P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com
Research Article https://doi.org/10.47278/journal.ijvs/2022.148

Sweet Orange (*Citrus sinensis*) Peel Powder with Xylanase Supplementation Improved Growth Performance, Antioxidant Status, and Immunity of Broiler Chickens

Rasha G Mohamed, Mohammed A Tony, Alzahraa M Abdelatty*, Maha M Hady and Esam Y Ismail

Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt
***Corresponding author:** raramehmet@gmail.com; alzahraa@cu.edu.eg

Article History: 22-517 Received: 29-Jan-22 Revised: 09-Mar-22 Accepted: 22-Mar-22

ABSTRACT

The global population is estimated to reach approximately 9.7 billion persons by 2050. To meet the demands of the growing population, highly valuable feedstuffs that have traditionally been included as staples in poultry diets (e.g., corn and soybeans) are being reallocated for human nutrition purposes. As the availability of conventional poultry feedstuffs has decreased, poultry nutritionists are investigating the potential use of nonconventional feedstuffs in poultry diets. The objective of this study was to investigate the effects of utilizing dried sweet orange (*Citrus Sinensis*) peel powder (with and without xylanase supplementation) as a nonconventional feedstuff on production and health parameters in broiler chickens. A total of 375-day-old male Cobb 500 chicks were weighed and blocked for their initial body weight and randomly assigned to 25 pens (15 birds/pen). Dietary treatments were randomly and independently applied to each pen (5 replicates/per treatment). The following five dietary treatments were investigated in this study: 1) CON: basal corn-soya diet, 2) OPP3: basal diet containing 3% orange peel powder, 3) OPP5: basal diet containing 5% orange peel powder, 4) OPP3ENZ: OPP3 diet with xylanase enzyme added (50g/metric ton) and 5) OPP5ENZ: OPP5 diet with xylanase enzyme added (50g/metric ton). Feed intake and body weight were recorded on a weekly basis. Blood samples were collected at the end of the trial and analyzed for antioxidant and immune-related metabolites. The weights of internal organs (including immunological organs) were collected and reported. The data were statistically analyzed using PROC MIXED in SAS (SAS 9.4). Regardless of xylanase supplementation, the OPP-fed birds had decreased feed intakes and improved feed efficiency than the birds that received the control treatment (P=0.01). Significantly reduced Malondialdehyde (MDA) levels in the blood in all OPP-fed groups (P=0.01) compared to the CON group, but relative thymus weight was increased in the OPP5ENZ group. In conclusion, the inclusion of OPP in the broiler chicken diet (up to 5% of DMI) with xylanase enzyme supplementation could improve antioxidant capacity and cellular immune responses, positively impacting production.

Key words: Nonconventional feedstuff, Production efficiency, Non-starch polysaccharides, Broiler chicken.

INTRODUCTION

By 2050, the global population is estimated to reach approximately 9.7 billion persons (Abdelatty et al. 2018). To help meet the nutritional demands of the growing global population, substantial quantities of conventional poultry feedstuffs, such as corn and soybeans, are being reallocated from animal production to human nutrition purposes (Eissa et al. 2021). With this shift, there has been an increased competition between livestock and poultry industries and human populations for these feedstuffs which has resulted in their limited availability and increased costs (Bakeer et al. 2021). In an effort to sustain poultry farming under these challenging conditions, the dietary inclusion of nonconventional feed ingredients in poultry diets has gained popularity.

Dried sweet orange peel (OPP) is becoming a popular nonconventional dietary ingredient in poultry diets (Ebrahimi et al. 2013; Alefzadeh et al. 2016). However, the effects of this dietary ingredient on production and general health parameters of chickens are not well understood.

The inclusion of sweet orange extract and waste products like its peel, or pulp has been shown to have a variable effect on broiler chicken growth performance. For example, the inclusion of fermented OPP was shown to negatively impact body weight gain (Ani et al. 2015).

Cite This Article as: Mohamed RG, Tony MA, Abdelatty AM, Hady MM and Ismail EY, 2023. Sweet orange (*Citrus sinensis*) peel powder with xylanase supplementation ımproved growth performance, antioxidant status, and ımmunity of broiler chickens. International Journal of Veterinary Science 12(2): 175-181. https://doi.org/10.47278/journal.ijvs/2022.148

Alefzadeh et al. (2016) demonstrated that the inclusion of dried OPP did not significantly impact key production parameters such as body weight gain and FCR. Lastly, the dietary inclusion of dried sweet orange pulp was shown to positively affect the following production parameters: body weight and body weight gain (Abbasi et al. 2015).

Citric acid, flavonoids, and phytochemicals are abundant in the orange peel, making it a powerful antioxidant (Arora and Kaur 2013; M'hiri et al. 2015). For example, the flavonoids and vitamin C contents of dried OPP are 110-128mg/g, (Hassan et al. 2021). The dietary inclusion of orange peel ethanolic extract enhanced the antioxidant capacity of growing rabbit (Hassan et al. 2021) and reduced the oxidative stress product MDA and lipid peroxidation in broiler chicken meat (Vlaicu et al. 2020).

At this current time, there is limited information regarding the effect(s) of OPP supplementation on cellular immunity and humoral immune response parameters in broiler chickens. In addition, the results that are available have demonstrated that the effects of OPP supplementation on immunological parameters is variable (Pourhossein et al. 2015, 2019). For example, Pourhossein et al. (2015 and 2019) demonstrated that OPP supplementation positively affected the Avian Influenza vaccine antibody titer but did not have any significant effects on the Hemagglutination inhibition test against Newcastle disease. Thus, additional research is needed to further investigate the effects of OPP supplementation of health and immunological parameters in broiler chickens.

Orange peel powder contains approximately 11.3% Xylan, which is a non-starch polysaccharide (Jang et al. 2021). Exogenous enzyme supplementation, such as xylanase, may increase the effects of OPP on production parameters in broiler chickens that consume diets high in fiber and non-starch polysaccharide content, as this enzyme increases the nutrient availability and absorption of the Xylan polysaccharide (Singh et al. 2021; Ismael et al. 2022).

The objective of this study was to investigate the effects of dietary OPP inclusion with or without xylanase supplementation on growth performance, antioxidant activity, and immune status parameters in broiler chickens. This research was conducted in an effort to evaluate the use of OPP as a potential nonconventional dietary ingredient in broiler chicken diets.

MATERIALS AND METHODS

Animal Welfare Statement and Ethical Approval

This study was conducted using the EU standards for the protection of animals used for scientific purposes and feed legislation. All procedures and bird-handling in the current study were approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine, Cairo University (IACUC approval number: VET CU/12/10/2021/352).

Sweet Orange Peel Powder and Non-starch Polysaccharide Enzyme

Dried, nonfermented orange peel powder (OPP) was purchased from the faculty of Agriculture, Cairo University, Egypt. The chemical composition of the OPP is presented in Table 1. The non-starch polysaccharide enzyme utilized in this study was xylanase 50,000U/g (Challen XY™, Beijing Challenge International Trade Co, Ltd, Beijing, China), and this enzyme added to the diet as a top-dressing at a rate of 50g/metric ton.

Table 1: Chemical composition of dried orange peel powder (OPP)[1]

Items	%
Dry matter	90.41
Crude protein	7.78
Crude fat	2.43
Ash	5.00
CF	16.60

[1]Metabolizable energy of OPP is 2500 Kcal/kg.

Experimental Design, Housing, and Diet

Diet ingredients and chemical composition are presented in Table 2. For this study, 375 one-day-old male Cobb 500 chicks were purchased from a local hatchery in West El-Badrashin, Giza, Egypt. The birds were randomly assigned to 25 pens (15 birds per pen). Dietary treatments were randomly and independently applied to each pen (5 replicates/per treatment). The following five dietary treatments were investigated in this study: 1) CON: basal corn-soya diet, 2) OPP3: basal diet containing 3% orange peel powder, 3) OPP5: basal diet containing 5% orange peel powder, 4) OPP3ENZ: OPP3 diet with xylanase enzyme added (50g/metric ton) and 5) OPP5ENZ: OPP5 diet with xylanase enzyme added (50g/metric ton). The experimental diets were fed to the broiler chickens for 35 days. At day 35, the broiler chickens were culled for data collection purposes.

Each dietary treatment consisted of five replicates (n=15 birds/replicate). Replicates were reared in pens, and the chicks were housed on a deep litter system with sawdust and free access to feed and water. Birds were vaccinated against Newcastle, Avian Influenza, and Gumboro diseases.

Growth Performance, Euthanasia and Sampling

Feed intake and body weight (BW; g/d) were recorded for each pen weekly. BW gain and feed conversion rate (FCR; feed intake (g/d)/BW gain (g/d)) were calculated for each pen accordingly. At the end of the experiment (day 35), 25 birds (of average group body weight) were selected from each treatment group (5 birds per replicate) for sampling. Birds were euthanized and blood samples were collected for serum separation according to Abdelatty et al. (2021).

Weight of hot carcass (grams) was recorded and dressing percentage was calculated. Liver, gizzard spleen, heart, visceral fat, bursa, and thymus weights (grams) were recorded, and the relative weight-to-live-body weight was calculated.

Serum samples were stored at -20°C until the time of analysis. Serum MDA and superoxide dismutase were measured using HPLC (Agilent HP1200 Series, CA, USA), analysis protocol and the HPLC conditions were detailed in Abdelatty et al. (2020a). The hemagglutination inhibition test was used to determine vaccine titer of Newcastle disease according to (Ebrahimi et al. 2015).

Table 2: Composition and chemical analysis of the experimental diets[1] (%, As-Is)

Ingredient	Starter[2]			Grower-Finisher[3]		
	CON[4]	OPP3[5]	OPP5[6]	CON	OPP3	OPP5
Yellow corn	52.86	50.35	49.35	56.45	53.69	52.14
CGM[7]	5	5	5	6	6	6
Soybean meal (46% crude protein)	35.5	35.25	34.3	30	29.75	29.3
OPP[8]	0	3	5	0	3	5
Oil	1.65	1.4	1.35	3	3	3
DCP[9]	2.2	2.2	2.2	2	2	2
Limestone	1.51	1.51	1.51	1.4	1.4	1.4
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Premix[10]	0.3	0.3	0.3	0.3	0.3	0.3
DL-Methionine	0.2	0.2	0.2	0.15	0.15	0.15
L-Lysine	0.25	0.25	0.25	0.18	0.18	0.18
L-Threonine	0.04	0.04	0.04	0.04	0.04	0.04
Choline Chloride	0.1	0.1	0.1	0.1	0.1	0.1
Sodium bicarbonate	0.1	0.1	0.1	0.1	0.1	0.1
Proximate composition[11]						
Dry matter	89.68	89.79	89.9	89.62	89.73	89.95
Crude protein	23.12	23.07	23.04	21.08	21.06	21.01
Ether extract	4.21	4.12	4.06	4.62	4.57	4.55
Crude fiber	2.69	2.79	2.85	2.42	2.79	2.85
Ash	4.51	4.55	4.58	4.52	4.68	4.71

[1]The diet ingredients and chemical composition of the other two dietary treatments (OPP3ENZ and OPP5ENZ) are similar to OPP3 and OPP5, respectively. The OPP3ENZ and OPP5ENZ dietary treatments included a top-dressing of the xylanase enzyme (50g/metric ton): [2]Starter period (d1-14): [3]Grower-finisher period (d 15-35): [4]Control basal soybean-corn diet: [5]Diet supplemented with 3% orange peel powder (OPP): [6]Diet supplemented with 5% OPP: [7]Corn gluten meal: [8]Orange peel powder: [9]Di-calcium phosphate: [10]Vitamin-mineral premix Supplied per kg diet: Mn 60 mg; Zn 50 mg; Fe 30 mg; Cu 4 mg; I 3 mg; Se 0.1 mg; Co 0.1 mg; vitamin A 10 000 IU; vitamin D_3 2000 IU; vitamin E 10 mg; vitamin K_3 1 mg; vitamin B_1 1 mg; vitamin B_2 5 mg; vitamin B_6 1.5 mg; biotin 0.05 mg; pantothenic acid 10 mg; folic acid 1 mg; nicotinic acid 30 mg: [11]Calculated.

Statistical Analysis

The PROC MIXED procedures of SAS 9.4 (SAS Institute Inc., Cary, NC, USA) was used to analyze the data. The difference between treatments were compared using the Duncan's method. Significance was declared at P≤0.05.

RESULTS

The effects of the dietary inclusion of OPP with or without xylanase enzyme supplementation on broiler chicken growth performance has been presented in Table 3. The inclusion of OPP in the broiler chicken diet decreased the daily feed intake (FI; P=0.01), regardless of the OPP inclusion rate or the addition of the xylanase enzyme. Additionally, the OPP inclusion in the broiler chicken diet improved feed conversion rate (FCR; P=0.01) and did not negatively impact the body weight gain (P=0.59), regardless of xylanase supplementation.

The effects of the dietary inclusion of OPP with or without xylanase enzyme supplementation on the dressing percentage and carcass traits in broiler chickens has been presented in Table 4. The inclusion of dietary OPP at a level of 3 or 5% did not alter the dressing percentage of the hot carcass (P=0.83), regardless of xylanase supplementation. Additionally, the weight of the internal organs relative to the live body weight were not affected by the inclusion of dietary OPP with or without xylanase enzyme supplementation (P>0.05).

As illustrated in Fig. 1, the inclusion of OPP at a rate of 5% with xylanase enzyme supplementation increased the relative thymus weight (relative to the live body weight) compared to the other groups (P=0.01), however, the bursa relative weight was similar between all dietary treatments (P=0.29).

The effects of dietary inclusion of OPP with or without xylanase on the hemagglutination inhibition test vaccine titer of Newcastle disease as well as the concentration of SOD and MDA antioxidants has been illustrated in Fig. 2. The inclusion of OPP with or without xylanase supplementation did not alter the Newcastle disease vaccine titer (P=0.37) by day 35 of the experiment. Additionally, the level of serum SOD was not negatively affected by any of the inclusion rates of OPP or the xylanase enzyme (P=0.53). The level of MDA was markedly decreased in birds that received OPP in their diet, regardless of its inclusion rate or the addition of xylanase enzyme (P=0.01).

DISCUSSION

In order to meet the demands of the growing global population, nutritionally valuable feedstuffs (such as corn and soybeans) are being reallocated from animal production to human nutrition purposes (Mandouh et al. 2020; Abdelatty et al. 2021). As a result, conventional feedstuffs that have traditionally been included in poultry diets have limited availability and increased feed costs (El-Attrouny et al. 2021). In order to promote sustainable poultry farming under these challenging conditions, novel, nonconventional feedstuffs are being investigated and included in poultry diets (Abdelatty et al. 2020b).

In recent years, orange peel powder (OPP) has become a popular alternative to conventional feedstuffs in poultry diets (Ebrahimi et al. 2013; Alzawqari et al. 2016). Previous research has shown that dietary OPP supplementation improved animal growth performance (Abbasi et al. 2015; Vlaicu et al. 2020). In this current study, OPP supplementation at a dose of 3% or 5% (with or without xylanase supplementation) resulted in a

Table 3: Effects of dried sweet orange peel (OPP) with or without xylanase on growth performance of broiler chickens[1,2]

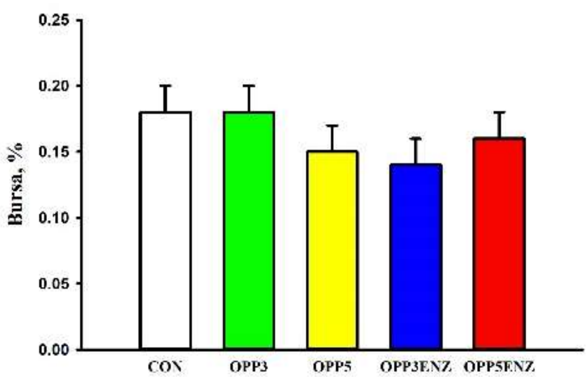
Parameter	CON[3]	OPP3[4]	OPP5[5]	OPP3ENZ[6]	OPP5ENZ[7]	SEM	*P*-Value
Initial BW, *g*[8]	47.31	47.17	46.93	46.87	46.87	0.26	0.66
BW gain, *g/d*[9]	57.42	56.81	56.66	56.28	56.27	0.55	0.59
Daily FI, *g/d*[10]	94.14[a]	86.94[b]	86.57[b]	85.41[b]	85.33[b]	1.18	0.01
FCR[11]	1.60[a]	1.55[b]	1.54[b]	1.53[b]	1.51[b]	0.02	0.01

[1]Values presented are Least Square Means (LSM): [2]Different superscript letters denote significant differences (P≤0.05) between treatments (based on Tukey's method): [3]Control basal diet: [4]Diet containing 3% of OPP: [5]Diet containing 5% of OPP: [6]Diet containing 3% of OPP and xylanase enzyme (50g/metric ton): [7]Diet containing 5% of OPP and xylanase enzyme (50g/metric ton): [8]Initial body weight (g): [9]Body weight gain (g/d): [10]Average daily feed intake per bird (g/d): [11]Feed conversion ratio (feed intake (g/d) divided by BW gain (g/d).

Table 4: Effects of dried sweet orange peel (OPP) with or without xylanase on dressing percentage and carcass traits (organ percentage[1]) of broiler chicken[2,3]

Items	CON[4]	OPP3[5]	OPP5[6]	OPP3ENZ[7]	OPP5ENZ[8]	SEM	P-Value
Dressing, %[9]	69.35	67.31	67.61	70.92	70.11	2.53	0.83
Liver, %	2.19	2.31	2.33	2.48	2.61	0.18	0.57
Visceral fat, %	0.83	0.86	1.23	1.21	1.24	0.21	0.38
Spleen, %	0.08	0.11	0.09	0.11	0.13	0.02	0.44
Gizzard, %	1.81	2.12	1.97	1.86	1.91	0.13	0.41
Heart, %	0.53	0.56	0.53	0.60	0.59	0.03	0.49

[1]The percentage of liver, visceral fat, spleen, gizzard, and heart are calculated relative to the live body weight of the bird: [2]Values presented are Least Square Means (LSM): [3]Different superscript letters denote significant differences (P≤0.05) between treatments (based on Tukey's method): [4]Control basal diet: [5]Diet containing 3% of OPP: [6]Diet containing 5% of OPP: [7]Diet containing 3% of OPP and xylanase enzyme (50g/metric ton): [8]Diet containing 5% of OPP and xylanase enzyme (50g/metric ton): [9]Percentage of hot carcass weight relative to live body weight.



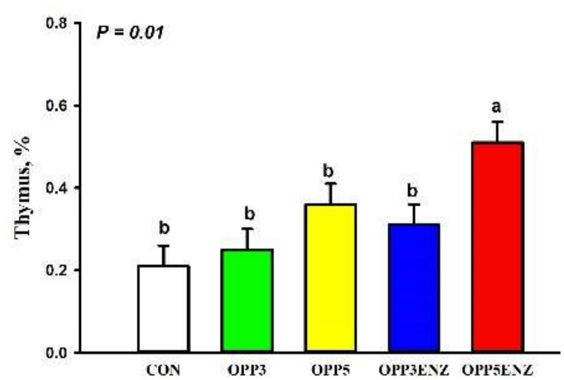


Fig. 1: Effect of dried sweet orange peel (OPP) with or without xylanase on immune organs of broiler chickens. The following five dietary treatments were investigated in this study: 1) CON: basal corn-soya diet, 2) OPP3: basal diet containing 3% orange peel powder, 3) OPP5: basal diet containing 5% orange peel powder, 4) OPP3ENZ: OPP3 diet with xylanase enzyme added (50g/metric ton), and 5) OPP5ENZ: OPP5 diet with xylanase enzyme added (50g/metric ton). Based on the results of these analyses, the dietary inclusion of sweet orange peel powder in broiler chicken diet at a rate of 5% with xylanase enzyme supplementation (OPP5ENZ; 50g/metric ton) significantly increased the relative weight of the thymus (P=0.01). Dietary OPP inclusion and/or xylanase supplementation did not significantly affect the relative weight of the bursa in broiler chickens.

significant decrease in feed intake and a significant increase in FCR. These results are consistent with the results observed by (Alefzadeh et al. 2016; Ebrahimi et al. 2013). However, it is important to note that OPP supplementation has also been shown to negatively impact growth performance in broiler chickens (Ebrahimi et al. 2013; Ani et al. 2015). Additionally, Alefzadeh et al. (2016) reported that OPP supplementation did not significantly affect growth parameters in broiler chickens. Due to the limited number of studies conducted and the discrepancies in results, additional research regarding the impacts of dietary OPP supplementation (with or without xylanase) on growth performance parameters in broiler chickens is needed.

The exact mechanism in which OPP supplementation affects feed intake is unknown; however, the mechanism may be influenced by a variety of factors. For example, Khosravinia (2007) reported that the appetite of broiler chickens may be dependent on the color of the feedstuff, such that broiler chickens consumed more feed containing green-colored ingredients and less feed containing orange and red-colored ingredients. As the OPP supplement is orange in color, this feedstuff characteristic may impact the broiler chickens' desire to consume the diet, subsequently resulting in decreased feed intake as seen in this study.

As OPP contains a high concentration of citric acid (Torrado et al. 2011), it may be possible that broiler chickens decreased their feed intake of OPP-supplemented diets based on the unpalatable taste of citric acid which was previously reported to be rejected by broiler chickens when offered in a two feeding choices trial (Niknafs and Roura 2018). In conclusion, it may be possible that the color and flavor profile of the OPP supplementation may contribute to the decreased feed intake observed within this current study and previously published reports.

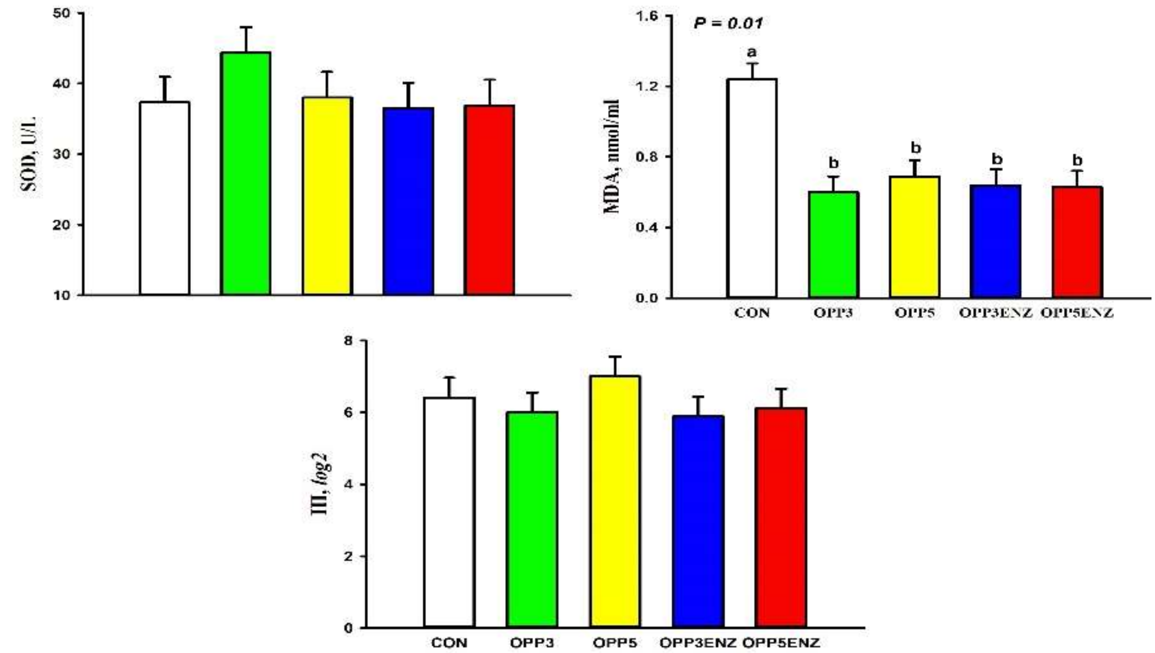


Fig. 2: Effect of dried sweet orange peel (OPP) with or without xylanase on antioxidant metabolites and hemagglutination inhibition titer against Newcastle disease of broiler chickens. The following five dietary treatments were investigated in this study: 1) CON: basal corn-soya diet, 2) OPP3: basal diet containing 3% orange peel powder, 3) OPP5: basal diet containing 5% orange peel powder, 4) OPP3ENZ: OPP3 diet with xylanase enzyme added (50g/metric ton), and 5) OPP5ENZ: OPP5 diet with Xylanase enzyme added (50g/metric ton). SOD=superoxide dismutase, HI=hemagglutination inhibition. Based on the results of these analyses, the level of MDA decreased (P=0.01) in birds fed a diet including OPP regardless of the inclusion rate of xylanase supplementation. Dietary OPP inclusion and/or xylanase supplementation did not significantly affect the SOD concentration or hemagglutination inhibition titer against Newcastle disease in broiler chickens.

It is important to note that the dietary OPP supplement utilized in the current study is dried, non-fermented, and does not contain the orange pulp (just the outer peel of the orange). In previous studies, other OPP supplements that have been investigated as nonconventional feedstuffs for broiler chicken diets were dried, fermented, and contained orange pulp (Ani et al. 2015). Differences in the physical and chemical composition of OPP supplements may explain the differences in the effects of dietary OPP inclusion on growth performance parameters between studies. For example, Ani et al. (2015) utilized a dried, fermented OPP supplement (which included pulp) and reported negative effects of OPP supplementation on growth performance parameters, which differed from the results reported in the current study. It may be possible that the type of dietary OPP supplement included in the diet may impact growth performance parameters in broiler chickens.

In the current study, dietary OPP supplementation (with or without xylanase supplementation) did not significantly affect any of the carcass parameters, including dressing percentage, in broiler chickens. These results were consistent with previously published research by ÇiFtçi et al. (2016). Due to its richness in phytochemicals and flavonoids (Arora and Kaur 2013), OPP supplements have high antioxidant properties (M'hiri et al. 2015; Alzawqari et al. 2016). This was confirmed in our study as dietary OPP supplementation (with or without xylanase supplementation) decreased the serum level of MDA (the end product of lipid peroxidation; as shown in Fig. 2). These results were consistent with previous research conducted by ÇiFtçi et al. (2016) in which OPP supplementation resulted in decreased concentrations of MDA in the liver and heart tissue of Japanese quail (compared to Japanese quail fed basal diets). These results suggest that the antioxidant metabolites of orange peel are functional nutrients that are highly absorbed at a cellular level and are utilized to support avian immune function. As OPP supplementation was shown to decrease MDA, it may be possible that OPP supplementation helps to protect feed lipids from peroxidation (Tavárez et al. 2011). As a result, an increased concentration of dietary lipids may be available and absorbed from the feed which may explain the decreased feed intake and increased feed efficiency observed in this study (Tavárez et al. 2011).

In the current study, OPP supplementation (with or without xylanase supplementation) did not significantly affect the HI against Newcastle disease antibody titer (as shown in Fig. 2). This finding was consistent with a previously published report by Pourhossein et al. (2019), in which OPP supplementation was shown to positively affect the Avian influenza titer, but it did not significantly affect the Newcastle disease titer. Although the antioxidant activity of OPP supplementation may play an important role in enhancing the humoral immune response through its antioxidant scavenging effect, its selective action on disease antibody titer is not well understood. Additional research regarding the effects of OPP supplementation on immunological response parameters in broiler chickens is needed. The bursa weight was not affected by the inclusion of dietary OPP (with or without xylanase supplementation; as shown in Fig. 1), and this result is consistent with previous research published by Pourhossein et al. (2019).

Lastly, in the current study, the relative thymus weight increased in birds that received the high dose of OPP (5%) supplementation combined with the xylanase enzyme supplementation compared to birds that received all other dietary treatments (Fig. 1). The inclusion of 3% OPP supplementation did not significantly impact the relative thymus weight, and these results are consistent with previous research published by Alefzadeh et al. (2016). In addition, previously published research by Attia et al. (2017) demonstrated that propolise supplementation (which is similar to OPP as it is rich in flavonoids and essential oils) increased the relative weight of immune organs when dietary propolise was added in the diet of broiler chickens. In this current study, it may be hypothesized that the interaction between the high concentration of OPP supplementation (5%) and the xylanase enzyme supplementation may result in higher concentrations of available antioxidant metabolites which would enhance the cellular immune response and increase the relative size of the thymus in broiler chickens. Due to limited research availability, additional research regarding the effects of OPP and/or xylanase supplementation on immunological response parameters in broiler chickens is needed.

Conclusion and Study Limitations

In conclusion, sweet orange peel powder (OPP) may be safely used as a nonconventional dietary ingredient (up to 5% inclusion of feed intake) in broiler chicken diets with a top-dressed xylanase enzyme (50g/metric ton) supplement included to enhance growth performance, immunity, and antioxidant potential of broiler chickens. Due to funding limitations, the effects of orange peel powder on gut morphometry and broiler chicken meat quality were not investigated in this study. Additional experiments are needed to further investigate the effects of orange peel powder on additional biological and production parameters.

Acknowledgments

The authors would like to acknowledge Dr. Samah Helmy for helping with sample analysis, and Dr. Omnia Adel for helping with statistical analysis and Dr. Marie Iwaniuk for helping with manuscript preparation and English editing.

Author Contributions

All authors contributed equally to this research study and manuscript. AM Abdelatty wrote the original manuscript draft.

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