Use of butyric acid glycerides as potential substitute for AGPs in poultry feed

Juan Antonio Javierre

Yu Peng

Yangyuan Li

Organic acids, and particularly butyric acid are proven potential AGP alternatives. However, it has to be stressed that other practices have to be put in place to guarantee success in commercial conditions, especially in older units in the tropics: diet digestibility, early feed intake, farm management, and biosecurity explain JUAN ANTONIO JAVIERRE, YU PENG and YANGYUAN LI*.

This paper was presented at the

broiler QC FEED QUALITY CONFERENCES

Bangkok, Thailand 21-22 August 2019
Jakarta, Indonesia 26-27 August 2019

Introduction

Since the ban of AGPs in many countries alternative strategies for control of intestinal health have become a focus of research. The task is now finding a way to maintain performance parameters at current levels, substituting AGP function, without so much increasing production costs. Among many proposed additives, butyrates seem to be a good alternative given their wide range of properties. As an intestinal bacterial fermentation product, butyrate plays a critical role on supplying ATP and ketone bodies for enterocyte growth, promoting of enterocyte proliferation and maturation, enhanced synthesis of mucus, pathogen control, regulation of inflammatory cytokines release and decreased apoptosis of healthy intestinal cells.

Sources of butyrates

Since free butyric acid or butyrate is characterized by a strong unpleasant, penetrating smell, it is almost impossible to be coped with in the feed manufacturing and this results in poor intake of the treated feed. Because of chemical make-up, or affinity, butyrate may impact releasing due to the pH conditions in the animal intestine. Moreover,

Table 1: Nutritional characteristics of the experimental diets.

	Phase I		Phase II	
	No AGP based diet	**Low AME diet**	**No AGP based diet**	**Low AME diet**
Main feed ingredient				
Corn	59.67	60.16	62.38	62.83
Soybean meal (46%)	28	28	27	27
Soybean oil	1	0.5	2.2	1.7
Fish meal	2.4	2.3	2	2
Corn gluten meal	5	5	3	3
Nutrient component				
ME, kcal/kg	3119	3086	3212	3182
Crude protein, %	20.55	20.51	19.46	19.46
Ether extract, %	4.00	3.51	5.11	4.64
Crude fiber, %	3.17	3.18	2.95	2.96
Lysine, %	1.22	1.22	1.09	1.09
Methionine, %	0.63	6.63	0.57	0.56
Available P, %	0.44	0.45	0.40	0.40
Calcium	0.83	0.84	0.76	0.76

The treatments used were: 1,000 ppm GTB, 1,500 ppm CSB, and 1,000 ppm GTB on the low-energy diet.

the main effect segment of butyrate is ileum and large intestine. Thus, distinguishing the release sites of different butyrate derivatives is one part to different their effectiveness.

Coated sodium butyrate (CSB) and glyceryl tributyrate (GTB) releasing rate were compared *in vitro* and *in vivo*. Ten mL of GTB were added in 50 mL PBS (pH 7.0-7.2) or simulated gastric juice (pH 2.0, 0.1% pepsin). After 4 hours incubation, the butyrate releasing rate from GTB were 0.77% and 0.68% in PBS and gastric juice, respectively. While the butyrate releasing rate from CSB were 12.55% and 27.94% were in PBS and gastric juice, respectively.

The releasing rate were also compared in several gastrointestinal segments, and relate it to the local pH conditions in the chicken. Fifteen percent of CSB or GTB were added in broiler feed. Three chickens from each group were slaughtered after 0.5, 1.0, 1.5 and 2.0 hours of feeding. pH and butyrate concentration were analyzed in crop, gizzard, ileum and cecum digesta. The results showed that CSB significantly decreased the crop digesta pH after 0.5, 1 and 1.5 hours of feeding, and significantly decreased the gizzard digesta pH after 1 and 1.5 hours of feeding. Different from CSB, the GTB significantly decreased ileum

Figure 1: Effect of GTB or CSB replace AGP in broiler feed on colonic bacterial counts.

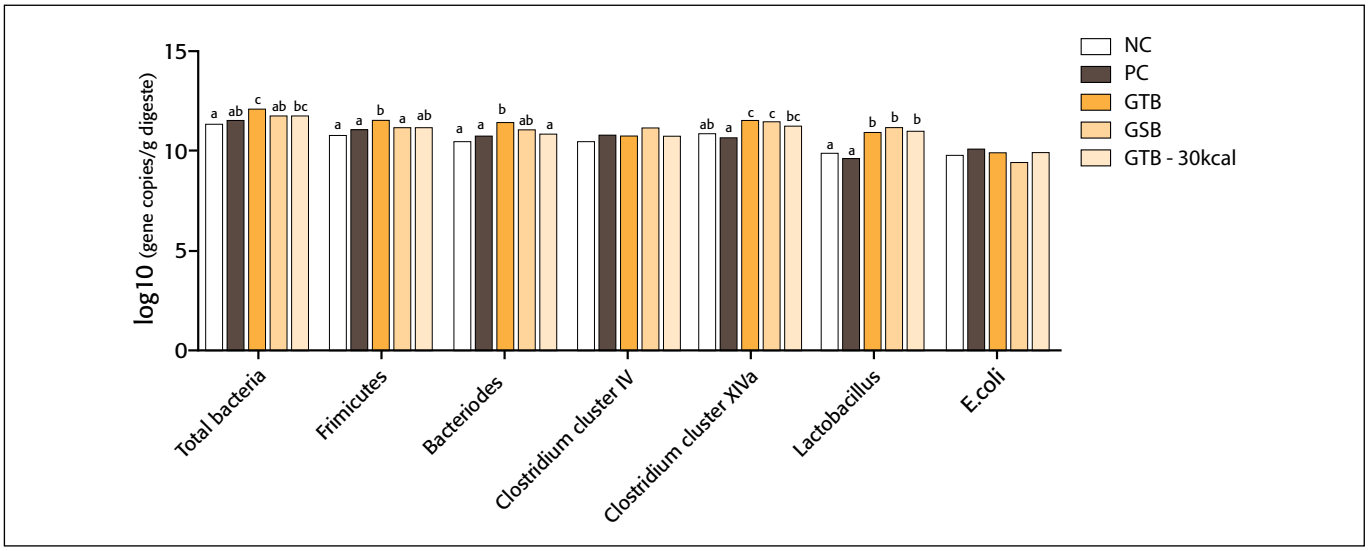

Table 2: Growth Performance of GTB or CSB replace AGP in broiler feed.

	NC	PC	GTB	CSB	GTB - LE
1-21 days					
Final weight, g	843.38^{a}	881.08^{b}	898.69^{bc}	849.29^{ab}	900.58^{bc}
ADG, g	38.38^{a}	40.16^{b}	41.00^{bc}	38.64^{a}	41.10^{bc}
ADFI, g	54.55	56.00	55.56	54.70	54.87
FCR	1.42^{c}	1.39^{c}	1.36^{ab}	1.41^{c}	1.33^{a}
22-42 days					
Final weight, g	2,640.36^{a}	2,702.23^{ab}	2,734.70^{ab}	2,725.22^{ab}	2774.26^{b}
ADG, g	85.29	86.71	90.03	97.33	95.35
ADFI, g	135.36^{a}	140.38^{ab}	141.14^{ab}	144.28^{c}	140.47^{ab}
FCR	1.53	1.62	1.58	1.66	1.51
1-42 days					
ADG (g)	61.97	63.44	64.22	63.99	65.16
ADFI (g)	95.85	98.19	98.35	99.27	97.64
FCR	1.55	1.55	1.53	1.55	1.50

Note: ^{abc}Values with non-common superscripts are statistically different ($p < 0.05$).

Table 3: Effect of GTB or CSB replace AGP in broiler feed on serum parameters and immune globulin.

	NC	PC	GTB	CSB	GTB-LE
Serum parameters (g/L)					
TP	28.58	29.08	28.74	28.12	32.56
ALB	13.16^{a}	13.06^{a}	13.12^{a}	12.70^{a}	14.50^{b}
GLB	15.42	15.84	15.62	15.48	18.42
Immune globulin (mg/mL)					
Ig A	0.93^{a}	0.89^{a}	0.94^{a}	0.95^{a}	1.08^{b}
Ig G	0.81	0.69	0.51	0.76	0.6
Ig M	23.72^{a}	32.09^{b}	30.03^{b}	33.23^{b}	41.78^{c}

Note: TP, Total protein; ALB, Albumin; GLB, Globulin

Values with non-common superscripts are statistically different ($p < 0.05$)

Table 4: Short chain fatty acids level in colonic digesta of GTB or CSB replace AGP in broiler feed.

	NC	PC	GTB	CSB	GTB-LE
Total SCFA	135.11^{a}	168.54^{ab}	216.15^{bc}	184.65^{abc}	205.14^{bc}
Acetate	95.94^{a}	119.69^{ab}	147.85^{bc}	125.07^{ab}	145.9^{bc}
Propionate	13.25^{a}	20.35^{ab}	33.95^{c}	27.62^{ab}	23.54^{b}
Butyrate	18.04	22	24.97	24.12	25.69
Valerate	2.00	2.28	3.15	2.66	3.39
BCFA	5.87	4.22	6.22	5.18	6.61
Isobutyrate	3.05	2.62	3.65	3.45	3.78
Isovalerate	2.82^{b}	1.59^{a}	2.57^{ab}	1.73^{a}	2.82^{b}

digesta pH after 1.5 and 2 hours feeding. The cecum digesta pH was not affected by CSB and GTB. In the crop, we found 0.5 pH unit difference between CSB and GTB. GTB requires lipase activity to split it into glycerol and butyric acid; additionally, unsplit GTB does not impact local pH. CSB causes higher pH because at local crop conditions it is almost 80% dissociated, providing alkali pH that increases that of crop. GTB is not split, does not release any butyric acid, and the local pH is the one normally found in crop in its physiological condition. Normal gizzard pH reaches 3.5; at this pH, CSB is 90% undissociated, therefore contributing to the acid environment; there is not enough lipase and local pH is unfavorable for lipase activity in the gizzard, so GTB does not release butyric acid here and does not modify local pH.

When measuring the local butyric acid concentration, one can see that butyric acid from GTB is maximized in jejunum and ileum, while CSB releases very little of this organic acid here. At jejunal pH, almost 90% of CSB is dissociated, and very little free acid is released. We see the data: after 1.5 hours of feeding, 111.9umol/g of butyrate were detected in ileum digesta for GTB, while the butyrate concentration

Table 5: Nutritional characteristics of the experimental diets.

	Phase I	Phase II	Phase III
ME, kcal/kg	3,000	3,100	3,200
Crude protein, %	23.0	21.5	19.5
Ether extract, %	4.8	6.4	7.5
Crude fiber, %	2.4	2.4	2.3
Lysine, %	1.2	1.15	1.03
Methionine, %	0.70	0.63	0.58
Available P, %	0.48	0.44	0.39
Calcium	0.90	0.85	0.80

The treatments used were: 500, 1,000 and 2,000 ppm GTB. There were 43 Ross 308 birds per repetition and 12 repetitions per treatments. Broilers were housed in floor pens. Table 6 shows the results from the experiment.

Table 6: Performance results from the traditional house experiment.

	Treatment	Final weight	Feed intake	FCR
Phase I	PC	322.7	261.8[a]	0.81
	NC	335.6	270.9[b]	0.81
	500ppm	337.8	272.5[b]	0.81
	1,000ppm	335.6	272.7[b]	0.81
	2,000ppm	335.6	271.4[b]	0.81
Phase II	PC	923.9[a]	1304.1[a]	1.42
	NC	658.3[b]	968.5[b]	1.48
	500ppm	891.8[a]	1279.0[a]	1.44
	1,000ppm	771.1[b]	1131.1[b]	1.47
	2,000ppm	712.8[b]	1081.5[b]	1.52
Phase III	PC	2664.1	4324.2	1.62
	NC	2449.0	4187.6	1.71[a]
	500ppm	2590.5	4218.4	1.64
	1,000ppm	2611.6	4161.5	1.60
	2,000ppm	2561.3	4010.8	1.57[b]

was just 4.68umol/g digesta for CSB. Therefore, we conclude that as delivery system, GTB is a more efficient vehicle for butyrate delivery into the intestine than CSB, because it does it right where is most needed. As a salt of a weak organic acid and a strong base, sodium butyrate has an alkali pH when dissolved in water. Chemistry taught us that the release of undissociated organic acid out from a solution is a pH-mediated reaction. PKa is the pH value in which a substance is 50% undissociated. For butyric acid, this value is about 5. This means that pH 5 or lower, increase the amount of free butyric acid, while pH above 5 reduce the amount of butyric acid released.

Broiler trials

Two *in vivo* broiler experiments with GTB were performed in very different housing conditions. One was run in an environment controlled house, and the other in a traditional house with just ventilation and fogging. In both cases, the experimental diets and the negative control diet had AGP's removed.

A total of 480, 1-day old broilers (Ross 308) were randomly divided into 5 groups, positive control (PC), negative control (NC), NC plus 1,000 ppm of GTB (GTB), NC plus 1,500 ppm CSB (CSB), PC plus 1,000 ppm of GTB and minus 30kcal/kg ME. The diet included corn, SBM, corn gluten, soy oil, and some fish meal, plus vitamins, minerals and amino acids. A positive control diet with enramycin, a negative control diet without antibiotic, and a positive control diet (30 kcal/kg ME less than positive controls). There were two phases, 1-21 days and 22-42 days. The main nutrients in the diets are set in Table 1.

Results

Environmentally controlled housing

Table 2 contains the results from the environmentally controlled experiment. Compared with NC group, AGP and GTB were significantly improved the 21 days final body weight and ADG. For 22-42days, AGP and GTB combination while decreasing 30kcal of ME significantly improved the body weight gain.

From the serum biochemical indices and immune globulin results showed that GTB added in PC diet while decreasing 30kcal ME significantly increased the albumin level and increased the IgM concentration. AGP, GTB and CSB were significantly improved IgM secretion.

The colonic bacterial counts were analyzed using GTB or CSB replace AGP in broiler feed. Compared with NC group, GTB and GTB-30 kcal groups were significantly increased the total bacterial counts. The Firmicutes, Bacteroides and *Clostridium* cluster XIVa counts were significantly improved in GTB group compared with NC and PC group. For *Lactobacillus*, chickens feed GTB, CSB and GTB – 30 kcal diets were higher than that feed NC and PC diets. From the results showed that GTB largely improved the colonic bacteria growth, especially the butyric acid producing bacteria and beneficial *Lactobacillus*.

The short chain fatty acids results are shown in Table 4. Compared with NC, GTB and GTB-LE diets were significantly increased the colonic digesta total SCFA, acetate and propionate concentration.

Open housing

The second experiment was performed in traditional house. Experimental diet included corn, SBM, corn gluten and palm oil, plus vitamins, minerals and amino acids. A positive control diet contained halquinol and avilamycin, and the negative control diet had no AGP. There were three phases, 1-11 days, 12-24 days and 25-42 days. The main nutrients in the diets are set into Table 3. Additionally, a coccidia challenge was used to create additional GI disturbance to the birds.

The bird's growth response was quadratic with the optimum level being 1,000 ppm GTB. The differences were significant during Phase II; at the end of the trial, however, there were no significant differences in body weight between treatments, although the negative control final weight was numerically much lower than any other group. FCR for the NC group was the worst, and the best, one was from 2,000 ppm GTB.

In that very hot and humid environment under the test conditions, GTB was unable to compensate totally AGP removal, resulting in final BW being about 2% lower than the positive controls, although significantly better than the negative diet.

Analysis of intestinal microbiota shows statistically significant differences in *Lactobacilli* counts for GTB at 1,000ppm in ileum at 28 days, and significant *E. coli* reduction at 28 days in ileum for 1,000 ppm. Counts did not differ between treatments at the end of the experiment. Nutrient digestibility and gut morphometry are still being processed.

Conclusion

The results from these two experiment suggest that AGP removal goes beyond simply exchanging additives. As these two trials demonstrate, environment conditions exert inordinate influence on the bird's performance once AGP have been removed from their diets. Therefore, we reason that environment control and management techniques, together with biosecurity and probably diet quality, have to become absolute requirements for technical and economic success when rearing poultry free of AGP, especially when doing it under tropical conditions. AP

Juan Antonio Javierre (j.javierre@tekzol.net) is Research & Development Director, Tekzol Research, Colombia. Yu Peng, Yangyuan Li are both with the Research & Development Center, Guangdong VTR Biotech Co., Ltd. Zhuhai, China.