



POTENTIAL OF BUTYRATE GLYCERIDES AS AN ALTERNATIVE TO DIETARY ANTIBIOTICS: A MECHANISTIC STUDY WITH BROILERS FROM NUTRITION PERSPECTIVES

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Introduction

Butyrate plays an important role in gastrointestinal health in broilers. Although very efficacious and possible to substitute for dietary antibiotics, pure butyrate has obvious limitations of smell and handling and is virtually absorbed in the upper digestive tract. Butyrate glycerides have no such limitations and their butyrate can be released by lipase in the small intestine, thus providing a novel delivery system to the chicken gut. In our previous pilot experiment, 0.3% butyrate glycerides supplementation in feed improved ($P < 0.05$) the body weight gain of young birds (up to 20 d) compared to the treatments with non-medicated and virginiamycin-medicated feed. We also observed a reduction of lipids deposition in abdominal and mesenteric fat. Therefore we speculated that the growth promoting and reduction of lipids deposition could be resulted from the energy redistribution. To verify, we have conducted a second chicken trial with a similar design, but reduced numbers of chickens. We have also explored possible mechanisms by examining gene expression profiles in the jejunum and liver of the chickens.

Materials and Methods

Animals and trial design

Forty newly hatched male chicks were equally divided into two groups: 1) basal diet (BD); 2) basal diet + butyrate glycerides (BG, 0.3% each; 0-7 d: Baby C4 + Mono C4, 7-20 d: Mono C4; SILO, Industria Zootechnica). The protocol for the trial was approved by the Animal Care and Use Committee of the University of Guelph.

Sample collection

All the birds were euthanized at 9:30 am after weighted on d 21. The whole small intestine, from proximal duodenum to the distal ileum, was collected and then the length was measured. The abdominal and mesenteric fat and the fat on the gizzard were isolated and weighted. After removal of digesta, the small intestine was washed three times with pre-cooled PBS and then paper towel dried prior to weighting. About 0.5 g liver and 1 cm jejunum collected from the middle part of the jejunum were cut into small pieces and stored in RNA later (Life Technologies Inc., Burlington, Canada) for gene expression analysis.

RNA extraction and transcriptome sequencing

Two birds from each of the BD and BG treatment groups were randomly selected for RNA extraction and transcriptome analysis. The RNA was extracted from the liver and jejunum of individual birds and then treated with DNase I using Ambion *mirVana* miRNA isolation kit. The mRNA-Seq libraries were constructed from 10 µg of total RNA of each tissue sample using the Illumina mRNA-Seq sample preparation kit. The liver and jejunum library samples (2 from each treatment) were individually sequenced using the Illumina Genome Analyze II system (Next Generation Sequencing Platforms Clinical Genomics Centre, Mount Sinai Hospital, Toronto, Canada).

Determining possible signaling and metabolic pathways

The data on gene expression profiles of liver and jejunum from the transcriptome analysis were subjected to statistical analyses by DESeq. The differentially expressed genes with NCBI gene ID, P value, and folder change were uploaded to the IPA online analysis system (http://www.ingenuity.com/products/pathways_analysis.html) for functional analysis. The genes expressed only in the samples from either BD or BG treated chickens were also uploaded to IPA online analysis system for functional analysis.

Statistical analysis

The data on body weight gain, lipids deposition, and intestinal development were analyzed by the GLM procedure of SAS with T test. A $P < 0.05$ was used to assess a statistical significance.

Results

Dietary supplementation with BG significantly increased the body weight, small intestine weight and length, the ratio of body weight to abdominal, mesenteric, and gizzard fat weight, and the ratio of small intestine weight to its length by 15.12%, 29.84%, 15.37%, 11.80%, and 12.69%, respectively, when compared with the control group of birds (fed BD only) (Table 1).

There were 32,209 genes available for *Gallus gallus*, of which 16,406 genes were identified through the transcriptome analysis. In the present study, there were 36 and 70 genes expressed differentially in the liver and jejunum between the two treatments, respectively (Table 2). Additionally, some unique genes (expressed only in either BG or BD-fed chickens, count number > 5) were also observed. Twelve and 4 unique genes were expressed in the liver and jejunum in BG-fed chickens, and 8 and 5 unique genes were expressed in the same organs of the control birds, respectively.

Twenty differentially expressed genes were identified, which were involved in 102 metabolic processes relating to lipid metabolism in the jejunum of BG-fed chickens. Among them, 11, 10, and 9 genes involved in 3 major processes, including regulation of

systemic lipid concentration, fatty acid metabolism, and lipid synthesis. Six out of the 20 differentially expressed genes involved in one of the most important lipid metabolism pathway (Figure 1). Ten differentially expressed genes were identified in the liver of BG-fed chickens, which were involved in 29 metabolic processes relating to lipids metabolism. In addition, 12 differentially expressed genes were involved in 5 metabolic processes relating to protein synthesis in the jejunum of BG-fed chickens.

Table 1. Growth performance, lipid deposition and small intestinal development in young birds

Item	Dietary Treatment	
	BG	BD
BW (g)	992.74±13.12 *	862.32±15.32
AMGF (g)	13.08±0.54	13.07±0.72
BW/AMGF (g/g)	76.37±2.79 *	68.31±2.73
WSI (g)	24.67±0.86 *	19.00±0.51
LSI (cm)	163.67±3.02 *	141.86±1.92
WSI/LSI (g/cm)	0.151±0.005 *	0.134±0.004

Note: * within a row indicated a significant differ ($P < 0.05$). n = 20.

AMGF, abdominal, mesenteric and gizzard fat; BD, basal diet; BG, butyrate glycerides; BW, body weight; WSI, weight of small intestine; LSI, length of small intestine.

Table 2. Co-expressed gene profiles in young broilers

Co-expressed gene	Organ	
	Liver	Jejunum
Function unknown, $P < 0.05$	52	161
Function identified, $P < 0.05$	36	70
No significant difference, $P > 0.05$	14722	14984

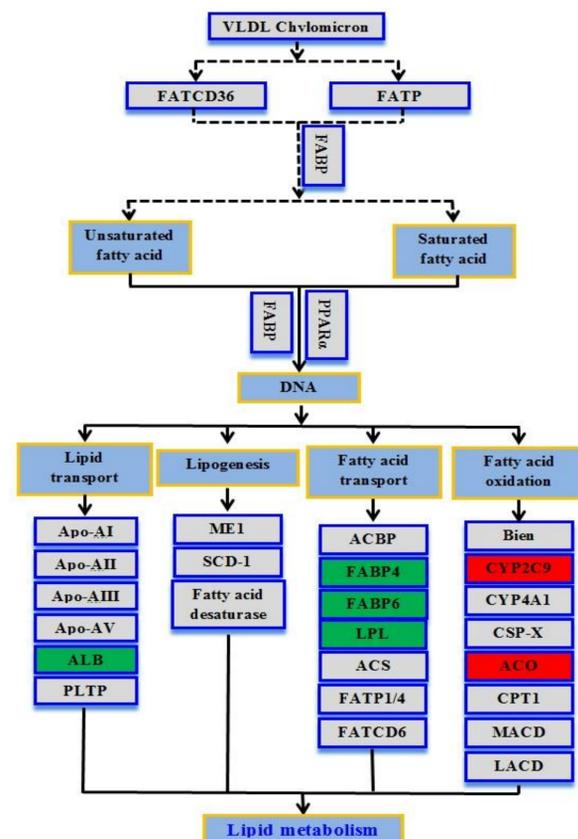


Figure 1. PPAR α pathway in lipid metabolism in response to butyrate treatment in young chickens. While 4 genes with the role in fatty acid transport are down-regulated (in green), 2 genes in fatty acid oxidation are up-regulated (in red).

Conclusion

BG treatment influenced the growth performance, lipid deposition, and small intestine development of young broilers. The effects on the later two were possibly achieved through the regulation of lipid metabolism and protein synthesis. The PPAR α pathway is the major one affected by the BG treatment.

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