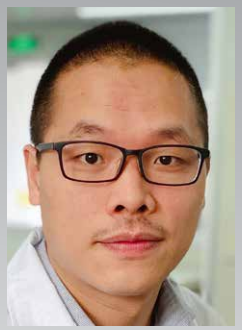
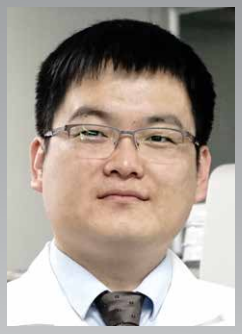

Yu Peng


Min Wang


Xipeng Cui


Yuguang Guo


Yangyuan Li

YU PENG, MIN WANG, XIPENG CUI, YUGUANG GUO and YANGYUAN LI* compare tributyrin to coated sodium butyrate as dietary sources of butyrate in layers.

Tributyrin improves performance, nutrient digestibility and microbial balance in laying hens

Research has shown that dietary supplementation butyrate feed additives had beneficial effects on growth performance, nutrient retention, intestinal microflora as well as immunity and gut mucosa. Compared with butyrate, butyric acid glycerides showed higher stability and facilitates passage to the lower gastrointestinal tract for release via lipase. In this study, the effects of tributyrin (TB) and coated sodium butyrate (CSB) on laying performance were investgated.

Table 1: Effects of TB[1] and CSB[2] on egg production of laying hens (n = 6).

Items	Egg production (%, hen-d)	Egg weight (g)	Feed consumption (g/hen/d)	Feed conversion feed:egg mass, g:g
Control	69.58 ± 1.01[a]	69.52 ± 0.47	116.08 ± 1.52	2.49 ± 0.04[b]
TB-500	75.15 ± 0.82[b]	68.96 ± 0.36	116.22 ± 2.01	2.32 ± 0.02[a]
TB-1000	77.22 ± 0.91[b]	69.66 ± 0.44	115.95 ± 1.68	2.30 ± 0.04[a]
TB-1500	77.31 ± 0.94[b]	68.99 ± 0.75	116.29 ± 2.30	2.29 ± 0.05[a]
CSB-500	72.84 ± 1.12[ab]	69.71 ± 0.78	116.42 ± 2.03	2.42 ± 0.04[b]
CSB-1000	74.09 ± 0.93[ab]	70.11 ± 0.45	116.04 ± 2.03	2.39 ± 0.01[ab]
CSB-1500	74.56 ± 0.95[b]	69.13 ± 0.29	117.03 ± 1.96	2.33 ± 0.02[a]

[a,b]Means in a row with no common superscript differ significantly ($p < 0.05$)
Note: [1]TB, tributyrin; [2]CSB, coated sodium butyrate

Materials and method

Eight hundred and forty laying hens (48 weeks of age; Babcock, brown egg) were assigned to 7 dietary treatments for a 10-week trail. Each treatment consisted of 6 replications and 10 cages each replication (two birds per cage). Following 1 week for feed adaptation, the hens were provided mash feed. Diets were formulated to have the similar nutrient value. TB and CSB were added to the control corn-soybean based diet (Table 1).

Results

Production performance

The effects of dietary supplementation of TB and CSB on productive performance of laying hens are shown in Table 1. Feeding 500ppm, 1000ppm and 1500ppm of TB significantly increased ($p < 0.05$) egg production and decreased ($p < 0.05$) feed conversion. Similarly, dietary inclusion of 1500ppm CSB significantly increased ($p < 0.05$) egg production and decreased ($p < 0.05$) feed conversion. However, dietary supplementation of 500mg and 1000ppm have no effects ($p > 0.05$) on egg production. The egg weight and feed consumption were not affected by TB and CSB.

Nutrient digestibility

The digestibility of DM was greater ($p < 0.05$) in hens fed on 1000mg and 1500ppm TB diets compared with those fed 500ppm TB and CSB diets. Supplemental TB significantly increased ($p < 0.05$) CP and GE digestibility. However, only diets supplemented with 1000ppm CSB increased ($p < 0.05$) CP digestibility and supplemented with 1000mg or 1500ppm CSB increased ($p < 0.05$) the GE digestibility in all CSB groups.

Microbial counts

Effects of dietary treatments on ileal and cecal microbial counts are shown in Figure 1 and 2, respectively. Ileal *Lactobacillus* counts were increased ($p < 0.05$), while the *E.coli* growth were inhibited ($p < 0.05$) by all treatments with TB and CSB. For *Clostridium* cluster XIVa counts, TB-1000 and TB-1500 significantly increased ($p < 0.05$) these bacteria counts. The *Salmonella* spp. bacteria were inhibited ($p < 0.05$) by medium (1000ppm) and high doses (1500ppm) of TB, and high dose (1500ppm) of CSB. In cecum, *Lactobacillus* were improved ($p < 0.05$) in all TB supplemented groups,

Table 2: Effects of tributyrin[1] and CSB[2] on nutrients total tract apparent digestibility (ATTD) of laying hens at 58 weeks (n = 6).

	DM (%)	CP (%)	EE (%)	GE (%)
Control	52.70 ± 0.83[a]	55.52 ± 0.87[a]	85.24 ± 1.35[a]	72.08 ± 1.02[a]
TB-500	55.65 ± 0.72[ab]	60.86 ± 0.76[b]	87.32 ± 2.21[b]	76.82 ± 1.03[b]
TB-1000	56.78 ± 0.7[b]	61.66 ± 0.64[b]	87.35 ± 1.04[b]	78.15 ± 1.18[b]
TB-1500	56.82 ± 0.74[b]	62.19 ± 0.75[b]	87.85 ± 1.97[b]	78.79 ± 1.12[b]
CSB-500	53.84 ± 0.82[ab]	58.71 ± 0.68[ab]	86.35 ± 1.76[ab]	74.12 ± 1.08[ab]
CSB-1000	54.79 ± 0.76[ab]	59.48 ± 0.75[ab]	86.21 ± 3.31[ab]	74.24 ± 1.05[ab]
CSB-1500	55.63 ± 0.81[ab]	60.71 ± 0.69[b]	86.35 ± 3.08[ab]	74.83 ± 0.96[ab]

[a,b]Means in a row with no common superscript differ significantly ($p < 0.05$)
Note: [1]TB, tributyrin; [2]CSB, coated sodium butyrate

while addition with 500ppm of CSB have no effects ($p > 0.05$). For harmful bacteria counts, 1000ppm and 1500ppm of TB significantly decreased ($p < 0.05$) the *E.coli* counts, while 500ppm of TB and CSB have no effects ($p > 0.05$) on *E.coli.* The *Clostridium* cluster IV bacteria only significantly increased ($p < 0.05$) in TB-1500 group, no changes were observed in other groups.

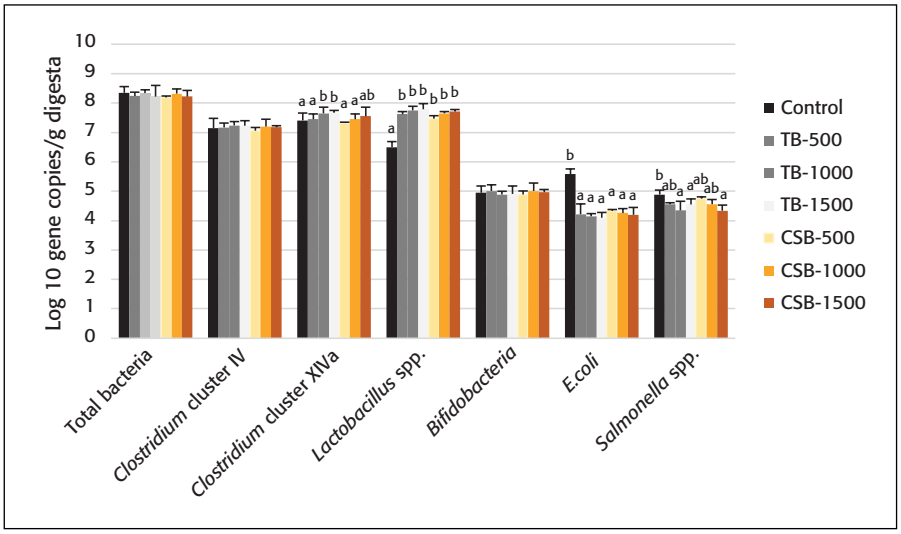
Discussion

Dietary SCFA, especially butyrate, have been demonstrated to promote production performance in poultry. As the prime enterocytes energy source, butyric acid attributes a growth promoting effect on beneficial intestinal microbes and serves as a cellular mediator regulating multiple gut functions, including epithelium cell differentiation, gut tissue development, immune modulation, oxidative stress reduction, and diarrhea control.

In this study, adding 500ppm, 1000ppm and 1500ppm tributyrin significantly increased egg production and decreased feed conversion, while significant results were only observed in CSB-1500 group. These results are similar to a previous study that found the inclusion of 500ppm of coated sodium butyrate had no effect on egg weight. Tributyrin had more advantage on production performance than coated sodium butyrate may be associated with the target butyrate release in the lower gastrointestinal. Our previous results showed that coated sodium butyrate release more than 25% of butyric acid in acid solution *in vitro.* And *in vivo* results also showed that more than 77.8 % to 97.8% of butyric acid was released from coated butyrate salt in proventriculus-gizzard. The results indicate that the gastrointestinal tract (GIT) segment where butyrate is released may modulate animal production performance.

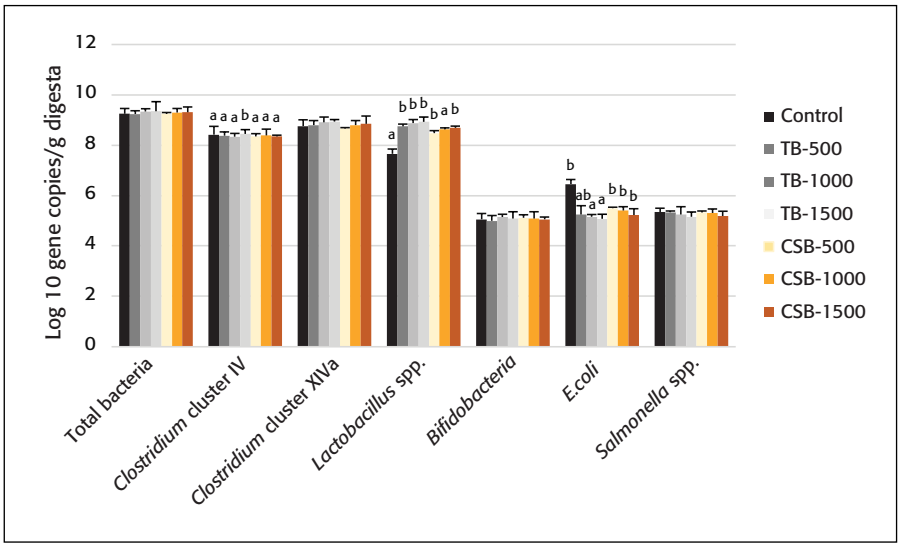
In the present study, tributyrin supplementation effectively increased nutrient digestibility, including dry matter (DM), crude protein (CP), ether extract (EE) and gross energy (GE). While in CSB supplementation groups, only

Figure 1: Effects of tributyrin[1] and CSB[2] on ileum microbial counts of laying hens.



[a,b] Within a row, values with different superscripts are different ($p < 0.05$); n = 6.
Note: [1]TB, tributyrin; [2]CSB, coated sodium butyrate

Figure 2: Effects of tributyrin[1] and CSB[2] on cecum microbial counts of laying hens.



[a,b] Within a row, values with different superscripts are different ($p < 0.05$); n = 6.
Note: [1]TB, tributyrin; [2]CSB, coated sodium butyrate

1500 ppm level supplementation had significantly effect on CP digestibility. It has been observed that supplementation 200ppm butyrate was significantly improved the apparent digestibility of crude fat and crude fiber in the broilers. It has also been reported that adding 1000ppm of sodium butyrate increased nitrogen and apparent digestibility of organic matter, but no improvement in apparent digestibility of crude fat. The differences of these studies may be related to the dosage and form of the butyric acid additive. The sodium butyrate coating is not completely and easily combined with acid ions in the gastrointestinal tract, and is consumed at the proximal GIT. That partly explains the lack of effect of coated sodium butyrate on apparent total tract nutrient digestibility. However, tributyrin as the precursor substances of butyric acid, slowly releases butyric acid through the decomposition of lipase in the intestine to regulate intestinal pH value to provide a suitable physiological environment for endogenous digestive enzymes, thereby improving digestion and absorption of nutrients in the digestive tract.

Lactobacilli, are known to

dominate the microbiota of the broiler GIT, in particular the proximal parts, and are considered crucial members of the commensal microbiota regarding health of the host. In present study, tributyrin and coated sodium butyrate supplementation improved *Lactobacillus* growth in ileum. Moreover, this beneficial bacteria in the GIT plays an important role in preventing colonization of the gut by pathogens, including *Salmonella* and *E. coli.* Thus, adding with tributyrin and coated sodium butyrate inhibited the proliferation of *Salmonella* in ileum and *E. coli* both in ileum and cecum. Free butyric acids which released from tributyrin or coated sodium butyrate can freely enter and exit the bacterial outer membrane and enter the cytoplasm. By dissociating H^+, the pH of the bacterial cytoplasm was reduced, the integrity of purines, bacterial DNA synthesis and proliferation were inhibited. Noticeably, the cecal *E. coli* inhibition effect was only observed in tributyrin supplemented groups. The possible reason may be associated with CSB releasing early in the gizzard and the small intestine due to incomplete coating. *Clostridium* cluster IV and *Clostridium* cluster XIVa is the main SCFAs producing bacteria cluster in the animal intestine, especially butyric acid. The increasing of *Clostridium* cluster XIVa in ileum by 1000 and 1500ppm tributyrin supplementation, and the increasing of *Clostridium* cluster IV in cecum by 1500ppm tributyrin supplementation indicated that the production of butyric acid may be improved. Butyric acid not only plays important role in hepatic circulation, but also provides energy for intestinal epithelial cells, and promotes nutrient absorption and utilization. In our results, DM, CP, EE and GE digestibility were significantly improved by tributyrin supplementation. These results suggest that the beneficial effects of tributyrin on layer hens' production performance could through the gut microbes' modulation, gut development and nutrients digestibility.

Conclusion

Tributyrin shows advantages in egg production, feed conversion, digestibility of CP, DM, EE and GE, the beneficial bacterial counts compared to coated sodium butyrate. The recommend dosage in laying hens was up to 1500ppm of coated sodium butyrate, and tributyrin was 1000 to 1500ppm. AP

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