



# Effect of Dietary Dried Orange (*Citrus sinensis*) Peel Powder and Exogenous Multi-Enzymes on Growth and Carcass Traits and Ileal Microflora of Broiler Chickens

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## ABSTRACT

The effects of dietary inclusion of dried orange peel powder (OPP) and supplementation of a commercial multi-enzymes (Natuzyne P50<sup>®</sup>) on the growth and carcass traits, gastrointestinal tract size and ileal microflora of meat-type broiler chickens were evaluated. Broiler chicks were assigned to treatments according to a completely randomized design by adding different levels of dried OPP (0, 1 and 4 g/kg, respectively) and multi-enzymes (0, 350 and 700 ppm, respectively) and their combination. On day 42, birds were slaughtered and samples collected. Growth traits varied marginally among dietary groups; however, multi-enzymes inclusion appeared to improve broilers weight gain without affecting feed efficiency. Dietary treatments did not affect the immunity-related organs weights. The multi-enzymes inclusion led a significant improvement of the gastrointestinal tract size as well as of gut microflora markers. In overall, our findings suggest that the inclusion of multi-enzymes in diet appeared to improve broilers performance; whereas supplementing dried orange peel powder has no effect.

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## Authors' Contributions

TA and AS conceived and designed the study. VL, VT, TA and AS wrote the article. TA and MB executed the experimental work.

## Key words

*Citrus sinensis*, enzyme, broiler, performance, gut microflora.

## INTRODUCTION

Plant waste products from human food industry may contain secondary compounds with valuable nutritional effects on growth performance and health in poultry. Advances in chemistry and the identification of plant compounds which are effective in improving performance and health have renewed interest in herbal medicines (Khan *et al.*, 2012; Dhama *et al.*, 2015).

Sweet oranges (*Citrus sinensis*) are produced in many tropical and subtropical climates. Traditionally, the orange peel has been used for ruminant nutrition, fertilizer, essential oils extraction, pectin extraction, industrial enzyme production, and single cell protein production (López *et al.*, 2010). Orange peels have been noticed in broilers (Ebrahimi *et al.*, 2013a,b, 2014), specifically as orange pulp (Abbasi *et al.*, 2015) and orange peel extract (Pourhossein *et al.*, 2015).

Exogenous enzymes are commonly added to broiler diets food or feed by-products to aid fiber digestion or to solubilize phytic phosphorus, thereby reducing their

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

However, researches to evaluate the effects of dried orange peel powder and enzymes on performance parameters and carcass characteristics, development of valuable parts as well as on immune system and microbial flora of broilers have not been yet published to the best our knowledge. Therefore, the objective of this study was to determine the effect of different dietary levels of dried orange peel powder with and without a commercial multi-enzymes supplement (Natuzyne P50<sup>®</sup>) on the performance traits of broiler chickens. Furthermore, the effect of dietary treatments on the gut microflora markers (*E. coli* and *Lactobacillae*) was also evaluated by quantitative culturing.

## MATERIALS AND METHODS

### Housing

The flock for this trial was kept in a commercial poultry house in Tahergourab, Guilan, Iran and at the

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Faculty of Agriculture, Islamic Azad University, Rasht Branch, Rasht, Iran. The facility was 6 × 23 m with two ventilators and six windows. The preparation of the rearing facilities was as reported by Laudadio *et al.* (2012) and Pourhossein *et al.* (2015). Briefly, prior to use, the units were thoroughly cleaned and properly disinfected including drinkers and feeders. Nonflammable parts were flamed up 2 days later, including the floor and metal walls of the pens. Walls were subsequently sprayed with water and lime. After drying, all joints, windows and ventilators was gasified with Formalex solution. During 48 h doors and windows remained closed. All equipment used during the rearing period including buckets, sandals, cardboard rolls, temperature gauges, and all drinkers and feeders were placed before gasification. Ventilation was turned on to optimize the climate 24 h before the broilers allocation.

#### *Rearing conditions*

A heater was used and temperature program was according to the instructions for Cobb-500 broilers. Air humidity was kept at 55 to 65% in the early growing period by spraying with water on the floor. Twenty watt lamps were installed at a height of 2 m above the floor. Twenty-one hours lighting was on and daily for three hours between 19:00 and 22:00 the house was left dark till slaughter at day 42.

The usual sanitation principles and health measures for raising chickens were applied as was described in the breeder's handbook. Drinker were washed and cleaned daily. After each vaccination, 1:1000 multi-vitamin plus electrolytes solution was mixed in the drinking water for 24 h. All feed remaining in feeders was weighed at the end of every week. The feeders were then cleaned thoroughly with a brush. Birds were vaccinated against the prevalent diseases. Birds were vaccinated at 1 and 8 days of age against Newcastle disease and Infectious bronchitis. Influenza vaccination was given at 1- day of age. Gumboro vaccination was given at 16-day, 19-day and 32-day. All vaccines were given by injection.

#### *Experimental design and dietary treatments*

The study was designed as a prospective trial with 9 treatments with 3 replicates per treatment. A total of 270 one-day-old mixed males and females chicks (50.7±0.85 g) of Cobb 500 strain were allotted to 27 groups of 10 birds each, such that mean group body weight was similar for each group. Environmental rearing conditions were similar for all treatments. Dried orange peel powder (OPP) (Khazarnoush Co, Chaboksar, Iran) and Natuzyme P50<sup>®</sup> (Bioproton Pty Ltd, Australia) were the experimental additives. Natuzyme P50<sup>®</sup> is a mixture

containing phytase: 1000,000 U/g, β glucanase: 700 U/g, α-amylase: 700 U/g, cellulase: 6,000 U/g, pectinase: 700 U/g, xylanase: 10,000 U/g, lipase: 30 U/g, protease: 3,000U/g, amilo-glucosidase, hemicellulase, pentosonase, acid phytase, and acid phosphatase. The dried orange peel powder contained: 3,124 kcal/g giving 2,511 kcal/kg of metabolisable energy, carbohydrates 63%, crude protein content 5.1%, dry matter 88%, ether extract 2.14%, calcium 4.15%, phosphorous 0.08%, ash 6.63% and crude fiber 10.96%.

The treatments were supplied to chicks from day 1 till day 42 (slaughtering age) as follows:

Treatment 1: basal diet (untreated control-group);

Treatment 2: basal diet included Natuzyme P50<sup>®</sup> multi-enzyme (350 ppm);

Treatment 3: basal diet included Natuzyme P50<sup>®</sup> multi-enzyme (700 ppm);

Treatment 4: basal diet included dried OPP (2.0%);

Treatment 5: basal diet included dried OPP (2.0%) and Natuzyme P50<sup>®</sup> (350 ppm);

Treatment 6: basal diet included dried OPP (2.0%) and Natuzyme P50<sup>®</sup> (700 ppm);

Treatment 7: basal diet included dried OPP (4.0%);

Treatment 8: basal diet included dried OPP (4.0%) and Natuzyme P50<sup>®</sup> (350 ppm);

Treatment 9: basal diet included dried OPP (4.0%) and Natuzyme P50<sup>®</sup> (700 ppm).

All chickens were fed according to the producer's feeding instructions. The nutrient composition of diets was similar for all treatments (Table I) and diets were isoenergetic and isonitrogenous. The diets met or exceeded Cobb-500 catalogue recommendations.

#### *Evaluated parameters*

Feed intake and weight gain were recorded weekly and feed conversion ratio was calculated. At the age of 42 days after 4 h of fasting to enable complete evacuation of the gut, one male bird from each replicate was selected and sacrificed. Care was taken to choose the most representative male birds with respect to body weight compared to the group mean body weight. Carcass yield and distribution of meat and weight of the gastrointestinal tract, lungs and immune organs were determined of these birds. Empty or edible carcass, breasts, drumsticks, wings, liver, abdominal fat, proventriculus, intestine, gizzard, pancreas, crop, thymus, spleen, bursa of fabricius were all weighed and recorded.

The ileal flora was characterized by numbers of *Escherichia coli* and *Lactobacillus* species after culturing on agar and subsequent colony counting. The MRS agar (Man Rogosa Sharpe Agar, 1.10660.500) was used to culture Lactobacilli, while eosin methylene blue (EMB, 1.01347.0500) was used to culture *E. coli*. Samples were

transferred to the laboratory and further processed according to the standards. Bacterial suspensions were prepared using phosphate saline (PBS). Serial dilutions were made ( $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$ , respectively). For the colony forming unit (CFU) counting 100 $\mu$ l was taken out of the  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  dilutions and poured into the Petri dish with a previously prepared medium. *Lactobacillae* were incubated at 37°C under anaerobic conditions for 72 h. Counting of the bacteria was done by colony counter. Bacterial counts were expressed as log<sub>10</sub> of bacterial counts per 1 g of sample.

**Table I.- Ingredients and nutrient analysis of the basal diet fed to broiler chickens.**

Ingredients (%)	1-21 days	22-42 days
Corn	49.50	53.91
Soybean meal (46% CP)	39.48	31.87
Mineral oysters	1.00	0.90
Corn oil	1.40	5.07
Ca% 22P% 18	3.37	3.00
NaCl	0.37	0.37
DL-Methionine	0.20	0.22
Lysine-Hydro-Chloride	0.08	0.06
Vitamin premix*	0.30	0.30
Mineral premix**	0.30	0.30
Zeolite/Orange peel powder	4.00	4.00
<b>Calculated analysis</b>		
Dry matter (%)	86.46	86.04
Metabolizable energy (kcal/kg)	2,900	3,000
Crude protein (%)	22.0	20.0
Ether extract (%)	3.5	3.5
Calcium (%)	1.0	0.9
Phosphorus (%)	0.5	0.46
Sodium (%)	0.12	0.12
Lysine (%)	1.3	1.2
Methionine (%)	0.5	0.5

\*Calcium pantothenate, 4 mg/g; niacin, 15 mg/g; vitamin B<sub>6</sub>, 13 mg/g; Cu, 3 mg/g; Zn, 15 mg/g; Mn, 20 mg/g; Fe, 10 mg/g; K, 0.3 mg/g; \*\*vitamin A, 5000 IU/g; vitamin D<sub>3</sub>, 500 IU/g; vitamin E, 3 mg/g; vitamin K<sub>3</sub>, 1.5 mg/g; vitamin B<sub>2</sub>, 1 mg/g.

#### Statistical analysis

Data were graphically analyzed by omnibus Mixed Linear Model with treatments as fixed effects, replicate as repeated measure and performance parameters as dependent variables. If the omnibus test showed a significant ( $P < 0.05$ ) difference, pairwise contrasts were tested with Bonferroni's test. The SPSS (1997) statistical software was used. If a significant interaction occurred between treatments, treatment groups were further analyzed with Student-Newman-Keuls test.

## RESULTS AND DISCUSSION

The growth parameters per dietary treatment group are shown in Table II. The evaluated traits varied marginally between the experimental groups. Feed conversion ratio was not significantly affected by Natuzyme P50<sup>®</sup> or dried OPP ( $P > 0.05$ ). This result suggested that growth or weight gain would not be different between treatment groups. Nevertheless, there was a significant effect on weight gain by treatment with Natuzyme P50<sup>®</sup> ( $P = 0.003$ ) and dried OPP ( $P = 0.006$ ). Table II shows also that birds in group 7 gained less than birds in the other groups, except for those in groups 4 and 8, respectively. If the single effects of treatment are more precisely analyzed for the valuable parts of the chicks, Natuzyme P50<sup>®</sup> appears to improved weight gain.

**Table II.- Growth performance of Cobb 500 broilers fed diets containing different levels of orange peel powder and multi-enzyme\*.**

Treatment		Cumulative feed intake (g/chick)	BW gain (g/chick)	FCR (g/g chick)
	0	4507 <sup>b</sup>	1859 <sup>b</sup>	2.28
Multi-Enzyme (ppm)	350	4629 <sup>a</sup>	1961 <sup>a</sup>	2.26
	700	4552 <sup>b</sup>	1956 <sup>a</sup>	2.26
SEM		18.75	20.26	0.05
P-value		0.001	0.003	0.959
Orange	0	4696 <sup>a</sup>	1999 <sup>a</sup>	2.37
Peel powder	2.0	4522 <sup>b</sup>	1938 <sup>b</sup>	1938 <sup>b</sup>
	4.0	4469 <sup>b</sup>	1839 <sup>c</sup>	2.25
SEM		18.02	20.85	0.09
P-value		<0.001	0.006	0.067
Orange (0)-Multi-enzyme (0)		4638 <sup>ab</sup>	1972 <sup>ab</sup>	2.42
Orange (0)-Multi-enzyme (350)		4724 <sup>a</sup>	4724 <sup>a</sup>	2.26
Orange (0)-Multi-enzyme (700)		4725 <sup>a</sup>	1980 <sup>ab</sup>	2.43
Orange (2.0)-Multi-enzyme (0)		4509 <sup>cd</sup>	1882 <sup>b</sup>	2.14
Orange (2.0)-Multi-enzyme (350)		4563 <sup>bcd</sup>	1972 <sup>ab</sup>	2.21
Orange (2.0)-Multi-enzyme (700)		4495 <sup>de</sup>	1958 <sup>ab</sup>	2.19
Orange (4.0)-Multi-enzyme (0)		4372 <sup>f</sup>	1722 <sup>c</sup>	2.28
Orange (4.0)-Multi-enzyme (350)		4600 <sup>bc</sup>	1866 <sup>b</sup>	2.31
Orange (4.0)-Multi-enzyme (700)		4436 <sup>ef</sup>	1930 <sup>ab</sup>	2.15
SEM		32.49	35.09	0.09
P-value		0.052	0.115	0.449

\* Means within each column of dietary treatments with no common superscript differ significantly.

Previous studies have reported the use of different citrus wastes as feed ingredients. Recently, 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

**Table III.- Economical carcass cuts weight (g) of Cobb 500 broilers fed diets containing different levels of orange peel powder and multi-enzyme\*.**

Treatment		Live BW	Empty abdomen carcass	Breast	Wings	Drumsticks
Multi-Enzyme (ppm)	0	1866 <sup>b</sup>	1198	398	102	349 <sup>b</sup>
	350	2002 <sup>a</sup>	1246	404	105	105
	700	1995 <sup>a</sup>	1248	422	108	385 <sup>a</sup>
SEM		9.21	7.56	3.31	2.15	3.12
Orange Peel powder	0	2054 <sup>a</sup>	1294	424	112	361 <sup>b</sup>
	2.0	1972 <sup>b</sup>	1240	422	103	378 <sup>a</sup>
	4.0	1836 <sup>c</sup>	1158	378	101	371 <sup>a</sup>
SEM		10.01	8.08	2.89	2.01	3.44
Orange (0)-Multi-enzyme (0)		2027 <sup>ab</sup>	1337	459	115	342 <sup>a,b</sup>
Orange (0)-Multi-enzyme (350)		2109 <sup>a</sup>	1290	397	114	367 <sup>a,b</sup>
Orange (0)-Multi-enzyme (700)		2025 <sup>ab</sup>	1255	417	106	374 <sup>a,b</sup>
Orange (2.0)-Multi-enzyme (0)		1931 <sup>bc</sup>	1234	411	100	370 <sup>a,b</sup>
Orange (2.0)-Multi-enzyme (350)		2001 <sup>abc</sup>	1247	415	101	385 <sup>a,b</sup>
Orange (2.0)-Multi-enzyme (700)		1983 <sup>abc</sup>	1239	441	107	379 <sup>a,b</sup>
Orange (4.0)-Multi-enzyme (0)		1639 <sup>d</sup>	1224	425	98	336 <sup>a</sup>
Orange (4.0)-Multi-enzyme (350)		1895 <sup>c</sup>	1200	401	99	375 <sup>a,b</sup>
Orange (4.0)-Multi-enzyme (700)		1975 <sup>bc</sup>	1249	409	111	402 <sup>a</sup>
SEM		10.22	7.95	3.04	2.17	3.01

\* Means within each column of dietary treatments with no common superscript differ significantly ( $P < 0.05$ ).

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.

As reported in Table III, the treatments 2, 5 and 8 led to a significant enhancement of broiler drumstick weight ( $P < 0.05$ ). In particular, adding 300 ppm of dried OPP improved drumstick weight by 27 g on average, while at 700 ppm dose improved drumstick weight by 36 g compared to Natuzym P50<sup>®</sup> treatment. Supplementing Natuzym P50<sup>®</sup> in diet however had no significant effect ( $P > 0.05$ ) on breast weight or empty carcass weight. In addition, dried OPP had no significant effect on broiler empty carcass and breast weights ( $P > 0.05$ ). Moreover, including 2% of OPP decreased the weight of most of the carcass valuable parts, while this was even worse at the 4% dose. Natuzym P50<sup>®</sup> and OPP had no significant effect on wing weight ( $P > 0.05$ ). In overall, broilers in group 7 (4% OPP) performed worse for most of the relevant performance parameters, indicating that the addition of Natuzym P50<sup>®</sup> in a diet containing dried OPP could compensate the negative effects of this diet on growth performance and development of valuable meat cuts.

In overall, birds' gut weight was not significantly affected by Natuzym P50<sup>®</sup>, whereas dried OPP

decreased gut weight in a dose-depend way ( $P < 0.05$ ; Table IV). The abdominal fat intestine weight were significantly decreased in the OPP supplemented broilers, often in a dose-depend way; whereas Natuzym P50<sup>®</sup> had no significant effect on these traits. The reason why the orange peel powder prevented maximal performance in the 42-day of rearing period is unknown. A metabolic effect does not seem to play a major role, since feed conversion ratio was not affected. Apparently, broilers gained less weight due to the less feed intake. It is possible that the dried OPP additive affected the appetite in a dose-depend way. None of the dietary treatments showed any significant effect on the mean weight of lungs, neck, head, heart and liver ( $P > 0.05$ ; Table V) as well as on the mean weight of the immunity related organs (thymus, spleen and bursa of Fabricius) ( $P > 0.05$ ; Table VI).

The results partly agreed with that of Ebrahimi *et al.* (2013a) 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.

**Table IV.- Gut organs weight (g) of Cobb 500 broilers fed diets containing different levels of orange peel powder and multi-enzyme\*.**

Treatment		Abdominal fat	Proventriculus	Intestine	Gizzard	Pancreas	Crop
Multi-Enzyme (ppm)	0	33 <sup>a</sup>	11	88 <sup>b</sup>	53	3	10
	350	35 <sup>a</sup>	10	98 <sup>a</sup>	60	4	10
	700	24 <sup>a</sup>	11	90 <sup>b</sup>	55	4	8
SEM		0.13	0.05	0.26	0.12	0.03	0.05
Orange Peel Powder	0	36 <sup>a</sup>	11	108 <sup>a</sup>	57	4	10
	2.0	37 <sup>a</sup>	11	90 <sup>b</sup>	59	3	10
	4.0	19 <sup>b</sup>	10	78 <sup>b</sup>	53	4	8
SEM		0.14	0.07	0.22	0.11	0.04	0.07
Orange (0)-Multi-enzyme (0)		51 <sup>b</sup>	11	96 <sup>a</sup>	55	3	8
Orange (0)-Multi-enzyme (350)		28 <sup>ab</sup>	12	130 <sup>a</sup>	65	5	9
Orange (0)-Multi-enzyme (700)		30 <sup>ab</sup>	11	98 <sup>a</sup>	53	4	8
Orange (2.0)-Multi-enzyme (0)		41 <sup>ab</sup>	12	90 <sup>b</sup>	57	3	9
Orange (2.0)-Multi-enzyme (350)		50 <sup>b</sup>	11	86 <sup>b</sup>	62	4	9
Orange (2.0)-Multi-enzyme (700)		21 <sup>ab</sup>	11	95 <sup>b</sup>	58	4	10
Orange (4.0)-Multi-enzyme (0)		7 <sup>a,c</sup>	9	79 <sup>b</sup>	48	4	9
Orange (4.0)-Multi-enzyme (350)		28 <sup>ab</sup>	8	77 <sup>b</sup>	53	3	10
Orange (4.0)-Multi-enzyme (700)		22 <sup>ab</sup>	11	78 <sup>b</sup>	53	4	7
SEM		0.15	0.06	0.23	0.16	0.05	0.06

\* Means within each column of dietary treatments with no common superscript differ significantly.

**Table V.- Organs weight (g) of Cobb 500 broilers fed diets containing different levels of orange peel powder and multi-enzyme\*.**

Treatment		Lungs	Head	Liver	Heart	Neck
Multi-Enzyme (ppm)	0	8 <sup>a</sup>	46 <sup>a</sup>	48 <sup>a</sup>	10 <sup>a</sup>	61
	350	9 <sup>a</sup>	47 <sup>a</sup>	51 <sup>a</sup>	11 <sup>a</sup>	65
	700	9 <sup>a</sup>	46 <sup>a</sup>	49 <sup>a</sup>	11 <sup>a</sup>	70
SEM		0.05	0.19	0.21	0.09	0.24
Orange Peel powder	0	10 <sup>a</sup>	50 <sup>a</sup>	60 <sup>a</sup>	10 <sup>a</sup>	61
	2.0	8 <sup>b</sup>	45 <sup>a</sup>	48 <sup>b</sup>	11 <sup>a</sup>	69
	4.0	9 <sup>a</sup>	44 <sup>a</sup>	39 <sup>b</sup>	11 <sup>a</sup>	67
SEM		0.06	0.20	0.22	0.11	0.25
Orange (0)-Multi-enzyme (0)		9 <sup>a</sup>	55 <sup>b</sup>	61 <sup>a</sup>	9 <sup>b</sup>	56
Orange (0)-Multi-enzyme (350)		10 <sup>a</sup>	44 <sup>a,b</sup>	63 <sup>a</sup>	10 <sup>a</sup>	60
Orange (0)-Multi-enzyme (700)		11 <sup>a</sup>	50 <sup>a,b</sup>	57 <sup>a</sup>	11 <sup>a</sup>	66
Orange (2.0)-Multi-enzyme (0)		8 <sup>a</sup>	43 <sup>a,b</sup>	46 <sup>a</sup>	11 <sup>a</sup>	65
Orange (2.0)-Multi-enzyme (350)		8 <sup>a</sup>	44 <sup>a,b</sup>	48 <sup>a</sup>	12 <sup>a</sup>	68
Orange (2.0)-Multi-enzyme (700)		9 <sup>a</sup>	47 <sup>a,b</sup>	51 <sup>a</sup>	10 <sup>a</sup>	75
Orange (4.0)-Multi-enzyme (0)		9 <sup>a</sup>	39 <sup>a,c</sup>	35 <sup>a</sup>	8 <sup>b</sup>	63
Orange (4.0)-Multi-enzyme (350)		9 <sup>a</sup>	52 <sup>a,b</sup>	43 <sup>a</sup>	9 <sup>a</sup>	68
Orange (4.0)-Multi-enzyme (700)		9 <sup>a</sup>	41 <sup>a,b</sup>	40 <sup>a</sup>	11 <sup>a</sup>	71
SEM		0.06	0.17	0.17	0.12	0.28

\* Means within each column of dietary treatments with no common superscript differ significantly.

**Table VI.- Immunity related organ weight (g) of Cobb 500 broilers fed diets containing different levels of orange peel powder and multi-enzyme\*.**

Treatment		Thymus	Spleen	Bursa of Fabricius
Multi-Enzyme (ppm)	0	5	2	2
	350	5	2	2
	700	6	3	2
SEM		0.22	0.09	0.05
Orange Peel powder	0	5	2	1
	2.0	5	2	1
	4.0	6	2	1
SEM		0.10	0.08	0.07
Orange (0)-Multi-enzyme (0)		5	2	1
Orange (0)-Multi-enzyme (350)		5	2	1
Orange (0)-Multi-enzyme (700)		5	3	2
Orange (2.0)-Multi-enzyme (0)		5	2	2
Orange (2.0)-Multi-enzyme (350)		5	3	2
Orange (2.0)-Multi-enzyme (700)		6	2	1
Orange (4.0)-Multi-enzyme (0)		6	2	2
Orange (4.0)-Multi-enzyme (350)		5	2	2
Orange (4.0)-Multi-enzyme (700)		6	2	2
SEM		0.08	0.05	0.06

\*Means within each column of dietary treatments with no common superscript differ significantly.

**Table VII.- Ileum microflora of Cobb 500 broilers fed diets containing different levels of orange peel powder and multi-enzyme\*.**

Treatment		Lactobacillus (log CFU/g)	E. coli (log CFU/g)
Multi-Enzyme (ppm)	0	6.5 <sup>a</sup>	8.2 <sup>a</sup>
	350	7.6 <sup>b,c</sup>	6.6 <sup>b</sup>
	700	7.2 <sup>a,c</sup>	7.1 <sup>b</sup>
SEM		0.11	0.14
Orange Peel powder	0	6.9 <sup>a,b</sup>	7.5 <sup>a</sup>
	2.0	7.7 <sup>a,b</sup>	6.5 <sup>b</sup>
	4.0	6.7 <sup>a,c</sup>	7.9 <sup>a</sup>
SEM		0.13	0.15
Orange (0)-Multi-enzyme (0)		6.3 <sup>a</sup>	7.7 <sup>a</sup>
Orange (0)-Multi-enzyme (350)		7.2 <sup>a</sup>	7.3 <sup>a</sup>
Orange (0)-Multi-enzyme (700)		7.2 <sup>a</sup>	7.5 <sup>a</sup>
Orange (2.0)-Multi-enzyme (0)		7.1 <sup>a</sup>	8.1 <sup>a</sup>
Orange (2.0)-Multi-enzyme (350)		8.0 <sup>a</sup>	5.2 <sup>a</sup>
Orange (2.0)-Multi-enzyme (700)		8.2 <sup>a</sup>	6.0 <sup>a</sup>
Orange (4.0)-Multi-enzyme (0)		6.2 <sup>a</sup>	8.8 <sup>a</sup>
Orange (4.0)-Multi-enzyme (350)		7.6 <sup>a</sup>	7.2 <sup>a</sup>
Orange (4.0)-Multi-enzyme (700)		6.2 <sup>a</sup>	7.7 <sup>a</sup>
SEM		0.09	0.15

\* Means within each column of dietary treatments with no common superscript differ significantly.

Both the gut microflora markers were significantly affected adding Natuzyme P50<sup>®</sup> at 350 ppm dose (Table VII). In particular, the *E. coli* count was increased ( $P<0.05$ ) as well as the Lactobacillae population

( $P<0.05$ ). The 2% OPP decreased *E. coli* count by 1 CFU/g compared to control diet and by 1.4 CFU/g compared to the 4% OPP determining a no-significant increase of the *Lactobacillae* count. There was no significant interaction between the two treatments (OPP and multi-enzymes) on broiler performance parameters. The physiological consequence of this finding was not clear, but did not appear to affect performance greatly. These results partly agreed with those of Pourhossein *et al.* (2012a,b,c) who found a positive effect of different levels of dried *C. sinensis* peel affecting on broiler gastrointestinal microbial population. Pourhossein *et al.* (2012a,b,c) found that the mean of Lactobacilli in the cecum on the postnatal 42 day showed no significant result, while the mean of *E. coli* in the ileum on the postnatal 42 day showed a significant difference from the control. The highest was produced in the control group and the lowest was related to the *C. sinensis* peel extract treatment of 1250 ppm up to the end of the experimental rearing period (Pourhossein *et al.*, 2012b).

## CONCLUSIONS

In overall, in the present trial the negative effects of supplementing dried OPP on growth parameters of broilers were shown. Some of these negative effects could be partially compensated by the inclusion of 350 ppm of Natuzyme P50<sup>®</sup> in the diet. Thus, the inclusion of Natuzyme P50<sup>®</sup> appeared to improve birds weight gain without affecting feed conversion ratio; whereas supplementing dried OPP has no beneficial effect. Moreover, taking into account the broiler rearing cost for 42 days, it can be stated that orange peel powder is not a beneficial additive for optimal growth in broilers. However, a larger field study should be performed to support this hypothesis.

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### Conflict of interests statement

None of the authors had a financial or personal relationship with other people or organizations that could inappropriately influence or bias the present paper.

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