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Effect of dried tangerine peel extract supplementation on the growth performance and antioxidant status of broiler chicks

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ABSTRACT

The aim of this study was to evaluate the effects of dried tangerine peel extract (DTPE) supplementation on the growth performance and immune and antioxidant status of broiler chicks. A total of 525 day-old, male, Arbour Acres (AA) broiler chicks were fed a basal diet (CTR) either with or without 80, 160, 240 and 480 mg DTPE/kg diet. The chicks were randomly assigned into five dietary groups for a 42-day experiment. There were seven replicates per group and 15 chicks per replicate. At 42 days of age, two birds from each cage were selected for blood sampling. The dietary DTPE supplementation linearly increased the body weight and the average daily gain ($p = .03$; $p = .03$) and quadratically increased the average daily feed intake ($p = .02$) during the starter period. In addition, the plasma lysozyme level in the 80 or 160 mg/kg DTPE group increased compared to the CTR group ($p = .02$ and .07, respectively). Chicks fed 80-mg/kg DTPE reduced the malondialdehyde concentration ($p = .02$) compared with birds fed the CTR diets. Moreover, the plasma glutathione peroxidase activities in the low DTPE dietary groups (80 and 160 mg/kg) were higher than in the CTR ($p < .01$) and 240 mg/kg DTPE group ($p < .01$ and $p = .02$, respectively). The results of the present study indicate that dietary DTPE with low supplemental dosage ($\leq$160 mg/kg) might positively modulate the systemic antioxidative defence property of chicks.

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Introduction

Due to increasing concerns about antibiotic-resistant bacteria, which might impact human health (Durso & Cook 2014), the interest in discontinuing in-feed antibiotics has been growing in recent years, which has greatly enhanced the search for alternatives, such as probiotics, vitamin-like compounds and plant polyphenols additives, to improve the immune system, antioxidant capacity and productivity of farm animals (Lessard et al. 2009; Zhang et al. 2014; Samuel et al. 2015). Flavonoids, a group of polyphenolic compounds found mainly in fruits and vegetables, are some of the widely researched alternatives to in-feed antibiotics in animal nutrition (Sugihara et al. 1999).

Dried tangerine peel (*Citri reticulatae pericarpium*) is commonly used in traditional Chinese medicine to treat a wide array of ailments, such as bronchial asthma, dyspepsia, and cardiac circulation (China Pharmacopoeia Committee 2010). Dried tangerine peel extract (DTPE) is rich in flavonoids, especially flavanone glycosides and polymethoxy flavone, which have antioxidant and anti-inflammatory activities (Tripoli et al. 2007). Hesperidin is the most predominant flavonoid in tangerine peel. Ho and Kuo (2014) observed that the potent anti-neuroinflammatory capacity of DTPE is attributed to the collective effect of hesperidin, nobiletin, and tangeretin. In addition, purified hesperidin supplementation to broiler chicks' diets was reported to increase total superoxide dismutase and reduce malondialdehyde production in plasma (Kamboh & Zhu 2013) and overcome the deleterious effects of persistent summer stress in broiler production (Kamboh et al. 2013).

Although DTPE has gained attention in medicine and health-related research in the last few decades, information on the efficacy of DTPE on aspects of

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economic importance in the broiler industry, such as growth performance and carcase yield, is scarce. Therefore, the objective of this study was to evaluate the efficacy of dietary DTPE supplementation on the growth performance, immune and antioxidant status of modern fast-growing broiler chicks. In addition, the dose responsive trial may contribute to the estimation of the optimal supplementation level of DTPE in the diet of broiler chicks, which may serve as a basis for further studies.

Materials and methods

The animal protocol for this research was approved by the Animal Care and Use Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences (FRI2012015).

Birds and treatments

A total of 525 day-old male broiler chicks of the Arbour Acres (AA) strain were obtained from Beijing Huadu Broiler Company and were weighed. The chicks were randomly assigned into five experimental dietary groups, which consisted of seven replicates. Each replicate contained 15 birds. The dietary groups were as follows: the control group (CTR) that was fed a basal diet without additive and the DTPE groups that were fed an 80, 160, 240 and 480 mg DTPE/kg diet. The DTPE product, which contained 88.9% hesperidin, was provided by Beijing Centre Biology Co., Ltd. (Beijing, China). The hesperidin content in the DTPE was analysed by a high performance liquid chromatography (HPLC) method (Sun et al. 2010). DTPE was primarily prepared to 1% premix with maize. Then, the mixture was slowly combined with the rest of the feed by constant mixing. The basal diet was a typical maize–soybean diet formulated to meet the nutrient requirements of broiler chicks (National Research Council 1994), for starter chicks from 0 to 21 days and for grower chicks from 21 to 42 days of age. The compositions of the basal diets and nutrient levels are presented in Table 1. Feed and water were provided *ad libitum* at all times during the experimental period. The management of the birds was in accordance with the guidelines of raising AA broilers. The lighting regimen and ventilation were continuously checked from day 0 to 42. The birds were raised in wire floor cages (cage size: $110 \times 100 \times 55\,cm^3$) in a four-level battery in an environmentally controlled room under continuous incandescent white light. During the experimental period, the room temperature was maintained at 33 °C for the 1st week and was then gradually decreased by 3 °C each consecutive week and kept at room temperature after 4 weeks. The birds were subjected to feed withdrawal overnight to allow gut clearance before sampling.

Table 1. Ingredient and chemical composition (g/kg) of the diets (as fed basis).

Item	Starter (day 0 to 21)	Grower (day 21 to 42)
Ingredients		
Maize	563.1	617.5
Soybean meal	337.6	282.2
Maize protein powder (50% CP)	40.0	40.0
Vegetable oil	17.3	23.1
Dicalcium phosphate	19.0	13.9
Limestone ($CaCO_3$)	13.6	14.3
Salt	3	3
DL-Methionine	1.6	0.9
L-Lysine HCl	1.4	1.7
Vitamin and mineral premix[a]	2.4	2.4
Choline chloride (50%)	1	1
Dried tangerine peel extract[b]	0/0.08/0.16/0.24/0.48	0/0.08/0.16/0.24/0.48
Calculated composition		
ME, MJ/kg	12.13	12.55
Crude protein	210	190
Calcium	10	9
Available phosphorus	4.8	3.8
Lysine	11	10
Methionine	5	4
Methionine + cysteine	8.2	7.2

[a]Premix supplied per kg of diet: retinyl acetate, 3.75 mg; cholecalciferol, 0.0625 mg; α-tocopherol acetate, 18.75 mg; menadione, 2.65 mg; thiamine, 2 mg; riboflavin, 6 mg; cobalamin, 0.025 mg; biotin, 0.0325 mg; folic acid, 1.25 mg; calcium pantothenate, 12 mg; niacin, 50 mg; manganese ($MnSO_4 \cdot H_2O$), 100 mg; copper ($CuSO_4 \cdot 5H_2O$), 8 mg; Zinc (ZnO), 75 mg; iron ($FeSO_4 \cdot 7H_2O$), 80 mg; selenium (Na_2SeO_3), 0.15 mg; iodine (iodised NaCl), 0.35 mg.
[b]The chicks fed the basal diet either without or with 0.08, 0.16, 0.24 or 480 g/kg DTPE.

Experimental observations and measurements

Body weight (BW) and feed intake were recorded at 21 and 42 days of age for each replicate to determine the average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). The dead chicks were removed, weighed, and recorded twice daily. The ADG, ADFI and FCR were corrected with dead birds.

At 42 days of age, two birds from each cage, close to the cage average weight, were selected and slaughtered by cutting the jugular vein. Blood samples were collected in heparinised test tubes to analyse the plasma antioxidant and lysozyme differences among dietary treatments and were immediately centrifuged at $3000 \times g$ for 10 min at 4 °C to separate the plasma. Then, the samples were stored at −20 °C until analysis. The plasma within the replicate was pooled and analysed in duplicate. The plasma parameters were

Table 2. Effect of DTPE supplementation on growth performance of broiler chicks[a].

		DTPE level, mg/kg					*p* Value		
Item	CTR	80	160	240	480	SEM	ANOVA	Linear	Quadratic
BW, g									
Day 21	682[c]	709	708	724[b]	721	11	.08	.03	.10
Day 42	2136	2127	2165	2190	2152	33	ns	ns	ns
ADFI, g/d									
Day 0 to 21	42.89[c]	45.65	46.37	46.65[b]	45.62	1.46	.10	ns	.02
Day 21 to 42	146	144	144	147	144	3	ns	ns	ns
Day 0 to 42	91.86	92.91	93.32	94.67	92.65	1.67	ns	ns	ns
ADG, g/d									
Day 0 to 21	30.34[c]	31.61	31.53	32.37[b]	32.19	0.75	.08	.03	.10
Day 21 to 42	66.56	66.28	67.65	66.51	65.29	2.39	ns	ns	ns
Day 0 to 42	47.57	48.28	48.84	48.64	48.04	1.20	ns	ns	ns
FCR									
Day 0 to 21	1.41	1.44	1.47	1.44	1.42	0.04	ns	ns	ns
Day 21 to 42	2.20	2.18	2.14	2.22	2.21	0.06	ns	ns	ns
Day 0 to 42	1.93	1.93	1.91	1.95	1.93	0.03	ns	ns	ns
Mortality, %	5.71	1.90	4.76	5.71	4.76	–	ns	–	–

CTR: basal diet without additive; DTPE: dried tangerine peel extract; BW: body weight; ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio; ns: not significant.
[a]$n = 7$ replicates/treatment.
[b,c]Means listed in the same row with different superscripts are tended to be different ($p \leq .10$).

measured spectrophotometrically with commercial assay kits according to the manufacturer instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Glutathione peroxidase (GSH-Px) activity was measured with 5, 50-dithiobis-p-nitrobenzoic acid, and the change in absorbance at 412 nm was recorded. The malondialdehyde (MDA) concentration was analysed with 2-thiobarbituric acid, and the change in absorbance was read at 532 nm. The total antioxidant capacity (T-AOC) was measured by a ferric reducing/antioxidant power assay (Benzie & Strain 1996). The plasma lysozyme activity was determined according to the method of Kreukniet et al. (1995) with *Micrococcus lysodeikticus* as a substrate.

At necropsy, the dressing percentage was calculated by dividing the eviscerated weight by the live weight. The breast muscle, leg muscle and abdominal fat were removed and weighed. The breast and leg muscles yield were calculated as a percentage of the eviscerated weight, whereas the abdominal fat was calculated as a percentage of the live weight (Samuel et al. 2015). The lymphoid organs (thymus, bursa of Fabricius and spleen) were also collected and weighed, and the relative weights of the organs were calculated as the ratio of the lymphoid organ weight (g) to the live weight (kg). The average value of the two birds was regarded as the replicate value.

Statistical analysis

All the experimental data were analysed as a completely randomised block design by one-way ANOVA using the SPSS analysis software package programme (SPSS 21.0, SPSS Inc., Chicago, IL). The model included the treatment effect, and the cage represented the experimental unit for growth performance, while the average value of the two birds was the experimental unit for carcase yield, immune organ index and plasma antioxidant and lysozyme activities. The treatment comparisons were performed using Tukey's honestly significant difference test for multiple testing, and the chi-square test was used to test mortality. Linear and quadratic contrasts of the DTPE effects were also computed. The treatment effects were considered significant at $p \leq .05$, whereas a trend for a treatment effect was noted for $p \leq .10$.

Results

Growth performance and carcase yield

The effect of the different levels of dietary DTPE supplementation on the growth performance is shown in Table 2. In the starter chick phase, the dietary DTPE supplementation linearly increased the BW on day 21 and ADG ($p = .03$; $p = .03$) and quadratically increased ADFI ($p = .02$). Likewise, the dietary DTPE supplementation tended to quadratically increase the BW and ADG ($p = .10$; $p = .10$). Moreover, dietary 240 mg/kg DTPE tended to increase the BW of chicks on day 21 compared with the CTR group ($p = .07$), and the ADFI and ADG of the chicks in the 240 mg/kg DTPE group had the tendency to be higher than the un-supplemented group from day 0 to 21 ($p = .10$; $p = .07$). However, dietary DTPE supplementation did not affect the growth performance in the grower chick phase and

Table 3. Effect of DTPE supplementation on carcase yield of broiler chicks[a].

		DTPE level, mg/kg					*p* Value		
Item	CTR	80	160	240	480	SEM	ANOVA	Linear	Quadratic
Carcase yield on day 42, %									
Dressing	80.26	79.37	79.58	79.14	79.15	0.85	ns	ns	ns
Breast muscle	25.55	27.14	27.51	25.84	26.52	1.14	ns	ns	ns
Thigh muscle	21.08	21.75	21.01	21.76	22.28	0.90	ns	ns	ns
Abdominal fat	1.62	1.46	1.76	1.67	1.48	0.21	ns	ns	ns

CTR: basal diet without additive; DTPE : dried tangerine peel extract; BW: body weight; ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio; ns: not significant.
[a]$n = 7$ replicates/treatment.

Table 4. Effect of DTPE supplementation on the relative immune organ index and plasma lysozyme level (on day 42) of broiler chicks[f].

		DTPE level, mg/kg					*p* Value		
Item	CTR	80	160	240	480	SEM	ANOVA	Linear	Quadratic
Immune organ index, g/kg BW									
Thymus	1.94	2.06	2.16	1.81	2.45	0.39	ns	ns	ns
Bursa	1.00	1.04	1.06	1.31	0.87	0.22	ns	ns	ns
Spleen	2.03	1.54	1.58	1.81	1.58	0.31	ns	ns	ns
PLZM, μg/mL	2.26[b,c,e]	2.86[a]	2.76[a,b,d]	2.07[c]	2.19[c]	0.18	<.01	<.01	<.01

CTR = basal diet without additive; DTPE = dried tangerine peel extract; PLZM = plasma lysozyme; ns = not significant.
[a–c]Means listed in the same row with different superscripts are significantly different ($p \leq .05$).
[d,e]Means listed in the same row with different superscripts are tended to be different ($p \leq .10$).
[f]$n = 7$ replicates/treatment.

overall period ($p > .05$). No dietary effect was observed on the eviscerated yield, breast muscle yield, leg muscle yield and abdominal fat ($p > .05$, Table 3).

Immune organ index and plasma lysozyme activity

The results of the immune organ (spleen, thymus and bursa of Fabricius) index and plasma lysozyme level at 42 days of age are presented in Table 4. The thymus, bursa and spleen indices were not affected by dietary DTPE addition ($p > .05$). However, dietary DTPE supplementation linearly and quadratically increased the plasma lysozyme concentration of the chicks ($p < .01$). Furthermore, chicks fed low dosages (80 and 160 mg/kg) of DTPE had a greater plasma lysozyme concentration than the CTR group ($p = .02$ and .07, respectively) and the high DTPE dosage groups at 240 mg/kg ($p < .01$) and 480 mg/kg ($p < .01$ and $p = .02$, respectively), whereas there were no differences between the high DTPE dosage groups (240 and 480 mg/kg) and the CTR group ($p > .05$).

Plasma antioxidant indices

The effect of DTPE supplementation on the plasma antioxidant indices of broiler chicks at 42 days of age is shown in Table 5. Dietary DTPE at 80 mg/kg reduced the MDA content compared with the CTR group ($p = .02$). In addition, all levels of DTPE supplementation increased the GSH-Px activity of chicks compared with the CTR group (ANOVA, linear and quadratic contrast, $p < .01$). Among the DTPE-added groups, chicks fed DTPE at 80 mg/kg showed a higher plasma GSH-Px activity than those fed high dosages (240 and 480 mg/kg) of DTPE ($p < .01$ and $p = .02$, respectively), and the 160 mg/kg DTPE group had a higher plasma GSH-Px activity than the 240 mg/kg DTPE group ($p = .02$). Moreover, DTPE supplementation tended to increase the plasma T-AOC of chicks (quadratic contrast, $p = .07$).

Discussion

The objectives of our study were to determine whether dried tangerine peel extract (DTPE) added to the diets of broiler chicks would improve growth performance and immune and antioxidant status and whether there would be an optimal supplementation level of DTPE. To the best of our knowledge, there is scarce information on the effect of DTPE on the growth performance and carcase yield of broiler chicks. In the present study, dietary DTPE linearly and quadratically increased the growth of chicks in the starter period, which may suggest a beneficial role of DTPE in broiler growth. Kamboh et al. (2013) reported that dietary purified hesperidin improved growth performance. In contrast, other authors did not demonstrate any effect of hesperidin supplementation on daily gain, feed intake, or FCR of broiler chicks (Simitzis et al. 2011; Kamboh & Zhu 2014). In the present study, the potential improvement in

Table 5. Effect of DTPE supplementation on plasma antioxidant activities (on day 42) of broiler chicks[e].

		DTPE level, mg/kg					*p* Value		
Item	CTR	80	160	240	480	SEM	ANOVA	Linear	Quadratic
MDA, nmol/mL	4.47[a]	2.88[b]	3.58	3.90	3.53	0.48	.04	ns	ns
GSH-Px, nmol/mL	960[d]	4483[a]	4140[a,b]	2967[c]	3279[b,c]	365	<.01	<.01	<.01
T-AOC, U/mL	9.74	12.68	13.72	12.75	11.98	1.89	ns	ns	.07

CTR: basal diet without additive; DTPE: dried tangerine peel extract; MDA: malondialdehyde; GSH-Px: glutathione peroxidase; T-AOC: total antioxidant capacity; ns: not significant.
[a–d]Means listed in the same row with different superscripts are significantly different ($p \leq .05$).
[e]$n = 7$ replicates/treatment.

growth performance of the DTPE chicks may be due to a modulated health status, as evidenced by the improved systemic lysozyme content and antioxidative capacity, although all the birds were fed under a standard condition. However, the results obtained in the present study indicated that dietary DTPE supplementation did not influence the carcase yield, which may be due to the lack of differences in the final live weight. Simitzis et al. (2011) observed that dietary supplementation with hesperidin did not influence the carcase yield and meat quality characteristics.

The anti-cancer, anti-atherogenic, anti-inflammatory and anti-neuroinflammatory properties of DTPE were demonstrated by several authors (Tripoli et al. 2007; Benavente-Garcia & Castillo 2008; Ho & Kuo 2014). Our current study found that plasma lysozyme activity significantly increased with a low dosage ($\leq$ 160 mg/kg) of dietary DTPE supplementation at the end of the trial. As an important humoral component of the innate immune system, lysozyme can attack the peptidoglycan layer in the cell wall of Gram-positive and partial Gram-negative bacteria. Likewise, the plasma lysozyme responds immediately to a pathogen challenge (Skouras et al. 2003). In addition, lysozyme is effective against certain parasites, such as coccidia (Sotirov & Koinarski 2003). The antibacterial activity of lysozyme may be attributed to its direct bacteriolytic action and its stimulatory effect on macrophage phagocytic function (Nyachoti et al. 2012). In our study, higher levels of plasma lysozyme activity indicated that dietary DTPE might have a beneficial effect on broiler chicks to improve innate immune and antibacterial capacity. However, no dietary effect on immune organs was observed in the present study, likely because those organs are less sensitive than plasma lysozyme for healthy chicks, which is in agreement with the finding by Samuel et al. (2015).

Free radicals are produced in normal and pathological cell metabolism. Reactive oxygen species (ROS) are the free radicals that damage biological systems and include superoxide, hydroxyl radical, hydrogen peroxide and nitric oxide (Wang et al. 2008). It has been suggested that the accumulation of ROS may induce numerous disorders and cause a variety of impairments to tissues (Zhao & Shen 2005). Abuelsaad et al. (2014) reported that hesperidin has a potential role in the suppression of the inflammatory response induced by *Aeromonas hydrophila* toxins through the down-modulation of ROS production. Antioxidants in blood, cells, and tissue fluids play an important role in neutralising the normal level of oxidative damage caused by the accumulated ROS (Saleh et al. 2010). The glutathione peroxidase (GSH-Px) activity and the malondialdehyde (MDA) content reflect the antioxidant and lipid peroxidation status of cultured cells and animal tissues (Efe et al. 1999). In the present study, we found reduced MDA production in plasma when the chicks were fed DTPE diets, mainly at the level of 80 mg/kg. Similar positive changes in plasma MDA levels, as observed in our study, were reported by the dietary bioflavonoids in broiler chicks (Kamboh & Zhu 2013) and citrus flavonoids in laying hens (Lien et al. 2008). Increased GSH-Px activity by the dietary DTPE supplementation was also observed in this study. Bentli et al. (2013) reported that hesperidin prevented oxidative damage caused by 2,3,7,8-tetrachlorodibenzo-p-dioxin via decreased lipid peroxidation and an increased antioxidant defence system and GSH level. Other studies also reported increased serum GSH-Px activity by supplementing nano-elemental selenium and sodium selenite to broiler chicks (Hu et al. 2012) and essential oil to weaned piglets (Zeng et al. 2015). Plasma T-AOC is mainly regarded as the representation of the *in vivo* balance between oxidising species and antioxidant compounds and may give more biologically relevant information than that obtained from measuring concentrations of individual antioxidants (Ghiselli et al. 2000). The tendency to increase the plasma T-AOC in this study may provide evidence that dietary DTPE could improve the antioxidative capacity to scavenge free radicals and decrease damage in the tissues or cells of chicks. Previous studies demonstrated that dietary flavonoid

supplementation resulted in a marked increase in the plasma T-AOC activity of broiler chicks (Kamboh & Zhu 2013). Moreover, the reduced MDA, which is the endogenous lipid peroxide, may also support this observation. In this study, the plasma GSH-Px activity increased as the dietary DTPE level reached a plateau at the DTPE concentration of 160 mg/kg. The effects of the DTPE level on the plasma GSH-Px activity demonstrated that the greatest response to dietary DTPE occurred with the 160 mg/kg dietary DTPE inclusion level, which suggests that the plasma GSH-Px activity, and probably its production, seemed to be reflective of its dietary DTPE level, while additional dietary DTPE did not stimulate further production and (or) activity of the enzyme. The results of MDA production and T-AOC activities also show the same trends, which may further suggest that the supplemental dietary DTPE requirement, when using the plasma antioxidant activity as the measurement criterion, would be no higher than 160 mg/kg for broiler chicks.

The positive effects of low dosage ($\leq$160 mg/kg) DTPE on the immunity and antioxidant property obtained in this study could support our hypothesis that the partially improved growth performance may be due to a better health status of the chicks; however, the improvement of the administered compounds on growth was not as significant as on the plasma parameters. This result may be due to the lack of a challenge in the experimental chicks, which could lead to the impaired health of chicks and enhance the positive effect of additives over the defence system more than the positive effect that was observed in the present trial, thus markedly increasing performance during a stressful event, such as oxidative challenge. Our results indicated that the effect of dietary DTPE was not dose-dependent, and the highest level (480 mg/kg) of DTPE addition was not as effective as the lower level ($\leq$ 160 mg/kg); the greatest response to dietary DTPE occurred at the 160 mg/kg dietary DTPE inclusion level. Although the dietary supplementation of DTPE did not significantly increase the growth performance of broiler chicks, the successful development of strategies that improve the health of broiler chicks will provide economic and welfare benefits. In addition, the low responsive dosage implied that DTPE might have the potential to improve the economic benefits for broiler industry. The present study showed how the administration of DTPE to broiler chicks could improve aspects of the defence system, such as immunity and antioxidant properties; however, further experiments should be performed to understand the possible beneficial effects on growth performance.

Conclusions

In conclusion, the supplementation of DTPE in broiler chicks' diet has the potential to increase the growth performance in the early feeding period of broiler chicks, and dietary DTPE at 80 or 160 mg/kg may improve the immune and antioxidant status of broiler chicks under a normal feeding environment without stress challenges. More studies on DTPE application may benefit the broiler industry.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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