Effect of dietary inclusion of dried citrus pulp on growth performance, carcass characteristics, blood metabolites and hepatic antioxidant status of rabbits

Jingzhi Lu a, Xianghua Long a,b, Zhifei He c, Yingchun Shen a, Yanhong Yang a, Yuanqing Pan a, Jiahua Zhang a and Hongjun Li c

a College of Animal Science and Technology, Southwest University, Chongqing, People’s Republic of China; b Agricultural Bureau of Shuicheng County, Guizhou, People’s Republic of China; c College of Food Science, Southwest University, Chongqing, People’s Republic of China

ABSTRACT
Citrus pulp is a major by-product of the citrus processing industry. A total of 192 post-weaning, 35-day-old male French Hyla rabbits were randomly divided into four groups. Each group contained four replicates and each replicate comprised 12 rabbits. The four groups were given 0 (control group), 7%, 14% and 21% citrus pulp in the feed. Results indicated no significant difference in the performance of rabbits (P >.118). Rabbits fed with increasing citrus pulp demonstrated an increase in hot carcass weight (linear, P =.041) and liver weight (linear, P =.015). An upward trend (linear, P =.087) was found in thymus with increasing citrus pulp. The experimental groups all displayed an increase of at least 8.28%, 11.7% and 5.07% in serum albumin (ALB), ALB:GLO ratio and calcium (Ca) levels, respectively, compared with the control group (P <.05), and the experimental groups also presented high serum P (linear, P =.023). Hepatic total antioxidant capacity activities in the experimental groups were significantly higher than those in the control group (P <.05), but no difference was found in glutathione peroxidase activities (P >.05). In summary, inclusion of increased citrus pulp did not impair growth performance, could improve immunization, serum Ca and hepatic antioxidant status of growing rabbits.

1. Introduction
Citrus pulp is a by-product of citrus juicing industries (Grasser et al. 1995). The use of agroindustrial by-product as feed may effectively lower the cost of waste processing and management (Salvador et al. 2014), which has been a hot topic in the livestock industry. Dried citrus pulp (DCP) is one of the by-products of citrus, which is a mixture of citrus peel, pulp and seeds. The ingredients of DCP include 20–40% sugar and 22–44% neutral detergent-soluble fibre carbohydrates (Sunvold et al. 1995; Hall et al. 2010), which can be used as a source of energy (Bampidis and Robinson 2006). In addition, flavonoids and vitamin C in DCP can present antioxidant properties (Williams et al. 2004; Santos et al. 2014), antibacterial (Nordi et al. 2014) and immune-stimulating activities (Lee et al. 2010; Ebrahimi et al. 2015; Pourhossein et al. 2015) and meat oxidative stability (Inserro et al. 2014). Currently, DCP is being commonly used as a common ingredient in the diet of domestic animals. 0–3% DCP had no effects on growth performance (Ebrahimi et al. 2013; Abbasi et al. 2015). DCP (Abbasi et al. 2015) or Citrus sinensis peel extract (Ebrahimi et al. 2014) can lower abdominal fat content of broiler chicken. DCP can also optimize the gastrointestinal microbiota of broiler chicken (Ebrahimi et al. 2015). However, Mourao et al. (2008) reported that 5–10% DCP in diets impaired the growth of broiler chickens. No side effects were observed on growth performance and egg quality by adding 6% DCP in the daily feed of laying quails (Florou-Paneri et al. 2001) and adding 10–12% DCP in the daily feeds of laying hens (Yang and Choung 1985; Nazok et al. 2009). In the study of Hon et al. (2009), no difference was found in growth performance after 20% dried sweet orange (Citrus sinensis) pulp meal was added in rabbit feeds. Moreover, fermentation of structural carbohydrates in citrus pulp can benefit the animal (Hernández et al. 2012). Information related to the effects of dietary citrus pulp on the performance, blood metabolites and antioxidant status of growing rabbits is limited.

In this experiment, we attempted to study the optimal fraction of citrus pulp in daily feed of rabbits and investigate how dietary citrus pulp could influence growth performance, serum biochemical indexes and immunization.

2. Material and methods
2.1. Rabbits, diets and experimental protocol
In this study, DCP was provided by Chongqing Three Gorge Fruit Industry Ltd (Huiyuan Juice Group Limited). After juice processing, fresh citrus pulps were dried by a hot breeze dryer. The chemical composition of DCP was 92.23% dry matter, 5.50% crude protein, 9.61% crude fibre, 17.70% neutral detergent fibre, 20.30% acid detergent fibre, 3.82% ash, 2.41% calcium and 1.29% phosphorus.

The experiment was conducted in accordance with the Animal Care and Use Guidelines of College of Animal Science and Technology, Southwest University, Chongqing, China. A total of 192 post-weaning 35-day-old male French Hyla
rabbits, with similar heredity, generation and body weight, were randomly divided into four groups, with each group containing four replicates, and each replicate comprising 12 rabbits. The rabbits were housed in galvanized mental wire cages. Natural lighting and automatic ventilation were used in the rabbit house. Of the four groups, the control group was not administered DCP. The other three groups received 7%, 14% and 21% DCP mixed into daily feeds. The feeding experiment lasted 35 days. The first 7 days was a pre-test, and the remaining 28 days was the formal experimental study. The rabbits were fed ad libitum during the 28-day experiment, and water was constantly available. Table 1 shows the diet composition and nutrient levels that were determined following rabbit nutrient demand suggested by France, as well as considerations of local feed sources in Chongqing. The feed was made into pellets approximately 4 mm in diameter and 10 mm long in length.

2.2. Experimental measurements

On the start and end of the experiment, rabbits were weighed before feeding in the morning. The feed intake was recorded daily.

At the end of the experiment, 12 rabbits were weighed and slaughtered. The carcasses were measured and divided, following the methods recommended by the World Rabbit Science Association (Blasco and Ouhayoun 1996). After slaughtering, the skin, head, distal part of legs, spleen and visceral organs (such as lung, genitals, urinary bladder and gastrointestinal tract) were removed. Hot carcass contained only meat, heart, liver, kidney, fat and bone. Dressing yield was the percentage of hot carcass weight divided by live weight. Liver, kidney, heart, spleen and thymus gland were weighed and expressed as the percentage of live weight, in which spleen- and thymus gland-to-body weights were immunization indexes.

2.3. Statistical analysis

Data were analysed using one-way ANOVA with SPSS 20.0. A completely randomized design was used for growth analysis with pen as the experimental unit. Contrast was established using linear, and quadratic polynomial methods to determine the effects of increasing citrus pulps. Multiple comparisons were made using the Turkey multiple range test. Differences were considered significant at $P < .05$.

3. Results

3.1. Growth performance

Performance data are presented in Table 2. Although fluctuations in live weight were observed at 70 days ($P < .05$, linear), the concentration of dietary citrus pulp did not affect the daily feed intake, average daily gain and feed conversion ratio ($P > .118$).

3.2. Carcass traits and internal organs

Rabbits fed with increasing citrus pulp exhibited increased hot carcass weight (linear, $P = .041$, Table 3) and liver weight (linear, $P = .015$). An upward trend (linear, $P = .087$) for thymus was observed with increasing citrus pulp. No difference was found in spleen ($P > .05$).

3.3. Serum metabolites and hepatic antioxidant enzyme activities

No significant difference was found in serum AST, TP, GLO, GLU and TC ($P > .05$). The experimental groups all displayed an increase of at least 8.28%, 11.7% and 5.07% in serum ALB, ALB:GLO ratio and Ca levels, respectively, compared with the control group ($P < .05$; Table 4). The experimental groups also...
presented high serum P (linear, \( P = .023 \)). Hepatic T-AOC activities in the experimental groups were significantly higher than those in control group (\( P < .05 \)), but no difference was found in GSH-Px activities (\( P > .05 \)).

### 4. Discussion

Feed intake slightly increased in the present study, because rabbits ate 30–40 meals a day, and their gastric volumes accounted for about 30% of their entire digestive tracts (Gidenne et al. 2009). Pascual and Carmona (1980) found that the digestibilities of dry matter, organic matter and fibre were improved with the increased addition of citrus pulp in the feed of 6-week-old New Zealand male rabbits. The inclusion of DCP in the daily feed improved the total tract apparent digestibility of ingredients such as dry matter, crude protein, ether extract and nonfibrous carbohydrates, which improved milking performance (Santos et al. 2014). The increased digestibility possibly accounted for the high live weight of rabbits in the current citrus pulp groups. In this study, utilization of DCP of up to 21% had no any adverse effects on feed intake, body weight gain and feed conversion ratio, which were similar to the results of Hon et al. (2009).

The weights of slaughtered rabbits were close to average weights, so live weight and hot carcass in the 21% citrus pulp groups were higher than those in the control, and

| Items                  | Control | 7% DCP* | 14% DCP | 21% DCP | SEMb  | \( P \) valuec |
|------------------------|---------|---------|---------|---------|-------|---------------|
| Blood metabolites      |         |         |         |         |       |               |
| AST (u/L)              | 68      | 64      | 69      | 63      | 6     | \( 0.803 \)    |
| AKP (u/L)              | 126a    | 174b    | 133a    | 143ab   | 9     | \( 0.795 \)    |
| TP (g/L)               | 50.5    | 53.0    | 50.6    | 51.7    | 1.3   | \( 0.878 \)    |
| ALB (g/L)              | 16.9a   | 19.6b   | 18.3b   | 18.5b   | 0.33  | \( 0.016 \)    |
| GLO (g/L)              | 33.68   | 33.48   | 32.34   | 33.14   | 1.08  | \( 0.565 \)    |
| ALB:GLO ratio          | 0.504a  | 0.584b  | 0.568b  | 0.563b  | 0.015 | \( 0.021 \)    |
| GLU (mmol/L)           | 7.72    | 7.49    | 7.78    | 7.96    | 0.16  | \( 0.173 \)    |
| TC (mmol/L)            | 1.43    | 1.58    | 1.29    | 1.39    | 0.11  | \( 0.424 \)    |
| Ca (mmol/L)            | 2.96a   | 3.13b   | 3.11ab  | 3.18b   | 0.04  | \( 0.002 \)    |
| P (mmol/L)             | 2.43    | 2.45    | 2.46    | 2.72    | 0.08  | \( 0.023 \)    |
| Hepatic antioxidant enzyme activities |         |         |         |         |       |               |
| T-AOC (U/mg prot.)     | 6.81a   | 9.01b   | 9.27b   | 9.17b   | 0.35  | \(< 0.001\)    |
| GSH-Px (U/mg prot.)    | 34.85   | 37.91   | 36.35   | 35.88   | 1.84  | \( 0.855 \)    |

Note: AST, aspartate aminotransferase; AKP, alkaline phosphatase; TP, total protein; ALB, Albumin g/L; GLO, globulin; GLU, glucose; TC, total cholesterol; Ca, calcium; P, phosphorus; T-AOC, total antioxidant capacity; GSH-Px, glutathione peroxidase.

*7% DCP, 14% DCP and 21% DCP groups had 7%, 14% and 21% citrus pulps in the feed, respectively.

**SEM, standard error of the means.

*Means within a row with a letter in common differ (\( P < .05 \)).
demonstrated the same weight trend at the end of experiment. The dressing yield, an important economic index for manufacturers, showed no difference from the control group.

Over 60 flavonoids have been found in citrus (Benavente-Garcia et al. 1997) and these compounds can be digested in the small intestine (Walsh et al. 2009); therefore, the level of these flavonoids can significantly increase in blood serum. Flavonoids have antioxidant, anti-inflammatory, antibacterial and immune-stimulating effects (Harborne and Williams, 2000). Pourhossein et al. (2015) found that C. sinensis peel extract significantly improves the serum levels of IgM, and IgG in broiler chickens. The immunization index, to some degree, indicates the immunization functionality of animals. In this experiment, the thymus indexes of the citrus pulp inclusion groups demonstrated an increased trend compared with those of the control group, which indicated that the dietary inclusion of citrus pulp benefitted the health of rabbits. AKP is a hydrolase that helps to improve the immunization of organisms (Ming et al. 2012). In this study, serum AKP levels in the citrus groups were higher than those in the control group. Hepatic T-AOC, the ferric reduction antioxidant power, increased in the citrus pulp groups. Zhu et al. (2002) demonstrated the relationship between the amount of total phenolic compounds and ferric reduction antioxidant power. This association was also supported by previous findings of Santos et al. (2014), who reported that the activity of ferric reduction antioxidant power in milk improves with the citrus pulp additions of 9% and 18%.

Serum AST is an indicator of liver function and health. Increased AST concentration is a reflective response of an organism (Ozardali et al. 2004). Research showed that the inclusion of citrus maxima peel power in the diet of mice with CC14-induced hepatic damage significantly reduced the AST concentration in serum and liver (Chowdhury et al. 2015). In our study, AST showed no change (P > .931), which indicated no adverse side effects on the liver. When evaluating the health status scores of cows during calving, Akbar et al. (2015) found that cows with high and medium scores showed high levels of ALB in serum. Daudu et al. (2013) reported high serum ALB levels in DCP-fed rabbits. Plasma proteins are part of the immune response, in which antibodies are made of ALB. ALB is the major protein in serum. In this study, the ALB and ALB:GLO ratio levels in the citrus groups were higher than those in the control group, which possibly indicated good overall health status of the rabbits in the citrus groups. Moreover, the dietary inclusion of DCP increased serum Ca concentration (P < 0.05), because DCP is a good source of Ca (Nazok et al. 2009). No difference in the plasma glucose was determined in lactating Holstein cows (Santos et al. 2014), demonstrating the same trend with this study. Although the concentration of phosphorus in DCP (Nazok et al. 2009) is low, phosphorus linearly improved in DCP diets in the current study. This improvement possibly contributed to the high phosphorus concentration in the experimental diet (0.7%).

5. Conclusion

The experimental results showed that citrus pulp could be used as an available feed resource for rabbits. Up to 21% DCP in diet of rabbits had no adverse effects on growth performance, and also helped to improve serum Ca concentration and liver antioxidant status.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Special Fund for Agro-scientific Research in the Public Interest of China under Grant [number 201303144]; and the Fundamental Research Funds for the Central Universities under Grant [number XDJK2013C096].

References

Abassi H, Seidavi AR, Liu W, Asadpour L. 2015. Investigation on the effect of different levels of dried sweet orange (Citrus sinensis) peel on performance, carcass characteristics and physiological and biochemical parameters in broiler chicken. Saudi J Biol Sci. 22(2):139–146.
Akbar H, Graha TM, Riboni MV, Cardoso FC, Verkerk G, McGowan J, Macdonald K, Webster J, Schutz K, Meier S, et al. 2015. Body condition score at calving affects systemic and hepatic transcriptome indicators of inflammation and nutrient metabolism in grazing dairy cows. J Dairy Sci. 98:1019–1032.
Bampidis VA, Robinson PH. 2006. Citrus by-products as ruminant feeds: a review. Anim Feed Sci Technol. 128:175–217.
Benavente-Garcia O, Castillo J, Marin FR, Ortuno A, Del Rio JA. 1997. Uses and properties of Citrus flavonoids. J Agr Food Chem. 45:4505–4515.
Blasco A, Ouhayoun J. 1996. Harmonization of criteria and terminology in rabbit meat research. World Rabbit Sci. 4:93–99.
Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72:248–254.
Chowdhury MRR, Sagor MAT, Tabassum N, Poto I MA, Hossain H, Alam MA. 2015. Supplementation of citrus maxima peel powder prevented oxidative stress, fibrosis, and hepatic damage in carbon tetrachloride (CCl4) treated rats. Evid Based Compl Alt Med. 2015:Article ID 598179. doi:10.1155/2015/598179.
Daudu OM, Sani RU, Adedibu II, Ademu LA, Bawa GS, Bawa GS, Olugbemi TS. 2013. Effect of sweet orange fruit waste diets and acidifier on haematological parameters in broiler chicken. J Appl Anim Res. 42(4):445–450.
Ebrahimii A, Qotbi AAA, Seidavi AR, Bahar B. 2014. The effects of dietary supplementation of Citrus sinensis peel extract on production and quality parameters of broiler chicken. J Appl Anim Res. 42(4):445–450.
Ebrahimii A, Qotbi AAA, Seidavi AR, Luddadio V, Tufarelli V. 2013. Effect of different levels of dried sweet orange (Citrus sinensis) peel on broiler chickens growth performance. Arch Tierzucht. 56(2):11–17.
Ebrahimii A, Santini A, Alise M, Pourhossein Z, Miraalami N, Seidavi AR. 2015. Effect of dried Citrus sinensis peel on gastrointestinal microbiota and immune system traits of broiler chickens. Ital J Anim Sci. 14(4):712–717.
Florou-Paneri P, Babidis V, Kufidis D, Christaki E, Spais AB. 2001. Effect of feeding dried citrus pulp on quail laying performance and some egg quality characteristics. Arch. FUR Gefluglkunde. 65:178–181.
Gidenne T, Combès S, Feugier A, Jehl N, Arveau P, Boisot P, Briens C, Corrent E, Fortune H, Montessuy S, Verdelhan S. 2009. Feed restriction strategy in the growing rabbit. 2. Impact on digestive health, growth and carcass characteristics. Animal. 3:509–515.
Grasser LA, Fadel JG, Garnett J, DePeters EJ. 1995. Quality and economic importance of nine selected by-products used in California dairy rations. J Dairy Sci. 78:962–971.
Hall MB, Larson CC, Wilcox CJ. 2010. Carbohydrate source and protein degradability alter lactation, ruminal, and blood measures. J Dairy Sci. 93:311–322.
Harborne B, Williams CA. 2000. Advances in flavonoid research since 1992. Phytochemistry 55:481–504.
Hernández J, Rojo R, Salem AZM, Mirzaei F, Gonzalez A, Vázquez JF, Montañez OD, Lucero FA. 2012. Influence of different levels of dried citrus pulp on in vitro ruminal fermentation kinetics of total mixed ration in goat rumen inocula. J Anim Feed Sci. 21:458–467.

Hon FM, Oluremi OLA, Anugwa FOI. 2009. The effect of dried sweet orange (Citrus sinensis) fruit pulp meal on the growth performance of rabbits. Pak J Nutr. 8:1150–1155.

Inserro L, Priolo A, Biondi L, Lanza M, Bognanno M, Gravador R, Luciano G. 2014. Dietary citrus pulp reduces lipid oxidation in lamb meat. Meat Sci. 96(4):1489–1493.

Lee SJ, Shin JH, Sung NJ. 2010. Comparison of physicochemical properties of Citron (Citrus junos Sieb ex TANAKA) from three different areas of Namhae. J Agr Life Sci. 44:81–90.

Maral J, Puget K, Michelon AM. 1977. Comparative study of superoxide dismutase, catalase and glutathione peroxidase levels in erythrocytes of different animals. Biochem Biophys Res Commun. 77:1525–1535.

Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci. 84:407–412.

Ming JH, Xie J, Xu P, Ge XP, Liu WB, Ye JY. 2012. Effects of emodin and vitamin C on growth performance, biochemical parameters and two HSP70s mRNA expression of Wuchang bream (Megalobrama amblycephala Yih) under high temperature stress. Fish Shellfish Immunol. 32:651–661.

Mourao JL, Pinheiro VM, Prates JAM, Bessa RJB, Ferreira LMA, Fontes CMG, Ponte PIP. 2008. Effect of dietary dehydrated pasture and citrus pulp on the performance and meat quality of broiler chickens. Poult Sci. 87:733–743.

Nazok A, Rezaei M, Sayyahzadeh H. 2009. Effect of different levels of dried citrus pulp on performance, egg quality, and blood parameters of laying hens in early phase of production. Anim Health Prod. 42:737–742.

Nordi ECP, Costa RLD, David CMG, Parren GAE, Freitas ACB, Lameirinha LP, Katiki LM, Bueno MS, Quirino CR, Gama PE, et al. 2014. Supplementation of moist and dehydrated citrus pulp in the diets of sheep artificially and naturally infected with gastrointestinal nematodes on the parasitological parameters and performance. Vet Parasitol. 205(3):532–539.

Ozardali I, Bitiren M, Karakılıçk AZ, Zerin M, Aksoy N, Musa D. 2004. Effects of selenium on histopathological and enzymatic changes in experimental liver injury of rats. Exp Toxicol Pathol. 56:59–64.

Pascual JM, Carmona JF. 1980. Citrus pulp in diets for fattening rabbits. Anim Feed Sci Technol. 5:23–31.

Pourhossein Z, Qotbi AAA, Seidavi A, LaudadioV CG, Tufarelli V. 2015. Effect of different levels of dietary sweet orange (Citrus sinensis) peel extract on rumoral immune system responses in broiler chickens. Anim Sci J. 86:105–110.

Salvador A, Igual M, Contreras C, Martinez-Navarrete N, del Mar Camacho MD. 2014. Effect of the inclusion of citrus pulp in the diet of goats on cheeses characteristics. Small Ruminant Res. 121:361–367.

Santos GT, Lima LS, Schogor ALB, Romero JV, De Marchi FE, Grande PA, Santos NW, Santos FS, Kazama R. 2014. Citrus pulp as a dietary source of antioxidants for lactating Holstein cows fed highly polyunsaturated fatty acid diets. Asian Australas J Anim. 27:1104–1113.

Sunvold GD, Hussein HS, Fahey GC, Marchen NR, Reinhart GA. 1995. In vitro fermentation of cellulose, beet pulp, citrus pulp, and citrus pectin using fecal inoculum from cats, dogs, horses, humans, and pigs and ruminal fluid from cattle. J Anim Sci. 73:3639–3648.

Williams RJ, Spencer JPE, Rice-Evans C. 2004. Flavonoids: antioxidants or signalling molecules? Free Radic Biol Med. 36:838–849.

Yang SJ, Choung CC. 1985. Studies on the utilization of citrus byproducts as livestock feeds, 4: feeding value of dried citrus byproducts fed to layers. Korean J Anim Sci. 27:239–245.

Zhu QY, Hackman RM, Ensunska JL, Holt RR, Keen CL. 2002. Antioxidative activities of oolong tea. J Agric Food Chem. 50:6929–6934.