j.Egypt.vet.med.Assoc 82, no 1. 101 - 113 (2022)

EFFECT OF DRIED ORANGE WASTE AND CARBOHYDRASE FEED EXOGENOUS ENZYME ON GROWTH PERFORMANCE, IMMUNITY AND ANTIOXIDANT STATUS OF BROILER CHICKENS

By

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ABSTRACT

This experiment was conducted to evaluate the effects of two levels (3 and 5%) of local produced dried orange peel powder (DOP) with or without supplementation of exogenous xylanase enzyme (Xylanase-50000®) on zootechnical performance, immunity and antioxidant status of broiler chickens. Five hundred 1-day-old broiler chicks of both sexes were used in this investigation. Chicks were weighed and randomly divided into 5 dietary treatments. Each treatment was subdivided into four replicates containing 25 chicks/replicate in 20 floor pens. The first group consumed the basal corn/soybean meal diets without supplementation and served as control group. Treated groups A and B were reared on the reformulated basal diets supplemented with 3% and 5% of dried orange peel powder (DOP) respectively. Groups C and D were fed on reformulated basal diets supplemented with 3% and 5% dried orange peel powder and fortified with 50 g Xylanase-50000®/ton feed. The control and treated experimental diets were formulated to be isocaloric and isonitrogenous. At the end of the experiment (Day 35) twenty birds from each group (5 birds/replicate) were selected randomly for blood samples collection and serum separation. Some immune parameters and antioxidant biomarkers were investigated. The collected birds were eviscerated after slaughtering to record some parameters of carcass yield. Results revealed that Supplementation of DOP to broiler diets improved significantly ($p<0.05$) final body weight and feed utilization. The best results recorded in group C which consumed 3% DOP and fortified with Xyalanse enzyme. DOP used significantly improves the antibodies titres against NDV ($p<0.05$). DOP fed broiler chickens showed significant increase in lysozymes and superoxide dismutase (SOD) activities ($p<0.05$). DOP supplemented groups recorded reduction significantly ($p<0.05$) in the metabolite malondialdehyde (MDA). Carcass weights, dressing percent, liver and abdominal fat weights were not affected significantly between groups. In conclusion, local Egyptian dried orange

powder (DOP) can be included safely in broiler chicken diets up to 5% as unconventional feed without any adverse effect. DOP has positive effects on broiler chicken immunity and antioxidant status.

Key words:

Dried Orange Peel (DOP), Broiler chickens, Xylanase, Immunity, antioxidant biomarkers.

INTRODUCTION

Production of poultry meat and eggs has increased consistently over the years, and this trend is expected to continue. This growth in poultry production is having a profound effect on the demand for feed and raw materials. Feed is the most important input for poultry production in terms of cost. The availability of low priced, high quality feeds is critical if poultry production is to remain competitive and continue to grow to meet the demand for animal protein **(Ravindran, 2013).** It is also becoming clear that, the requirements for the traditional feed ingredients in Egypt -maize, soybean meal, and corn gluten meal, cannot meet the gap between local and/or imported supply for demand of these traditional ingredients. Feed diversification in the poultry diet is one of many attempts to reduce the cost of feed for poultry industry in Egypt. Feeding food waste to poultry and animals has been an important component of livestock production and provides a competitive alternative to more traditional feed grains and protein sources. Alternative feedstuffs are often referred as"non-traditional feedstuffs" because they have not traditionally been used in poultry and animal nutrition **(Alshelmani *et al*., 2016).** According to the United States Department of Agriculture (USDA), in 2016/17 Egypt was the sixth largest orange producer in the world. Several orange varieties are produced in Egypt.

The six dominant types are Navel, Baladi, Sukkari, Valencia, Khalily and Blood Orange **(El-Kholei, 2014).** Orange peel is by-product produced by the orange fruit processing and juice industry, so attempts were made to use the waste orange peel as a natural feed material and even as medicinal supplement for animals **(Callaway *et al.*, 2008).** Orange peels contain high concentrations of phenols, especially flavonoids **(Manthey, 2004).** The health benefits of orange fruit have mainly been attributed to the presence of bioactive compounds, such as phenolic compounds **(Ross *et al.*, 2000),** vitamin C **(Nagy, 1980)** and vitamin E **(Craig, 1997).** Studies have found that, the peel has the highest levels of vitamins C and E followed by the pulp then the juice. Thus, orange peel could enhance performance;

increase the production of antibodies and improving antioxidant status of broiler chickens.

Exogenous carbohydrate degrading enzymes are commonly added to broiler diets feed to aid

fiber and non-starch polysaccharides (NSP) digestion, thereby reducing their negative effects on broiler performance **(Choct *et al.*, 1999; Tufarelli *et al.*, 2007).** Moreover, dietary enzymes supplementation has been shown to improve the feeding value of feed by disrupting the water holding capacity of NSP resulted in improving nutrient digestion **(Choct, 2006).**

In Egypt researches to evaluate the effects of dried orange peel (DOP) powder with or without enzymes on performance parameters, carcass characteristics, immune system and antioxidant status of broiler chickens have not been yet fully investigated or published. Therefore, the objective of this study was to determine the effect of two dietary levels of local Egyptian dried orange peel powder (3 and 5%) with and without a commercial Xylanase enzyme supplement (Xylanase-50000®) on performance of broiler chickens. Furthermore, the effect of dietary treatments on immunity and antioxidant status of broiler chickens was also evaluated.

MATERIAL AND METHODS

Ethical approval:

This study was approved by the Institutional Animal Care and Use Committee (IACUC), Cairo University, Egypt (Vet., CU/II/F/69/21). *Dried orange peel Powder (DOP).* Dried orange peel powder (DOP) was obtained locally from Egypt under supervision of Agricultural Research Centre (ARC), Egypt. The obtained orange peels were from the local Egyptian orange breeds (Navel, Baladi, Sukkari, Valencia, Khalily and Blood Orange). Processing, drying and grinding were done in Egyptian Veterinary Industries Company (EVICO). Samples from the processed DOP were analysed according to AOAC, 2000 to determine its nutritive value (Table 1).

Table (1): Dried orange peel (DOP) chemical composition

Parameter	Percent
Moisture	**9.60**
Dry matter (DM)	**90.40**
Crude protein (CP)	**7.70**
Ether extract (EE)	**2.43**
Crude fiber (CF)	**16.64**

Total Ash	**15.55**
Nitrogen free extract (NFE)	**48.08**
Calculated metabolizable energy (ME) (kcal/kg)*	**1766.59**

***Calculated ME according to Poultry NRC, 1994.**

Xylanase Exogenous Feed Enzyme:

Xylanase-50000® is a commercial xylans degrading enzyme (50,000 U/g) produced by Challenge International Trade Co., Ltd., Beijing, China and distributed in Egypt by A2M Egypt Import and Export Co.

Experimental Birds, house and diets:

Five hundred 1-day-old broiler chicks of both sexes (Commercial Cobb) were obtained from a local hatchery (Coop Society of Animal Wealth and Poultry, West El-Badrashin). Chicks were weighed and randomly divided into 5 dietary treatments. Each treatment was subdivided into four replicates containing 25 chicks/replicate in 20 floor pens at the Poultry Rearing Center, Faculty of Veterinary Medicine,Cairo University, Egypt.All experimental birds were vaccinated against avian influenza, Newcastle, Infectious Bronchitis and Gumboro diseases according the vaccination program of Poultry Rearing Center, Faculty of Veterinary Medicine, Cairo University, Egypt. The first dietary treatment consumed the basal corn/soybean meal diets without supplementation and served as control group. Treated groups A and B were reared on the reformulated basal diets supplemented with 3% and 5% of dried orange peel powder (DOP) respectively. Groups C and D were fed on reformulated basal diets supplemented with 3% and 5% dried orange peel powder and fortified with 50 g Xylanase-50000®/ton feed.

The control and treated experimental diets were formulated to be isocaloric and isonitrogenous.

Two phases feeding regime consisting of starter (1-14 days) and grower-finisher (15-35 days) was used in the study. Corn/Soybean meal based diets were formulated to meet the nutrient requirements of Cobb broilers **(Cobb manual Catalogue, 2015).**

Diets in the form of mash and water were provided *ad-libitum* during the 35 days experimental period. The individual body weight as well as the rest of feed was recorded weekly. Body weight gain and feed conversion were calculated.

(Tables 2, 3) show the composition and proximate nutrients profile of the control and treated

starter diets. Tables (4 and 5) illustrate the control and treated grower-finisher diets and its proximate analyses.

Table (2): Feed ingredients of the control and treated starter diets (1-14 days of age).

Ingredients %	Control	Group A	Group B	Group C	Group D
Yellow corn	52.5	50	49	50.395	49.345
SBM (46% CP)	35.5	35.25	34.3	35.25	34.3
Corn gluten meal	5	5	5	5	5
Soya oil	1.65	1.4	1.35	1.0	1.0
DCP (Dicalcium Phosphate)	2.2	2.2	2.2	2.2	2.2
Limestone	1.5	1.5	1.5	1.5	1.5
DOP (dried orange peel)	-	3	5	3	5
Xylanase-50000®	-	-	-	0.005	0.005
Broiler premix*	0.3	0.3	0.3	0.3	0.3
Choline chloride	0.1	0.1	0.1	0.1	0.1
DL-Methionine	0.2	0.2	0.2	0.2	0.2
L-lysine	0.25	0.25	0.25	0.25	0.25
L- therionine	0.04	0.04	0.04	0.04	0.04
Common salt	0.3	0.3	0.3	0.3	0.3
Sod. bicarbonate	0.1	0.1	0.1	0.1	0.1
Feed additives	0.36	0.36	0.36	0.36	0.36
Total	100	100	100	100	100

***Per kg premix: 1 200 000 IU vit. A, 350 000 IU vit. D_3, 4 000 mg vit. E, 250 mg vit. B_1, 800 mg vit. B_2, 600 mg vit. B_6, 3.2 mg vit. B_{12}, 450 mg vit. K_3, 4.5 g nicotinic acid, 1.5 g Ca-pantothenate, 120 mg folic acid, 5 mg biotin, 55 g choline chloride, 3 g Fe, 2 g Cu, 10 g Mn, 8 g Zn, 120 mg I, and 40 mg Co.**

Table (3): Proximate analysis of the control and treated starter diets (1-14 days of age).

Parameter %	Control	Group A	Group B	Group C	Group D
Moisture	10.32	10.21	10.10	10.21	10.10
Dry matter (DM)	89.68	89.79	89.90	89.79	89.90

Crude protein (CP)	**23.12**	**23.07**	**23.04**	**23.12**	**23.03**
Ether Extract (EE)	**4.21**	**4.12**	**4.05**	**3.78**	**3.81**
Crude fibre (CF)	**2.69**	**2.79**	**2.85**	**2.77**	**2.85**
Nitrogen free extract (NFE)	**55.16**	**55.26**	**55.38**	**55.58**	**55.61**
Total Ash	**4.50**	**4.55**	**4.58**	**4.54**	**4.60**
Calculated metabolizable energy (ME) (kcal/kg)*	**2965.84**	**2963.76**	**2961.95**	**2908.32**	**2912.18**

***Calculated ME according to Poultry NRC, 1994 included the enzyme matrix.**

Table (4): Feed ingredients of the control and treated grower-finisher diets (15-35 days of age).

Ingredients %	Control	Group A	Group B	Group C	Group D
Yellow corn	**56.145**	**53.395**	**51.845**	**53.89**	**52.34**
SBM (46% CP)	**30**	**29.75**	**29.3**	**29.75**	**29.3**
Corn gluten meal	**6**	**6**	**6**	**6**	**6**
Soya oil	**3**	**3**	**3**	**2.5**	**2.5**
DCP (Dicalcium Phosphate)	**2**	**2**	**2**	**2**	**2**
Limestone	**1.4**	**1.4**	**1.4**	**1.4**	**1.4**
DOP (dried orange peel)	**-**	**3**	**5**	**3**	**5**
Xylanase-50000®	**-**	**-**	**-**	**0.005**	**0.005**
Broiler premix*	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**
Choline chloride	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**
DL-Methionine	**0.15**	**0.15**	**0.15**	**0.15**	**0.15**
L-lysine	**0.175**	**0.175**	**0.175**	**0.175**	**0.175**
L- therionine	**0.03**	**0.03**	**0.03**	**0.03**	**0.03**
Common salt	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**
Sod. bicarbonate	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**
Feed additives	**0.3**	**0.3**	**0.3**	**0.3**	**0.3**
Total	**100**	**100**	**100**	**100**	**100**

***Per kg premix: 1 200 000 IU vit. A, 350 000 IU vit. D_3, 4 000 mg vit. E, 250 mg vit. B_1, 800 mg vit. B_2, 600 mg vit. B_6, 3.2 mg vit. B_{12}, 450 mg vit. K_3, 4.5 g nicotinic acid, 1.5 g Ca-pantothenate, 120 mg folic acid, 5 mg biotin, 55 g choline chloride, 3 g Fe, 2 g Cu, 10 g Mn, 8 g Zn, 120 mg I, and 40 mg Co.**

Table (5): Proximate analysis of the control and treated grower-finisher diets (15-35 days of age)

Parameter %	Control	Group A	Group B	Group C	Group D
Moisture	**10.38**	**10.27**	**10.25**	**10.27**	**10.25**

Dry matter (DM)	**89.62**	**89.73**	**89.75**	**89.73**	**89.75**
Crude protein (CP)	**21.08**	**21.06**	**21.01**	**21.02**	**21.03**
Ether Extract (EE)	**4.62**	**4.57**	**4.55**	**3.82**	**3.75**
Crude fibre (CF)	**2.42**	**2.51**	**2.63**	**2.51**	**2.63**
Nitrogen free extract (NFE)	**56.98**	**56.91**	**56.85**	**57.68**	**57.61**
Total Ash	**4.52**	**4.68**	**4.71**	**4.70**	**4.73**
Calculated metabolizable energy (ME) (kcal/kg)*	**3155.81**	**3152.92**	**3156.08**	**3110.37**	**3115.14**

***Calculated ME according to Poultry NRC, 1994 included the enzyme matrix.**

Blood samples and immunological parameters:

At the end of the experiment (Day 35) twenty birds from each group (5 birds/replicate) were selected randomly for blood samples collection and serum separation. Detection of antibodies titer to Newcastle disease virus vaccine (NDV) using Haemagglutination Inhibition test (HI) according to **Alazawy and Ajeeli (2020)** with chicken RBCs and 4 units of NDV antigen, then geometric mean titers were calculated. Lysozyme activity was measured by agarose gel plate lyses assay according to **(Tony *et al.* 2014).** Lysoplates were prepared by dissolving 1% agarose in 0.06 mPBS at pH 6.3 in which Micrococcus lysodeikticus (50 mg/100 ml agarose) had been dispersed. Then 25 µl of serum samples and standard lysozyme were added in each well. After 18 hours the cleared zones diameter were measured. The concentration of lysozyme was obtained from logarithmic curve prepared using standard lysozyme solution.

Serum antioxidant parameters:

Directly after serum separation without delay, serum was used for estimation of superoxide dismutase (SOD) and malondialdehyde (MDA). SOD was assayed by inhibition and auto oxidation of adrenaline method. The estimation of MDA in the serum was done by thiobarbituric acid reactive species method according to **(Tejasvi *et al.*, 2014).**

Carcass yield:

At the end of the experiment, twenty birds /group (5 birds/replicate) were randomly selected from all groups. Feed was withdrawn 12 h before slaughter. Birds were defeathered and eviscerated after slaughtering by bleeding the jugular vein. Liver, abdominal fat were excised and weighed. After removal of head, shanks and offal, ready to cook carcass was obtained. The ready to cook carcass weight was then determined, and the carcass yield percentage (dressing %) was calculated according to **(Abdel Razek and Tony 2013).**

Statistical analysis:

All data were statistically analyzed using IBM SPSS® version 19 software for personal computer-2010. Means were compared by one way ANOVA ($p<0.05$) using Post Hoc test and least significant difference (LSD) according to **(Petrie and Watson 1999).**

RESULT AND DISCUSSION

Zootechnical performance:

Body weight development, feed intake and feed conversion ratio (FCR) in response to feeding a diet supplemented with dried orange peel powder (DOP) with or without Xylanase enzyme are tabulated in (Table 6).

Supplementation of DOP to broiler diets improved significantly ($p<0.05$) final body weight and feed utilization. The best results recorded in group C which consumed 3% DOP and fortified with Xyalanse enzyme. All treated groups regardless the utilisation of Xylanase enzyme showed more efficient utilization of feed, reduced feed to gain ratio and did not negatively affect weight gain. These results are consistent with the results observed by **(Ebrahimi *et al.* 2013 and Alefzadeh *et al.* 2016).** Previous studies have reported the use of different citrus wastes as feed ingredients. **Heuzé *et al.* (2012)** reported that even at inclusion low levels, *C. sinensis* by-products have low value in poultry diets since they reduced growth performance in chickens. However, **Oluremi *et al.* (2006)** and later **Agu *et al.* (2010)** replaced up to 15 or 20%, respectively, of the corn content in broiler diets with dried *C. sinensis* peel and found no negative influence on broiler productive traits. Furthermore, **Mourao *et al.* (2008)** demonstrated that adding of citrus pulp in chicken diet resulted in higher feed efficiency compared to birds fed diet including up to 10% of citrus pulp.

Table (6): Growth performance, feed utilisation and feed conversion of broiler chickens fed diets supplemented with dried orange peel powder (DOP) with or without Xylanase enzyme.

Parameter	Control[1]	Group A 3% DOP	Group B 5% DOP	Group C 3% DOP+Xylanase	Group D 5% DOP+Xylanase
Initial body Weight (g)	**47.2 ± 0.71[a]**	**48.5 ± 0.56[a]**	**46.8 ± 0.62[a]**	**45.9 ± 0.91[a]**	**47.5 ± 0.56[a]**
Final body weight (g)	**2061.1 ± 13.68[a]**	**2070.97 ± 10.81[b]**	**2066.37 ± 15.30[b]**	**2103.60 ± 13.30[c]**	**2110.32 ± 11.88[c]**
Weight gain (g)	**2013.9 ±**	**2022.47 ±**	**2019.57 ±**	**2057.7 ±**	**2062.82 ±**

	14.32[a]	**10.32[b]**	**10.85[b]**	**10.21[c]**	**10.95[c]**
Feed intake (g)	**3162.81 ± 42.18[a]**	**3135.80 ± 35.37[b]**	**3145.62 ± 41.53[b]**	**3164.27 ± 48.25[c]**	**3197.92 ± 39.12[c]**
Feed conversion ratio (FCR)	**1.57**	**1.55**	**1.56**	**1.54**	**1.55**

[1]Control group fed basal diets without supplementation.

Different subscripts within the row indicate significant effect (P< 0.05)

Values are expressed as Mean ± SE (Standard error).

Immunological parameters and antioxidant biomarkers:

The results of antibody titers to Newcastle disease virus vaccine (NDV) using Haemagglutination Inhibition test (HI) are shown in (Table 7). DOP used significantly improves the antibodies titres against NDV ($p<0.05$). The highest antibodies titers showed in treated groups which consumed feed supplemented with 5% DOP regardless of Xylanase enzyme added.

Table (7): HI titer of NDV vaccine, lysozymes activity, superoxide dismutase (SOD) and malondialdehyde (MDA) in serum of broiler chickens fed diets supplemented with dried orange peel powder (DOP) with or without Xylanase enzyme.

Parameter	**Control[1]**	**Group A 3% DOP**	**Group B 5% DOP**	**Group C 3% DOP+Xylanase**	**Group D 5% DOP+Xylanase**
HI titer of NDV (Log2)	**6.4 ± 0.67[a]**	**6.5 ± 5.8[a]**	**7.0 ± 0.58[b]**	**6.8 ± 0.67[b]**	**7.01 ± 0.32[b]**
lysozymes activity (µg/ml)	**12.12 ± 2.97[a]**	**31.16 ±13.3[b]**	**27.01 ± 5.0[b]**	**27.03 ± 5.0[b]**	**28.20 ± 4.5[b]**
SOD (µmol/ml)	**33.19 ± 4.34[a]**	**46.45 ± 3.49[b]**	**48.00 ± 4.37[b]**	**46.49 ± 2.83[b]**	**48.31 ± 5.45[b]**
MDA (µmol/ml)	**1.00 ± 0.25[c]**	**0.69 ± 0.09[b]**	**0.69 ±.052[b]**	**0.73 ± 1.09[b]**	**0.63 ± 0.06[a]**

[1]Control group fed basal diets without supplementation.

Different subscripts within the row indicate significant effect (P< 0.05).

Values are expressed as Mean ± SE (Standard error).

The results of lysozymes activity are shown in (Table 7). DOP fed broiler chickens showed significant increase in lysozymes activity at the end of the experimental period. All treated group with and without Xylanse supplementation recorded high values of lysozymes activity compared with the control group. Antioxidants are abundant in fruits and vegetables and have the ability to neutralize free radicals and convert them into harmless molecules **(Leonard *et***

***al*., 2002).** Therefore, according to **Byer *et al.* (2001),** the increase of antioxidant levels decreases free radical reactions which may have beneficial effects on cell activity. **Mona and Hanan (2007)** showed in laying hens that *Citrus sinensis* peel can improve immune system activities due to their antioxidant properties. According to **Kayvan and Eshtiyaghi (1992),** the living environment is full of pathogens, but the animal and human bodies have the ability to protect themselves with the help of the immune system.

Results of superoxide dismutase (SOD) and malondialdehyde (MDA) illustrated in (Table 7). The activities of SOD were improved significantly in all treated groups in comparison with the control group. On the other hand, MDA recorded high value in the control group. DOP clearly improved the antioxidant status of broiler chickens regardless of Xylanase used.

Orange peels contain high concentrations of active compound as phenolic compound and ally flavonoids. These bioactive compound enhance the immune system and improve antioxidant satus of the body **(Manthey, 2004).** The health benefits of orange fruit have mainly been attributed to the presence of bioactive compounds, such as phenolic compounds **(Ross *et al*., 2000),** vitamin C **(Nagy, 1980)** and vitamin E **(Craig, 1997).** Studies have found that the peel has the highest levels of vitamins C and E followed by the pulp then the juice. Thus, orange peel could enhance performance; increase the production of antibodies and improving antioxidant status of broiler chickens.

Carcass yield:

Carcass weights, dressing percent, liver and abdominal fat weights were not affected significantly by either DOP or Xylanase supplementation (Table 8).

Table (8): Carcass yield of broiler chickens fed diets supplemented with dried orange peel powder (DOP) with or without Xylanase enzyme.

Parameter	Control[1]	Group A 3% DOP	Group B 5% DOP	Group C 3% DOP+Xylanase	Group D 5% DOP+Xylanase
Live weight (g)	**2065.5**	**2073.5**	**2070.4**	**2094.9**	**2098.5**
Carcass weight (g)	**1466.5**	**1476.33**	**1496.98**	**1491.57**	**1489.64**
Dressing %	**71**	**71.2**	**72.3**	**71.2**	**70.98**

Liver weight (g)	**4.5**	**4.7**	**4.5**	**4.3**	**4.5**
Abdomen fat (g)	**2.35**	**2.26**	**2.19**	**2.21**	**2.12**

[1]Control group fed basal diets without supplementation.

All data are not significantly different from each other.

Values are expressed as Mean ± SE (Standard error).

The abdominal fat in all treated groups were decreased that reported in the control group in a dose-depended way. It is possible that the dried orange peel additive used affected the appetite in a dose-depend way. The results partly agreed with that of **Ebrahimi *et al.* (2013)** who stated that the effect of different treatments supplemented with dried *C. sinensis* peel on final body weight, and carcass yield percentage of broilers was not significantly different from the control groups, but those of different treatments supplemented with dried *C. sinensis* peel on carcass characteristics and the jejunum and ileum were significantly different from the control groups.

CONCLUSION

In conclusion, local Egyptian dried orange powder (DOP) can be included safely in broiler chicken diets up to 5% without any adverse effect on zootechnical performance and carcass yield. DOP can improve immunity and antioxidant status in broiler chickens. Xylanase exogenous feed enzyme improves the utilization of DOP for broiler chickens due to reduction of non-starch polysaccharides and viscosity in broiler chickens' intestinal tract. Further studies should be investigate in detail the effect of DOP on birds intestinal integrity and the metabolism of phenolic compounds and vitamins which present in DOP on the immunity and antioxidant biomarkers of broiler chickens.

Acknowledgements:

This manuscript was obtained from MVSc Thesis of Rasha G. Mohamed at Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Cairo University, Egypt.

The authors thank A2M Egypt Import and Export Co. for supplying Xylanase-50000® used in this study.

The authors thank Dr. Abd-Elrahim A. A. Rehan, Agriculture Research Center, Institute of Animal Production Research, Ministry of Agriculture, Egypt for his help in preparation and supply of local dried orange peel (DOP) used in this study.

Conflict of interest:

The authors declare that they have no conflict of interest.

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United States Department of Agriculture (USDA), (2016): Agricultural Research Service. Food Data Central, fdc.nal.usda.gov.