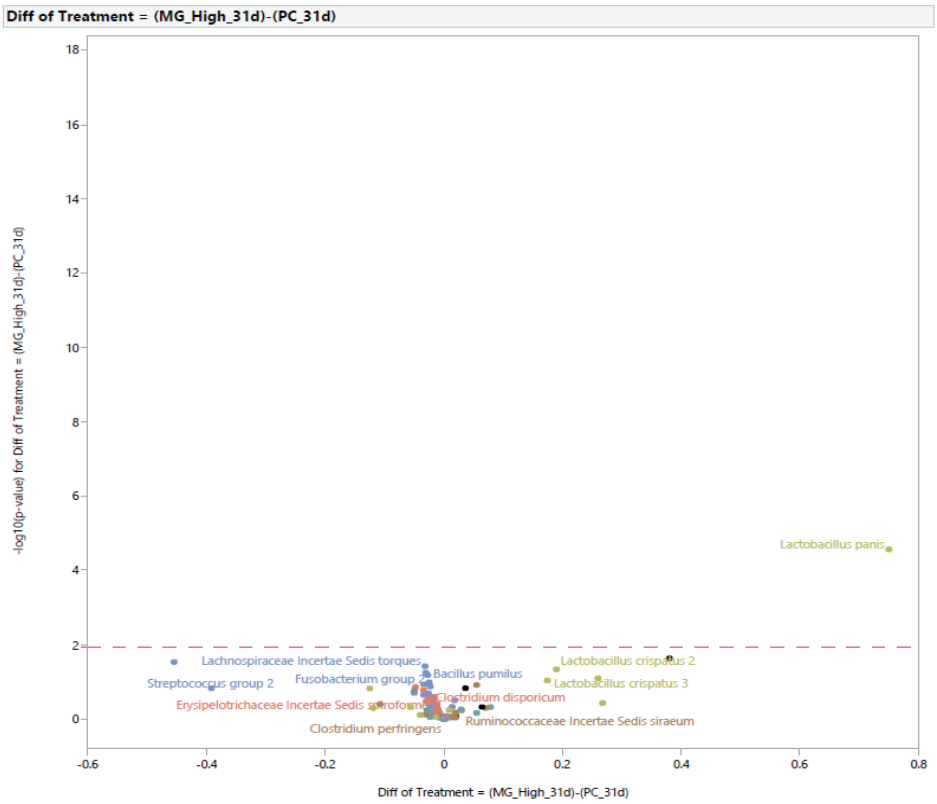

Figure 6: Effects of dietary supplementation with medium chain glucomannans (MG) at 0.20% of the diet on intestinal microbial population in comparison with turkey females from positive control treatment (PC) at 31 days of age (after stress).

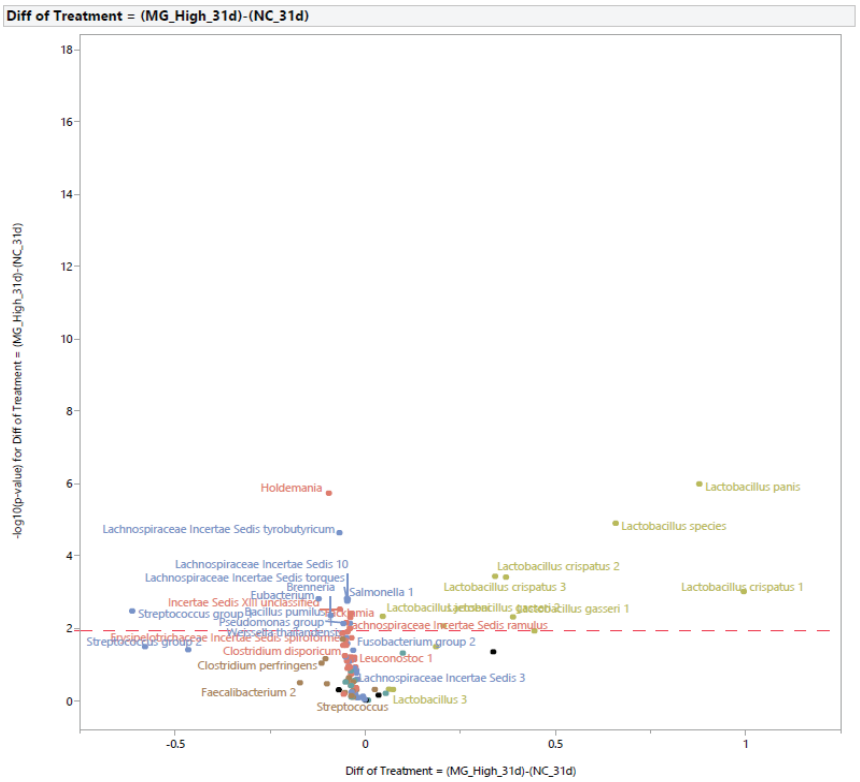

Figure 7: Effects of dietary supplementation with medium chain glucomannans (MG) at 0.20% of the diet on intestinal microbial population in comparison with turkey females from negative control treatment (NC) at 31 days of age (after stress).

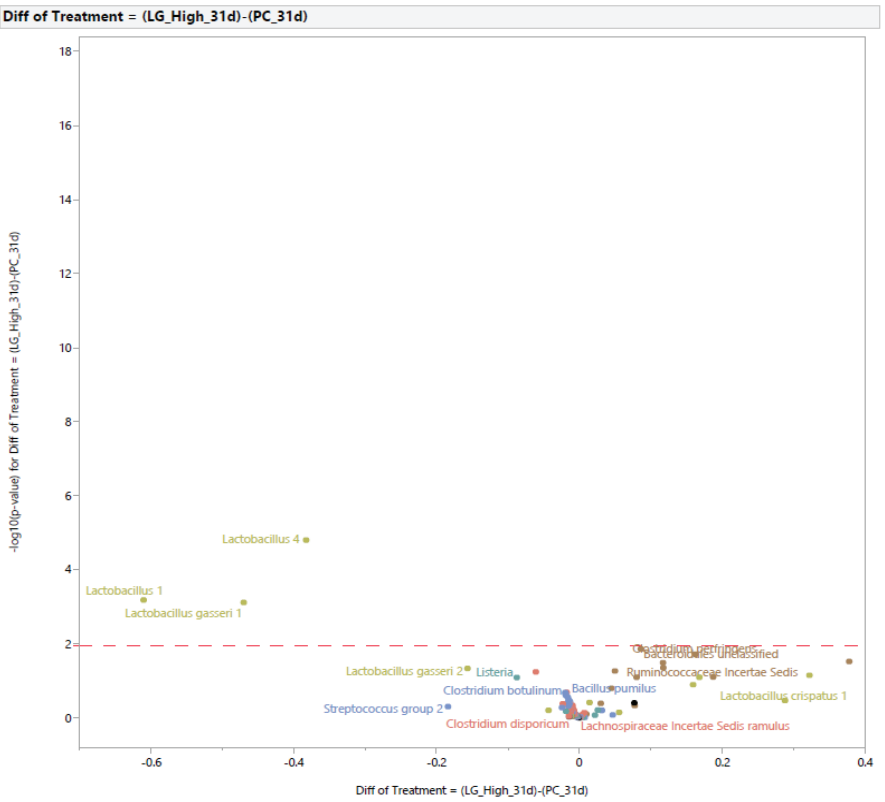

Figure 8: Effects of dietary supplementation with long chain glucomannans (LG) at 0.20% of the diet on intestinal microbial population in comparison with turkey females from positive control treatment (PC) at 31 days of age (after stress).

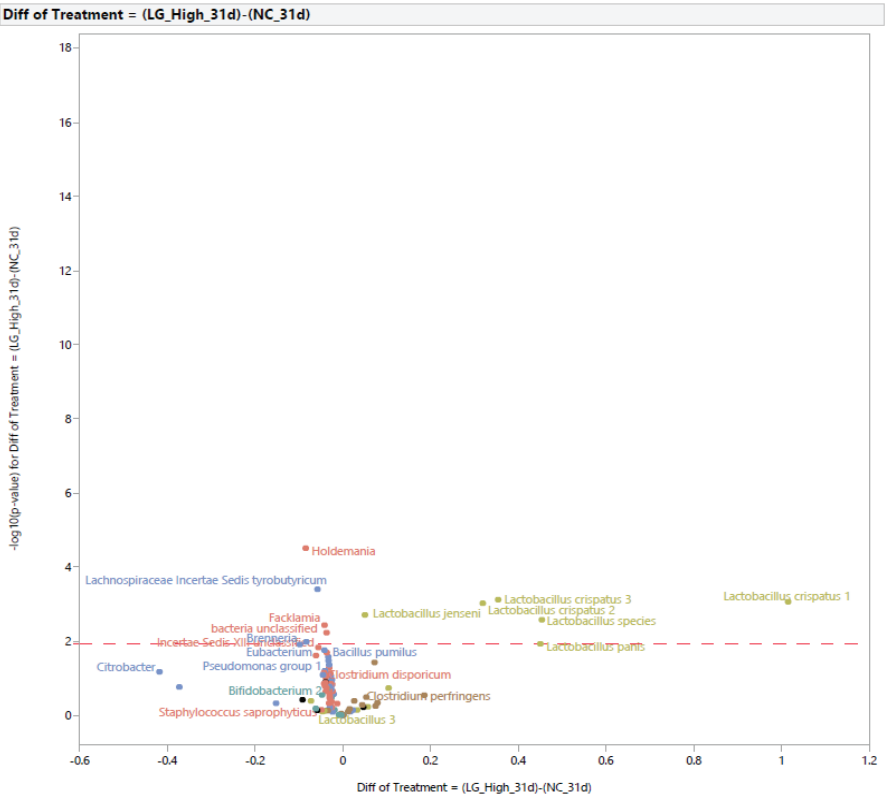

Figure 9: Effects of dietary supplementation with long chain glucomannans (LG) at 0.20% of the diet on intestinal microbial population in comparison with turkey females from negative control treatment (NC) at 31 days of age (after stress).

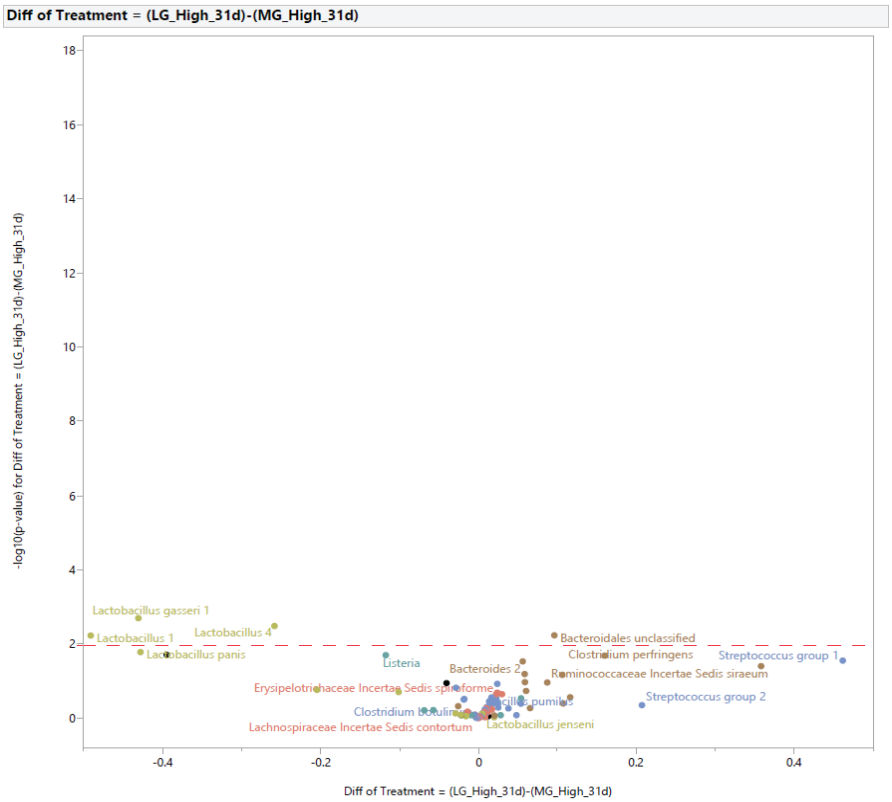

Figure 10: Effects of dietary supplementation with long-chain glucomannans (LG) or medium-chain glucomannans (MG) at 0.20% of the diet on intestinal microbial population at 31 days of age (after stress).

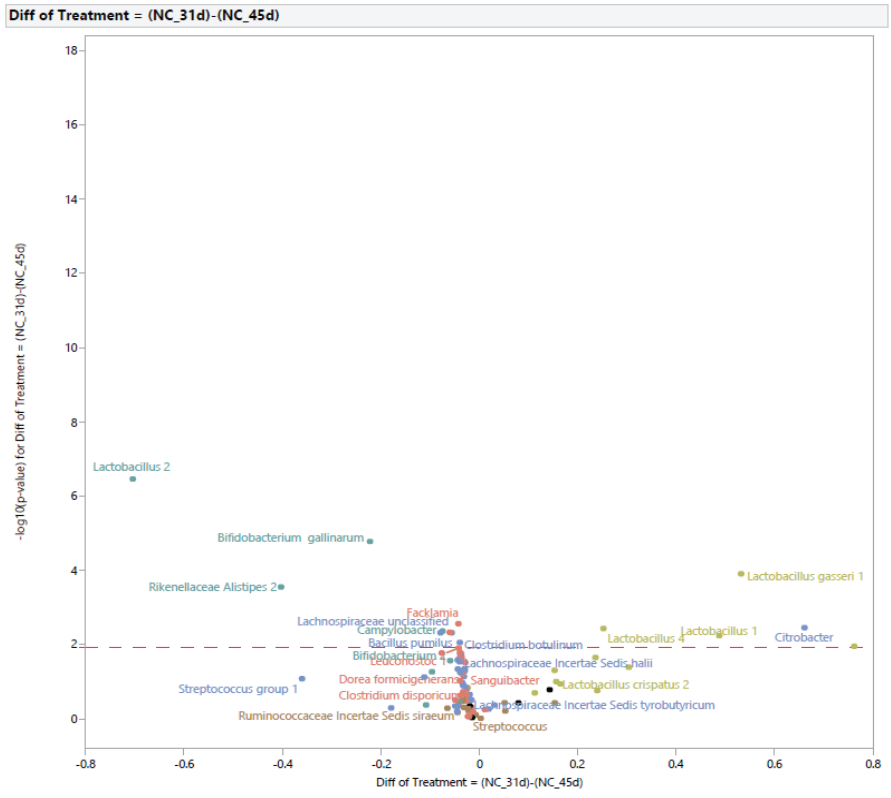

Figure 11: Age effect on the development of intestinal microbial population of turkey females within negative control treatment (NC) from 31 (after stress) to 45 days of age.

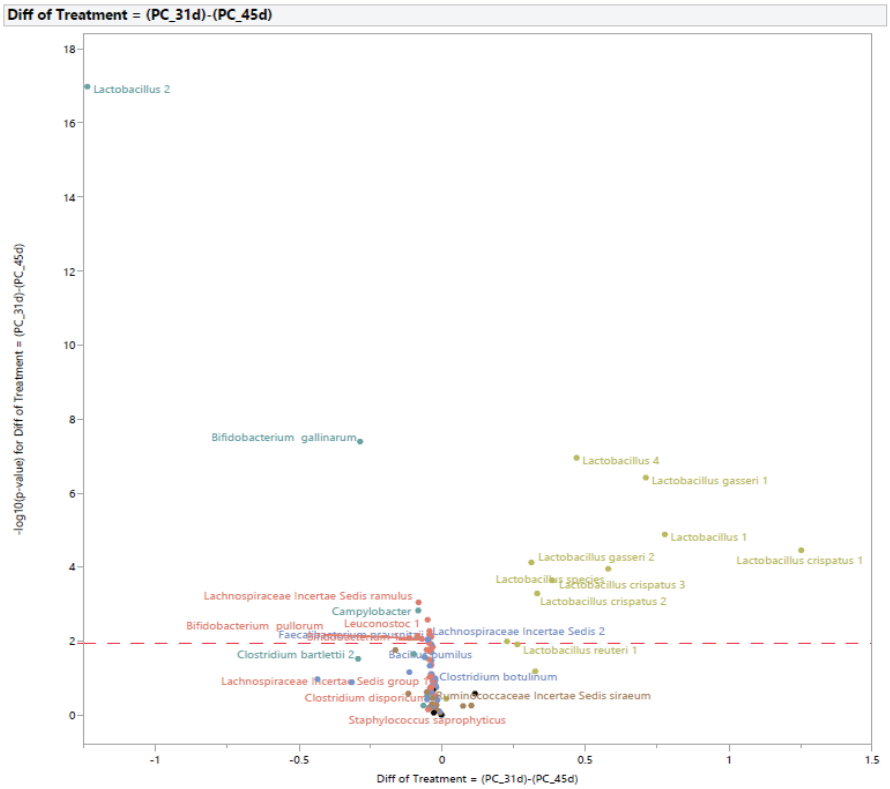

Figure 12: Age effect on the development of intestinal microbial population of turkey females within positive control treatment (PC) from 31 (after stress) to 45 days of age.

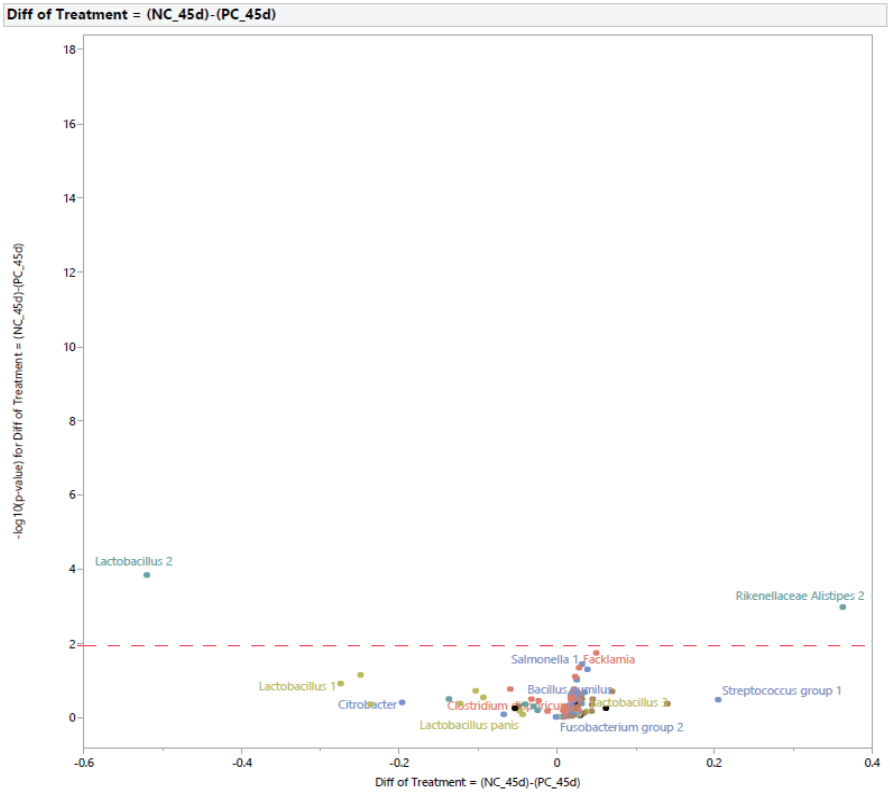

Figure 13: Effects of simulated transportation challenge on intestinal microbiota of turkey females at 45 days of age (after stress).

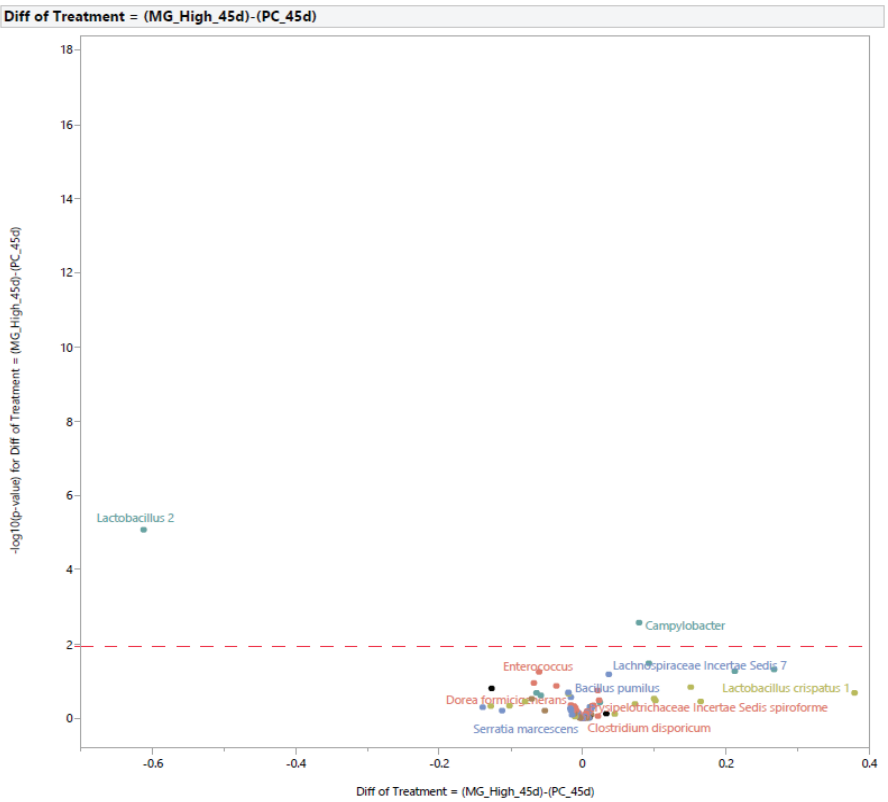

Figure 14: Effects of dietary supplementation with medium chain glucomannans (MG) at 0.20% of the diet on intestinal microbial population in comparison with turkey females from positive control treatment (PC) at 45 days of age (after stress).

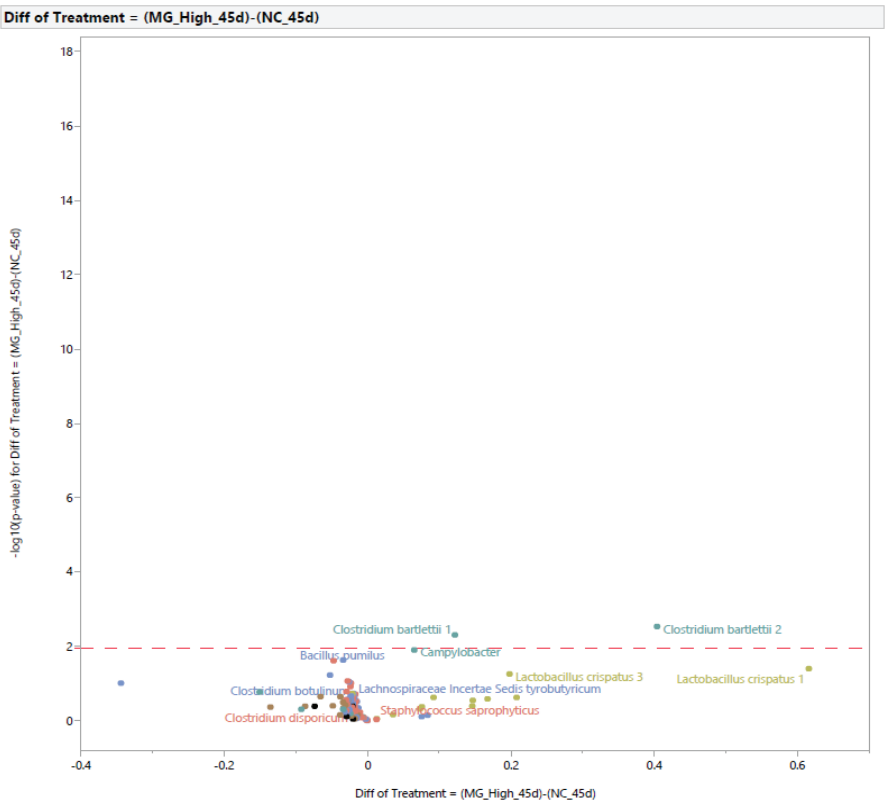

Figure 15: Effects of dietary supplementation with medium chain glucomannans (MG) at 0.20% of the diet on intestinal microbial population in comparison with turkey females from negative control treatment (NC) at 45 days of age (after stress).

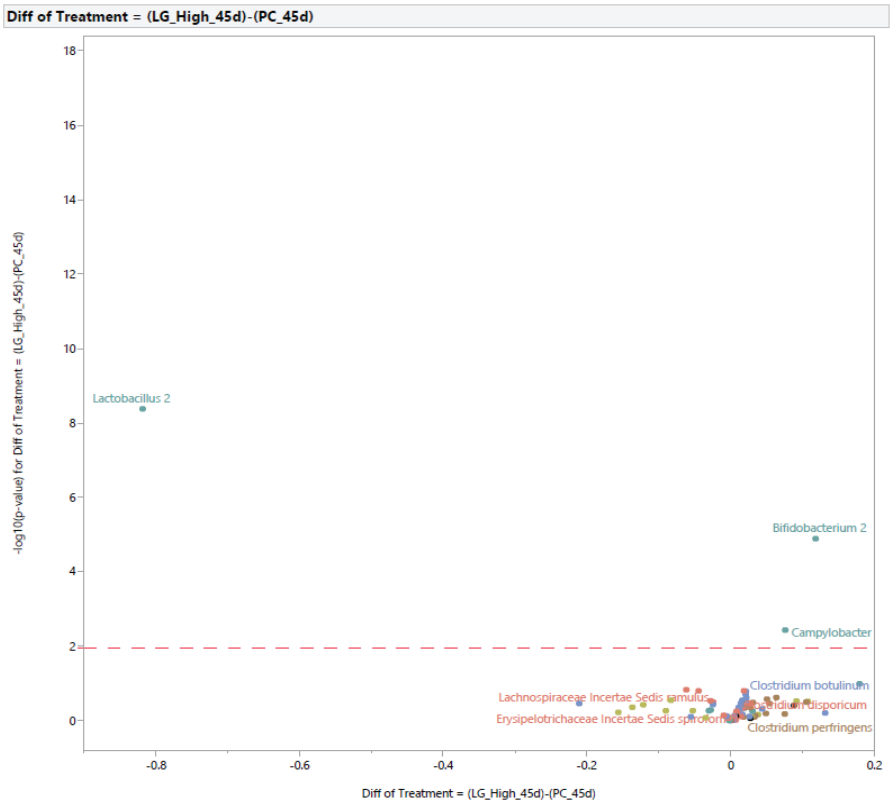

Figure 16: Effects of dietary supplementation with long chain glucomannans (LG) at 0.20% of the diet on intestinal microbial population in comparison with turkey females from positive control treatment (PC) at 45 days of age (after stress).

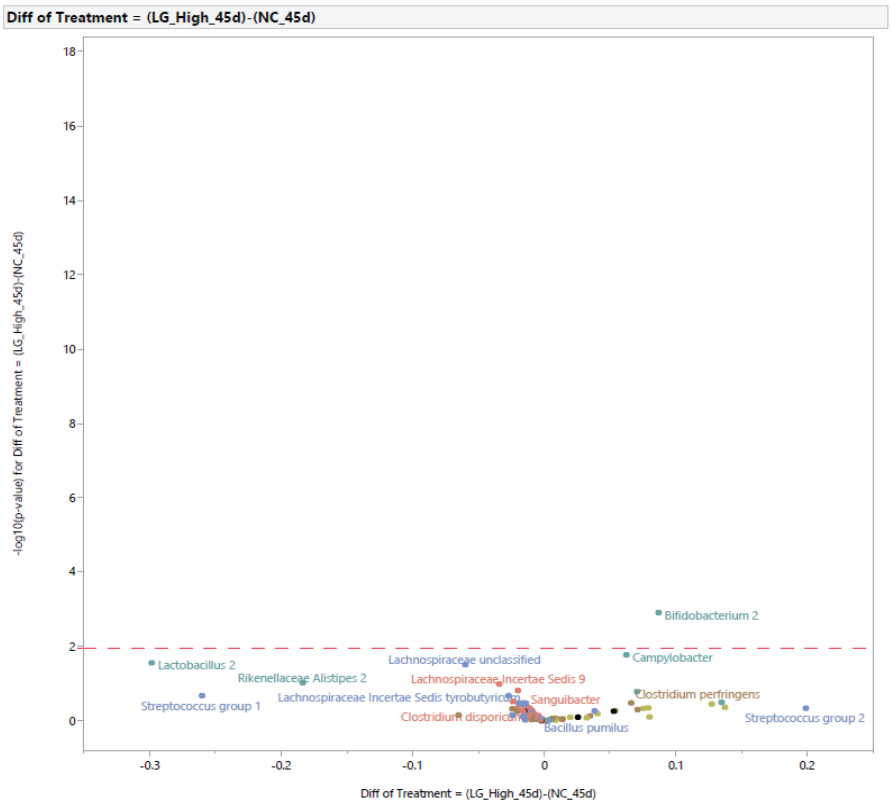

Figure 17: Effects of dietary supplementation with long chain glucomannans (LG) at 0.20% of the diet on intestinal microbial population in comparison with turkey females from negative control treatment (NC) at 45 days of age (after stress).

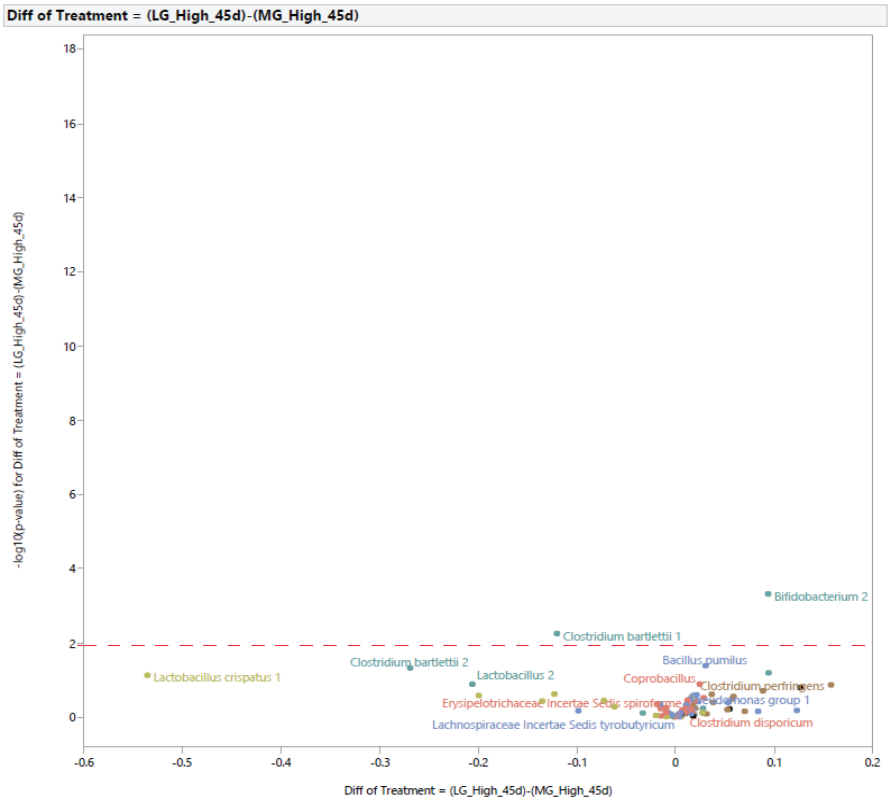

Figure 18: Effects of dietary supplementation with long-chain glucomannans (LG) or medium-chain glucomannans (MG) at 0.20% of the diet on intestinal microbial population at 45 days of age (after stress).

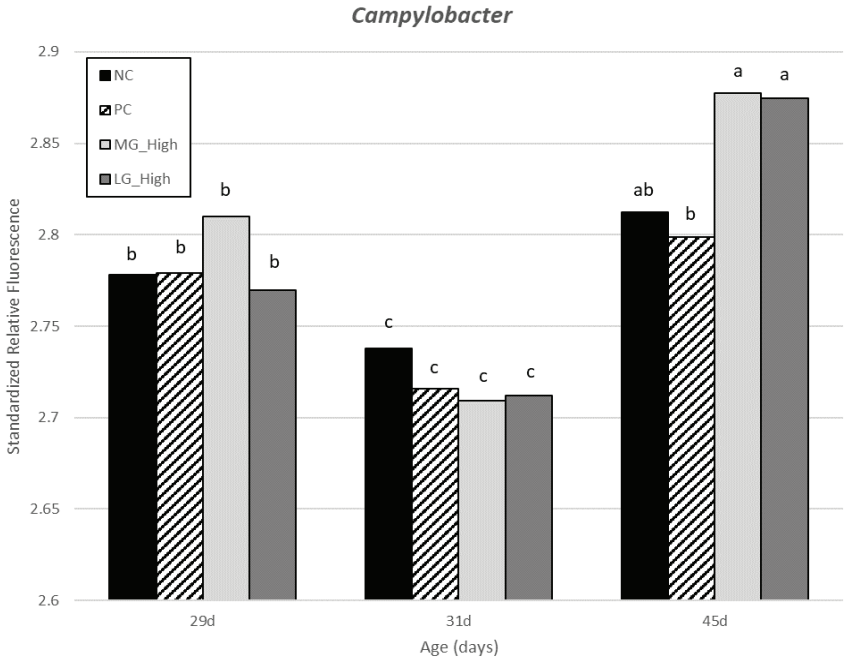

Figure 19: Effects of simulation of transport challenges and dietary treatments on *Campylobacter* abundance detected in cloacal samples from turkey females.

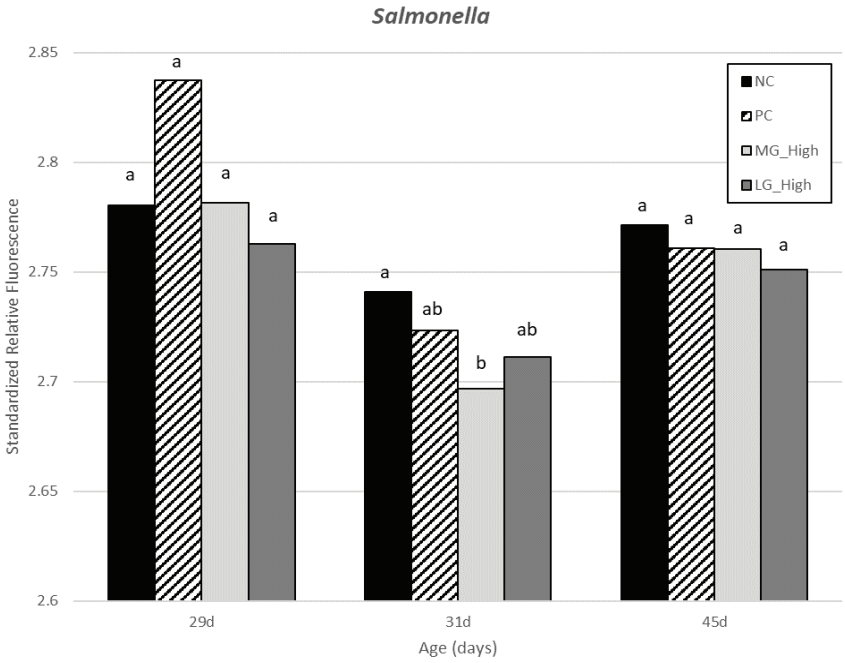

Figure 20: Effects of simulation of transport challenges and dietary treatments on *Salmonella* abundance detected in cloacal samples from turkey females.

performance measurements at 31 d showed that only birds from PC and LG High groups were not able to achieve similar BW to birds not exposed to STC (NC group). In addition, the only treatment group which provided similar BWG to the NC group from d 29 to 31 was MG High, mainly driven by the FI, with the greatest cumulative value (1.74 *vs*. 1.71 kg/bird) at d 31.

Overall, at the end of the trial (d 45), birds seem to recover the negative impacts of STC on BW and BWG after one acute stressor as the conditions applied in this study. However, recovery in performance might be hindered by a greater number of stressors or repeated stressors. Huff et al. [16] applied feed and water restriction combined with *E. Coli* challenge at 6 wk old female turkey and reported a BW recovery after the induced stress. However, birds were stressed two more times at 12 and 16 wk of age, resulting in an overall decrease in BW and increased FCR compared to the control birds. In another study, Bartz et al. [8] measured birds that did not recuperate on performance after a heat application and feed and water restriction. It is then essential to do more experiments to determine if dietary glucomannans can aid birds when they are exposed to repeated stressors. On the other hand, the MG High group was the only treatment with greater FI at 45 d, indicating that in a scenario with more acute stress events, birds may benefit from a greater FI as a limitation for better growth performance.

No statistical differences were observed in growth performance due to treatments at 45 d of age. Similar results were observed in other studies in which prebiotics influenced growth performance when birds were 15 or 18 wks old [17], indicating that the beneficial effects of prebiotics may be at longer term.

A stress response in poultry can be induced by many stressors including transportation, transportation processes, environmental factors, restricted feeding, and disease challenges [18,19]. When birds are stressed, they produce stress hormones, such as corticosteroids, which directly affect the immune system [9]. Birds may respond to stressful conditions by decreased performance, decreased livability, alterations in the immune system, and impaired reproduction [8].

Several authors have studied how stressors can affect bird performance and have measured stress biomarkers such as corticosterone. In contrast to mammals, birds produce corticosterone instead of cortisol as a stress hormone. Corticosterone is the primary adrenal glucocorticoid in birds [20]. This hormone is vital for the bird's metabolic, cardiovascular, immune, and behavioral processes [20]. When the effect stressors increase, corticosterone secretion increases by activating the hypothalamic-pituitary-adrenal axis [21]. This effect was observed in the study herein where birds not subjected to the simulation of transport conditions had a significantly lower corticosterone level than birds subjected to the applied simulation of transport conditions. These results are similar to the ones reported by Bartz et al.[8] when birds subjected to similar conditions had greater blood corticosterone levels than birds from the control group. In the study herein, birds fed diets with glucomannans at the greatest level (0.20%), regardless of the glucomannan source, had greater corticosterone levels than the control groups and glucomannans at 0.02% of the diet.

The analyses of the microbial population were performed with birds from 29 d of age as a starting point, prior to STC exposure. Therefore, a comparison between the microbiota of birds from NC and PC at this age should be similar. The results demonstrated that a small difference in abundance of five different bacteria species was present at 29 d of age, which might be explained by initial bacteria colonization in the gut at earlier age, either at hatchery or allocation in different pens in the research facility, considering that diets were similar and stress challenge was not applied until 31 d of age.

Overall, the shifts in the gut microbiota population were first driven by the diet composition, either with or without glucomannans included

at 0.20% in the diets, regardless of the type of glucomannan at 29 d of age, prior to the exposure to STC (Figure 1). However, dietary change and exposure to STC led to shifts in the microbiota at 31 d of age, resulting in microbiota population being more similar between NC and LG high or between PC and MG high treatment groups. Therefore, demonstrating that glucomannans, especially LG may provide a more stable gut microbiota even under acute stress events at least for 2 wks after the stress event. The shifts in the microbiota population might be partially explained by the differences in feed intake either at 4 or 6 wks of age, after STC was applied. In addition, glucomannans have been reported to provide a prebiotic effect modulating the microbiota population due to being a substrate for fermentation by specific bacteria species, preventing bacteria attachment to the gastrointestinal mucosa, or activation of the immune system [13]. The prebiotic effect may be larger with feeding long-chain glucomannan due to a lower concentration of free mannose and longer chain, leading to lower gastrointestinal breakdown until reaching the large intestine.

Cloacal swab samples contain microbiota from the small and large intestine sections of the gastrointestinal tract of birds. Therefore, microbes which are usually present in specific gastrointestinal tract sections may be present in the samples analyzed. *Lactobacillus* is an example of bacteria typically colonizing the small intestine. On the other hand, *Bifidobacteria* or *Lachnospiracea* commonly colonize the large intestine. Considering these assumptions, the greater abundance of either *Bifidobacteria* or *Lachnospiracea* instead of *Lactobacillus* in the sample may be used as an indication of earlier maturation of the gastrointestinal tract, more specifically the development of the fermentative large intestine. In agreement, Gonzales-Ortiz et al. [22] reported that as animal ages and the microbiome matures, a reduction in the lactic acid production is observed at the expense of other SCFA, which may occur in later age in turkeys in comparison with broilers. The slower gut and microbiota maturation can be observed based on the microbiota development within NC group from 29 to 31 d of age, with abundance of *Lactobacillus* species still being increased at later age (Figure 5). Consequently, a feed additive that promotes a reduction of *Lactobacillus* species in turkey females up to 31 d of age is not necessarily the most desirable, however, the feed additive should maintain the microbiota as similar as possible to the microbiota in the NC group. Results from 31 to 45 d of age showed that at later stage, *Lactobacillus*, which metabolizes readily digestible carbohydrates was reduced, while *Rikenellaceae Alistipes 2*, which ferment fiber, was increased (Figure 12), confirming the slower intestinal maturation in turkey females in comparison with broilers.

Immediately after STC exposure, at 31 d of age, the effect of stress challenge was observed with an increase in abundance of *Lactobacillus* species, indicating a possible negative effect on the development of the intestinal microbiota. The inclusion of either MG or LG did not reduced this trend in the microbiota shift. However, LG supported a more subtle change by reducing *Lactobacillus* in comparison either with PC or MG at 0.20% of the diet, reducing the negative effects of STC.

Feed supplementation with MG at 0.20% of the diet reduced *Salmonella 1* at 31 d of age compared to turkey females from NC group. This indicated that right after STC this prebiotic shows potential to, at this dose, create an intestinal environment unfavorable to *Salmonella* to rise due to stress.

In conclusion, young female turkeys subjected to STC at 4 wk of age experienced impaired performance and increased blood corticosterone levels indicating a stress response. During acute stress situations, MG supplementation at 0.20% is recommended to support the growth of turkey females, mainly driven by increased feed intake. Although both medium- and long-chain glucomannans modulated changes in the gastrointestinal microbial profile of turkey females, long-chain glucomannans provided a more stable and similar microbiota population compared to turkey females reared under conditions with lower stress. Glucomannan levels should be further analyzed with repeated or longer applied stressors to determine their efficacy in extreme production conditions.

Declarations of Interest

None.

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References

1. Erasmus MA (2018) welfare issues in turkey production. In: Mench JA (ed) Advances in poultry welfare. Woodhead Publishing, 263-291.
2. Erasmus MA (2017) A review of the effects of stocking density on turkey behavior, welfare, and productivity. Poult Sci. 96: 2540-2545.
3. Noll SL, el Halawani ME, Waibel PE, Redig P, Janni K (1991) Effect of diet and population density on male turkeys under various environmental conditions. Turkey growth and health performance. Poult Sci 70: 923-934.
4. Calik A, Emami NK, White MB, Walsh MC, Romero LF, et al. (2022) Influence of dietary vitamin E and selenium supplementation on broilers subjected to heat stress, Part I: Growth performance, body composition and intestinal nutrient transporters. Poult Sci 101: 101857.
5. Zaboli G, Huang X, Feng X, Ahn DU (2019) How can heat stress affect chicken meat quality?-a review. Poult Sci 98: 1551-1556.
6. Kabir SML (2009) The role of probiotics in the poultry industry. Int J Mol Sci 10: 3531-3546.
7. St-Pierre NR, Cobanov B, Schnitkey G (2003) Economic losses from heat stress by us livestock industries. J. Dairy Sci 86: E52-E77.
8. Bartz BM, McIntyre DR, Grimes JL (2018) Effects of Management Related Practices on Turkey Hen Performance Supplemented With Either Original XPC™ or AviCare™. Front Vet Sci 5: 185.
9. Koutsos EA, Klasing KC (2014) Factors modulating the avian immune system. In: Schat KA, Kaspers B, Kaiser P (eds) Avian immunology (second edition) Academic Press, Boston, 299-313.
10. Yaqoob MU, Abd El-Hack ME, Hassan F, El-Saadony MT, Khafaga AF, et al. (2021) The potential mechanistic insights and future implications for the effect of prebiotics on poultry performance, gut microbiome, and intestinal morphology. Poult Sci 100: 101143.
11. Ashraf S, Zaneb H, Yousaf MS, Ijaz A, Sohail MU, et al. (2013) Effect of dietary supplementation of prebiotics and probiotics on intestinal microarchitecture in broilers reared under cyclic heat stress. J Anim Physiol Anim Nutr 97: 68-73.
12. Lee C, Kim JH, Kil DY (2022) Comparison of stress biomarkers in laying hens raised under a long-term multiple stress condition. Poult Sci 101: 101868.
13. Meijerink N, de Oliveira JE, van Haarlem DA, Hosotani G, Lamot DM, et al. (2021) Glucose Oligosaccharide and Long-Chain Glucomannan Feed Additives Induce Enhanced Activation of Intraepithelial NK Cells and Relative Abundance of Commensal Lactic Acid Bacteria in Broiler Chickens. Vet Sci 8: 110.

14. Meijerink N, de Oliveira JE, van Haarlem DA, Lamot DM, Velkers FC, et al. (2022) Long-chain glucomannan supplementation modulates immune responsiveness, as well as intestinal microbiota, and impacts infection of broiler chickens with *Salmonella enterica* serotype Enteritidis. Vet Res 53: 9.

15. van der Hoeven-Hangoor E, van der Vossen JMBM, Schuren FHJ, Verstegen MWA, de Oliveira JE, et al. (2013) Ileal microbiota composition of broilers fed various commercial diet compositions. Poult Sci. 92: 2713-2723.

16. Huff GR, Huff WE, Jalukar S, Oppy J, Rath NC, et al. (2013) The effects of yeast feed supplementation on turkey performance and pathogen colonization in a transport stress/*Escherichia coli*. Poult Sci 92: 655-662.

17. Sims MD, Dawson KA, Newman KE, Spring P, Hoogell DM (2004) Effects of dietary mannan oligosaccharide, bacitracin methylene disalicylate, or both on the live performance and intestinal microbiology of turkeys. Poult Sci 83: 1148-1154.

18. Huff G, Huff W, Rath N (2009) Nutritional immunomodulation as an approach to decreasing the negative effects of stress in poultry production. J Arkansas Academy of Sci 63: 87-92.

19. Huff GR, Huffx WE, Rath NC, de los Santos FS, Farnell MB, et al. (2007) Influence of hen age on the response of turkey poults to cold stress, Escherichia coli challenge, and treatment with a yeast extract antibiotic alternative1. Poult Sci 86: 636-642.

20. Smith SM, Vale WW (2006) The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. Dialogues Clin. Neurosci. 8: 383-395.

21. Cockrem JF, Candy EJ, Castille SA, Satterlee DG (2010) Plasma corticosterone responses to handling in japanese quail selected for low or high plasma corticosterone responses to brief restraint. Br. Poult. Sci 51: 453-459.

22. González-Ortiz G, Olukosi OA, Jurgens G, Apajalahti J, Bedford MR (2020) Short-chain fatty acids and ceca microbiota profiles in broilers and turkeys in response to diets supplemented with phytase at varying concentrations, with or without xylanase. Poult Sci 99: 2068-2077.