

Effect of trehalose supplementation on growth performance and intestinal morphology in broiler chickens

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ABSTRACT

Trehalose (Tre) is a natural disaccharide. A laboratory-scale investigation showed that Tre supplementation increased the growth rate in juvenile chicks, possibly via the improvement of innate intestinal immune responses. In this study, two trials were conducted to evaluate the growth-promoting effect of Tre supplementation in broiler chickens. In experiment-1, two thousand day-old male and female broiler chicks (Ross) were fed 0 (control), 0.25, 0.50, and 0.75% Tre-supplemented pellet-form diets from d 1–17, and subsequently, they were provided grower (d 18–30) and finisher (d 31–37) diets without Tre supplementation. Over the trial period, there was no significant difference in body weight (BW), feed intake and feed conversion ratio (FCR) between chickens in the control and Tre-fed groups. Tre treatment increased villus height (VH)/crypt depth (CD) ratio and villus surface in jejunum; decreased CD and increased VH/CD ratio in ileum on d 17, when these results were compared to the control group. In experiment-2, two hundred day-old female broiler chicks were fed an antibiotics-free and mash-form diet supplemented with 0.5% Tre from d 1–21, before being fed a non-supplemental diet until d 43. There was no difference in BW on d 21 between the control and Tre-0.5% groups; however, from d 22–43, Tre-0.5% group showed significantly higher BW gain and lower FCR compared to the control group. From these results, we suggest that Tre feeding can be beneficial for intestinal morphology and growth performance in broiler chickens. However, these outcomes did not occur in parallel owing to the different feeding conditions observed.

1. Introduction

Antimicrobial growth promoters (AGPs) are commonly incorporated in poultry diets for the promotion of growth and the control of infectious diseases. Owing to the risk of possible drug resistance in human pathogenic bacteria, the use of AGPs has been limited globally. Therefore, research on alternatives to AGPs has been intensively conducted, wherein most attention is focused on intestinal conditions such as villus and crypt morphological indices, bacterial numbers, and inflammatory status. Oligosaccharide, butyrate, fibre, glycerol, yeast cell product (Amerah et al., 2009; Ozdogan et al., 2014; Markazi et al., 2017; Sikandar et al., 2017; Walugembe et al., 2015; Xu et al., 2003), and diet-form (Qaisrani et al., 2014; Qaisrani et al., 2015; Zaefarian et al., 2016) have recently been shown to contribute to the improvement of intestinal conditions as well as the growth performance of chickens.

Trehalose (Tre) is a glucose-glucose disaccharide linked by an α,α -1,1-glycoside bond; it is ubiquitous to diverse organisms, including bacteria, yeast, fungi, and invertebrates. Tre has garnered attention for its ability to mitigate protein aggregation by stimulating cellular autophagy and suppressing hepatic inflammatory cascade (DeBosch et al., 2016; Minutoli et al., 2008; Pagliassotti et al., 2017). A few investigations focusing on animal production have been conducted, and they showed that antioxidant activity and lipid peroxidation were improved in the milk of cows fed with Tre supplemented diet (Aoki et al., 2010; Aoki et al., 2013). Moreover, our previous investigation using broiler chickens showed that post-hatch Tre feeding increased the growth rate, possibly via the improvement of innate immune responses, such as down-regulation of toll-like receptors and inflammatory cytokines in the duodenum of the juvenile chickens (Kikusato et al., 2016).

These beneficial outcomes were obtained under laboratory conditions, while the Tre effect needed to be validated in a field-scale

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Table 1
Diet compositions (experiment 1).

Ingredient (%)	Starter (d 1–17)	Grower (d 18–30)	Finisher (d 31–37)
Corn	56.180	58.730	58.780
Dehulled soybean meal	30.380	22.320	22.320
Full fat soybean	5.000	8.000	8.000
Wheat	3.000	5.000	5.000
Soybean oil	1.720	2.820	2.820
Mono/dicalcium phosphate	1.257	0.916	0.916
Limestone	0.976	0.857	0.857
Salt	0.236	0.250	0.250
Sodium bicarbonate	0.065	0.043	0.043
Choline chloride	0.052	0.061	0.061
DL-methionine	0.261	0.214	0.214
L-lysine HCl	0.226	0.188	0.188
L-threonine	0.085	0.042	0.042
BS Premix	0.200	0.200	0.200
Pellet binder (Pelex Dry)	0.300	0.300	0.300
Sacox (Salinomycin 12%)	0.050	0.050	-
Quantum Blue 5 G*	0.010	0.010	0.010
<i>Nutritional values</i>			
Crude protein (CP), %	22.3	19.8	19.8
Metabolizable energy (ME), kcal/kg	3,051	3,187	3,187

* 66 ppm as final concentration.

† Reformulated using Quantum Blue (matrix)

investigation for practical application. Therefore, in the present study, two field-scale feeding trials were conducted to evaluate the beneficial effect of Tre supplementation on broiler chicken production.

2. Material and methods

2.1. Animal experiment design -experiment 1

Two thousand one-day-old broiler chicks (*Gallus gallus domesticus*, Ross 308) were obtained from Phanat Poultry Group Co., Ltd, Chonburi, Thailand. They were divided into 4 dietary treatment groups containing 10 replicates of 50 birds (25 males and 25 females per replicate). In the experiment, three pellet-form basal diets were prepared according to the growing stages: starter (d 1–17), grower (d 18–30), and finisher (d 31–37), and each nutritional composition is shown in Table 1. In the starter stage, chicks were fed a basal diet (control) and the diets supplemented with 0.25, 0.5, or 0.75% Tre (Tre-0.25, Tre-0.5, and Tre-0.75, respectively). Hayashibara Co., Ltd. manufactured the Tre used in this study. During the grower and finisher stages, all birds were fed with the basal diet without Tre supplementation. Chickens were reared under a 23:1 light-dark cycle and were allowed free access to water and diet during the feeding stages. All diets were formulated according to Ross 308 recommendations and manufactured at Bangkok Animal Research Center. The body weight (BW) was recorded on d 1, 7, 17, 30 and 37, and the feed intake (FI) was recorded for each replicate. At the end of the experiment, 4 birds from each replicate (2 males and 2 females) were randomly selected for carcass trait measurements.

In the morphological analysis, 2 birds (1 male and 1 female) from each replicate were sacrificed on d 17, and a 2 cm segment of the midpoint of the jejunum and ileum was dissected to evaluate intestinal morphology. The segments were fixed in 10% buffered formalin for 24 h and then embedded in paraffin. The fixed segments were sliced to obtain a 5 µm section, which was then placed on a glass slide and stained with haematoxylin and eosin. Slides were viewed with an upright microscope. The morphology was determined as previously described (Brunsgaard, 1998) and illustrated in Fig. 1; villus height (VH) was measured from the brush border membrane to the basolateral membrane, and the crypt depth (CD) was measured from the basement membrane to the region of transition between the crypt and the villus.

On d 37, birds from each replicate were randomised for faecal scoring using the Waltham faeces scoring system (Moxham, 2001). This

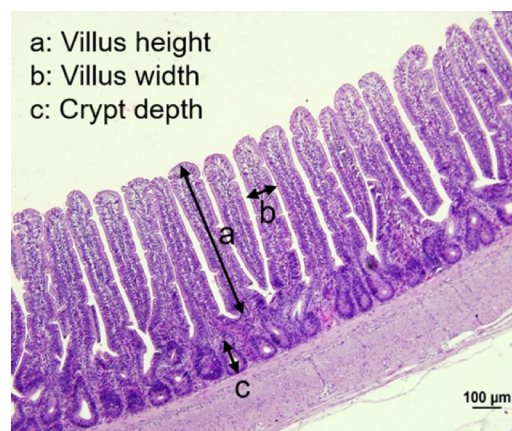


Fig. 1. Cross-section of the intestine stained with haematoxylin-eosin, displaying villus height (a), width (b), and crypt depth (c). These parameters and villus surface area were determined, as previously described (Brunsgaard, 1998).

system was used following the five-point scale scoring system, which is based on the severity of the faeces form consistency; hard-dry and crumbly (score 1), well-formed (score 2), moist and beginning to lose form (score 3), major or total loss of form (score 4) or watery diarrhoea (score 5).

The experiment was conducted at the Poultry Research and Development Center, Kasetsart University, and was approved by the committee of ethics and animal experimentation of the University.

2.2. Animal experiment design -experiment 2

Two hundred day-old female broiler chicks (Ross 308) were obtained from Komatsu hatchery farm (Matsumoto, Nagano, Japan). They were divided into 2 dietary treatment groups consisting of 4 replicates of 25 birds that showed similar average body weight. In the experiment, two mash-form diets were prepared according to the growing stage; grower (d 0–21) and finisher (d 22–43). The diets mainly consisted of 60–64% grain mixture (corn, sorghum, brown rice) and 29–36% protein source (soybean, fish, canola and sesame oil meals), whose nutritional values were CP 22%/ME 3,100 kcal/kg (a grower diet) and CP 18%/ME 3,250 kcal/kg (a finisher diet). Tre was added into only the grower diets at 0.5%, and chickens were allowed free access to water and diets during the feeding stages. BW and FI for each replicate were recorded weekly. The procedures were approved and conducted according to the guidelines of Yamanashi Prefectural Livestock Dairy Technology Center.

2.3. Statistical analysis

Statistical analyses for the effect of Tre on growth performance in the trials were performed using R 3.4.0 software (R Core Team). The following linear mixed model was applied in this study.

$$y_{ik} = \mu + diet_k + time_l + diet_k \times time_l + u_i + e_{ik}$$

where y_i is the observation of animal i for diet period k ; μ is the total mean; $diet_k$ is the fixed effect of diet k (two classes; control, Tre); $time_l$ is the fixed effect of time l (two classes; starter, finisher); $diet_k \times time_l$ is the interaction between diet and time; u_i is the random effect of animal i , and e_{ik} is the residual random effect. The *lmer* function was used in the R package *lme4* (Bates et al., 2015) for fitting the above linear mixed model, and an analysis of variance (ANOVA) was then performed for the fixed effects with the *Anova* function, using type III SS in the R package *car* (Fox & Weisberg, 2011). Faecal score and intestinal morphologic indices were analysed using a one-way analysis of variance followed by Tukey's multiple comparison test.

Table 2
Morphological indices on the effects of Tre supplementation on the jejunum and ileum in broiler chickens on d 17.

	Cont	Tre-0.25	Tre-0.5	Tre-0.75	P-value	SEM
<i>Jejunum</i>						
Villus height (VH) (µm)	918	935	998	1,022	0.16	0.02
Villus width (µm)	128	128	130	140	0.14	0.002
Villus surface area (mm ²)	0.37 ^b	0.36 ^b	0.41 ^{ab}	0.43 ^a	< 0.01	0.008
Crypt depth (CD) (µm)	133	123	122	123	0.33	0.002
VH/CD ratio	7.14 ^b	7.86 ^{ab}	8.32 ^a	8.46 ^a	< 0.01	0.12
<i>Ileum</i>						
Villus height (VH) (µm)	550	527	587	567	0.23	0.011
Villus width (µm)	126	131	134	137	0.16	0.002
Villus surface area (mm ²)	0.22	0.22	0.25	0.24	0.11	0.005
Crypt depth (CD) (µm)	136 ^a	113 ^b	115 ^b	112 ^b	< 0.01	0.003
VH/CD ratio	4.25 ^b	4.76 ^{ab}	5.24 ^a	5.30 ^a	< 0.001	0.07

^{ab} Mean values in a same row with no common superscripts are significantly different (*P* < 0.05).

3. Results

3.1. Intestinal morphology, growth performance, and faecal score (experiment 1)

The effects of Tre on jejunum and ileum morphology of broiler chickens at d 17 were evaluated first. In the jejunum, VH, villus width and CD showed no considerable difference in the control and Tre-fed groups (Table 2). Meanwhile, Tre supplementation significantly affected villus surface area and VH/CD ratio, with the effect increasing with an increase in Tre supplementation. The morphological parameters in the jejunum were increased considerably in the Tre-0.75 group compared to those of the control group (^{ab}*P* < 0.05). Tre supplementation incredibly affected CD and VH/CD ratio but not VH or villus width and surface area in the ileum. CD and VH/CD ratio were changed depending on the Tre supplemental concentration, with Tre-0.75% showing lower CD and higher VH/CD ratio compared to those of the control group (^{ab}*P* < 0.05).

Table 3 shows the results of BW gain, FI, FCR, and mortality in chickens fed with Tre. The data were analysed using a two-way repeated measure ANOVA to evaluate the impact of diet (Tre supplementation) or time (stage) as the main effect or together as the interaction effect. There was no significant effect of the diet on BW gain, FI, FCR, and mortality between the groups. All the parameters were significantly affected by time; however, the significant interaction effect due to time and diet was not observed.

Tre supplementation did not affect faecal score at d 37 or carcass traits such as de-feathering, BW, the percentage of breast, drum stick, wing, leg, thigh or abdominal fat per chilled carcass weight (data not shown).

3.2. Growth performance (experiment 2)

The study further evaluated the effects of Tre 0.5% supplementation on growth performance of broiler chickens fed with a mash form diet,

Table 3
The effects of Tre supplementation on growth performance of broiler chickens at the starter, grower and finisher stages.

Parameters	Starter (d 1-17)				Grower (d 18-30)				Finisher (d 31-37)				SEM	Significance of effects		
	Cont	Tre-0.25	Tre-0.5	Tre-0.75	Cont	Tre-0.25	Tre-0.5	Tre-0.75	Cont	Tre-0.25	Tre-0.5	Tre-0.75		Diet	Time	Diet × Time
BW gain (g/bird)	670	668	672	672	1,142	1,128	1,145	1,146	531	551	534	529	30.0	0.474	< 0.001	0.557
FI (g/bird)	817	819	820	823	1,687	1,696	1,700	1,727	1,082	1,090	1,090	1,104	26.4	0.987	< 0.001	0.992
FCR (g/g)	1.22	1.23	1.22	1.22	1.48	1.50	1.49	1.51	2.05	1.99	2.05	2.10	0.016	0.348	< 0.001	0.410
Mortality (%)	4.20	5.20	4.40	4.40	0.00	0.43	0.83	1.25	0.21	0.21	0.42	0.00	0.25	0.576	< 0.001	0.522

Data were means from 10 replicates (50 birds per replicates). Abbreviations: BW, body weight; FI, feed intake; FCR, feed conversion ratio.

which was used in the previous investigation exhibiting the growth-promoting effect of Tre supplementation (Kikusato et al., 2016). While BW gain was significantly affected by time and not diet, the interaction effect was observed (Table 4). There was no significant effect observed on FI between the control and Tre-0.5 groups, while FCR had a statistically similar result to that of BW gain. BW gain and FCR at the grower and finisher stages were significantly improved by Tre supplementation.

4. Discussion

The present study demonstrated that Tre supplementation resulted in the improvement of intestinal epithelial morphology (experiment 1) and growth rate (experiment 2) in broiler chickens. We hypothesised that the growth-promoting effect of Tre supplementation might be attributable to the improved intestinal functions including villus development and innate immunity (Kikusato et al., 2016), as the morphological changes were indicative of gut health and nutrient absorption (Jamroz et al., 2006; Xu et al., 2003). However, the Tre-fed chickens that exhibited morphological improvements did not yield the enhanced growth output (experiment 1). Meanwhile, Tre supplementation was able to improve the growth rate in a different diet form (experiment 2). From these results, we need to re-consider the possible mechanism inducing growth rate and intestinal morphological alterations in broiler chickens and the reason the promoting effect was not obtained in experiment 1.

For the growth-promoting mechanism, we focused on metabolizable energy (ME) content in the diet supplemented with Tre. In experiment 2, the supplementation of 0.5% Tre yielded +16.6 kcal/kg diet ME content, if the supplemented Tre was completely hydrolysed and metabolised in the intestinal tract. However, the total ME values are almost equivalent between the basal (3,100–3,250 kcal/kg) and Tre-0.5% (3,101–3,250 kcal/kg) diets; this fact applied to the diets used in experiment 1. Based on this information, it is unlikely that ME from Tre-diet contributes to growth promotion in broiler chickens. Further, we considered intestinal morphological parameters such as VH/CD ratio, as they are used as indicative factors for growth rate in chickens. The reason why the morphological changes due to Tre supplementation did not improve the growth rate in experiment 1 remains unclear, and it could be suggested that the morphological alterations in machinery might be attributable to the autophagy-promoting effect of Tre (DeBosch et al., 2016). Intestinal epithelial cells are renewed within 2–5 days from intestinal stem cells in the steady-state (Barker et al., 2008), and a recent study found that intrinsic autophagy is vital for the maintenance of intestinal stem cells (Asano et al., 2017). For Tre to exert the above effect in chicken intestinal epithelial cells, the disaccharide needs to be delivered into the cell interior with the structure intact. Tre is hydrolysed by trehalase, whose activity is expressed from embryonic day 18 to 7 days post-hatch in isolated enterocytes obtained from the jejunum and ileum of embryonic and post-hatch chicks, with the activity gradually reducing depending on the ages (Chotinsky et al., 2001).

Meanwhile, it has been reported that Tre uptake occurs through simple diffusion in chicken brush-border membrane vesicles lacking the disaccharidase activity (Brot-Laroche and Alvarado, 1984). From these

Table 4

The effects of Tre 0.5% supplementation on growth performance of broiler chickens at the starter and grower/finisher stages.

Parameters	Starter (d 0-21)		Grower/finisher (d 22-43)		SEM	Significance of effects		Diet × Time
	Cont	Tre-0.5	Cont	Tre-0.5		Diet	Time	
BW gain (g/bird)	820 ^c	829 ^c	1,777 ^b	1,847 ^a	13.2	0.616	< 0.001	0.023
FI (g/bird)	1,195	1,232	3,658	3,702	29.4	0.375	< 0.001	0.811
FCR (g/g)	1.46 ^c	1.48 ^c	2.06 ^b	2.00 ^a	0.015	0.190	< 0.001	< 0.001

Data were means from 4 replicates (25 birds per replicates). Means within a row marked with different superscripts are significantly different (^{abc} $P < 0.05$). Abbreviations: BW, body weight; FI, feed intake; FCR, feed conversion ratio.

findings, one might assume that Tre incorporates into intestinal epithelial cells to modulate cell renewal, possibly via induction of autophagy. For future investigations, it is crucial to address this action of intestinal epithelial cells in chickens fed with Tre.

The previous investigation suggested that Tre supplementation enhances the growth rate, possibly via the improvement of intestinal innate immune function in juvenile chicks (Kikusato et al., 2016). The present study examined the faecal score, which is one factor for evaluating intestinal health conditions; however, these results showed that the values were not changed by feeding chicken with Tre, suggesting that the disaccharide might not improve intestinal functions. In the present study, the discrepancy in growth outcome due to Tre supplementation between the experiments needs to be discussed. To this end, we focused on the several differences between the experiments, such as the often-used sex of chickens and diet composition and form. In experiment 1, each replicate consisted of half males and females, while only female birds were used in experiment 2. One might assume that the difference in sex ratio might be the cause of the discrepancy between the experiments, although it is unclear whether or not Tre affects the growth performance of male broiler chickens.

Moreover, it is also possible that the different diet forms might be associated with the inconsistent results on growth output between the experiments, given that Tre gives *Lactobacillus acidophilus* tolerance against acidic conditions (Wang et al., 2018). The diets used in experiment 1 were steamed in the pelletising process, and the diets were, therefore, partly sterilised. While this heating process does not destroy Tre because of its thermal stability, Tre-related bacterial modulation effect might be ineffective. Moreover, the diet in the previous study (Kikusato et al., 2016) and experiment 2 did not contain any antibiotics, suggesting that Tre supplementation might serve as an alternative to antibiotic growth promoters. From the points, it could be assumed that the diet-making process and/or the presence or absence of antibiotics might influence the growth outcome due to Tre supplementation.

Insects have Tre as a major carbohydrate storage capable of maintaining glucose via enzymatic degradation due to trehalase. Recently, insect meal has been attempted to be used as an alternative protein source in animal diets. It has been reported that a replacement of dietary protein source with *Hermetia illucens* larvae meal decreases plasma cholesterol, triglyceride and urea levels, and increases total caecum short-chain fatty acid concentrations in laying hens (Bovera et al., 2018; Moniello et al., 2019). The availability of insect meals has been gradually investigated, and it could be interesting to consider a possible involvement of Tre in insect meal, creating a new diet for chickens.

5. Conclusions

The present study found that Tre supplementation resulted in the improvement of intestinal morphology (exp. 1) and growth performance (exp. 2). At the same time, these effects did not occur in parallel, likely owing to the different feeding conditions used. Several factors may be associated with the discrepancy in the growth promotion relating to Tre supplementation, which in turn suggests that the disaccharide may adequately act as a growth-promoting compound in broiler production once appropriate feeding conditions are maintained.

Further investigations are required to obtain mechanistic insights into how Tre supplementation improves the growth output in broiler chickens.

Declaration of Competing Interest

The authors have declared that no competing interests exist.

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