Research Article

Oregano (*Origanum vulgare* Linn.) Powder as Phytobiotic Feed Additives Improves the Growth Performance, Lymphoid Organs, and Economic Traits in Broiler Chickens

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Abstract | Antibiotics have long been recognized as growth promoters and disease controllers in poultry production. However, due to rising public health concerns over antibiotic-resistant bacteria, the use of antibiotics in livestock and poultry feed has been banned in the European Union since 2006. Thus, this study was conducted to determine the effects of oregano powder (OP) as phytobiotic feed additives on the growth performance and cell-mediated immunity of broiler chickens. A total of sixty-day-old Cobb-broiler chickens were used in the study and arranged in a Completely Randomized Design experimental set-up with four dietary treatments. Each treatment was replicated three times, having five birds in every replication. The experimental rations containing graded levels of OP (0%, 1%, 3%, and 5%) were formulated and fed *ad libitum* in 42 days feeding trial. The results indicated that broiler chickens fed with OP showed a significant increase ($p<0.05$) in the final weight, body weight gain, voluntary feed intake, and feed conversion ratio. The cell-mediated immunity showed no significant difference ($p>0.05$) among treatment means. However, the results revealed that the immune organ indicators of broiler chickens fed with OP were higher than those birds without OP in the diet. On the other hand, the total expenses of broiler chickens fed with 5% oregano powder were reduced by 5.81% of the total inputs, and the income generated increases as high as 30.86% of the return above feed and chick cost. In conclusion, 5% oregano powder can be incorporated without adverse effects on the production performance and cell-mediated immunity.

Keywords | Broiler chicken, Oregano powder, Lymphoid organs, Cell-mediated immunity, Feed additives

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INTRODUCTION

Chicken meat has grown increasingly popular worldwide due to its beneficial nutritional qualities, such as high protein, low fat, and comparatively high polyunsaturated fatty acid (PUFA) concentrations compared to beef or pork (Suiryanrayna and Ramana, 2015; Brenes and Roura, 2010; Ahmed et al., 2013). Moreover, the protein that comes from poultry meat is of good quality, and it is frequently regarded as a benchmark against which other meats are measured (Atteh, 2004). This is because broiler chicken grows fast and produces soft meat for quick and easy home cooking, which is very important in modern countries where people spend less time preparing meals at home (Petracci et al., 2013)

Recently, most poultry growers have been using synthetic antibiotics to boost production performance and meet the demand for poultry meat and by-products (Mathew et al., 2009; Castanon, 2007). Moreover, the antibiotic growth

promoters (AGP) have been very useful in enhancing the growth performance and improving the feed efficiency in livestock (Dibner and Richards, 2005; Kalia et al., 2017). However, antibiotic immunity is a significant threat to human health because antibiotic-resistant bacteria associated with animals may be dangerous to humans, quickly transmitted through food chains to humans, and widely spread via animal waste (Abudabos et al., 2017; Lagua and Ampode, 2021). Hence, antibiotics as a growth promoter were banned in the European Union in January 2006 due to growing public health concerns (Murugesan et al., 2015).

As a result, interest in the effects of different phytobiotic plants on animal health and their performance increases. The positive effects of phytobiotic feed additives (PFA) have been reported (Abudabos et al., 2017; Amad et al., 2013). Spices and herbs stimulated endogenous enzyme secretion, antimicrobial effect, and feed intake antioxidant leading to improved nutrient absorption (Lee et al., 2015; Ri et al., 2017). Many researchers have been interested in phytobiotic sources as an alternative to chemical antibiotics as a proven growth promoter for many years ago. The phytobiotic plants such as cinnamon, cloves (both appetite and digestion enhancers), oregano (which has antibacterial qualities), and red pepper (which has anti-diarrheal and anti-inflammatory potential) are the most commonly studied herbs utilized in animal nutrition today (Kamel, 2000). Furthermore, sources of phytogenesis have been investigated as an alternative to antibiotics used in poultry and animals (Hashemi and Davoodi, 2010).

Oregano (*Origanum vulgare* Lin.) is an aromatic herb with a high concentration of active chemical compounds (De Falco et al., 2013; Ri et al., 2017). This plant has been used to replace chemical antibiotics in poultry and livestock (Ri et al., 2017; Ertas et al., 2005), and its effect as a feed additive has been investigated in past research. Oregano contains antibacterial, antioxidant, antiviral, immunomodulatory, and antiparasitic properties as a feed additive (Alagawany et al., 2018). The potential benefits of oregano extracts in chicken diets are improved feed intake and conversion, enhanced digestion, increased productive performance, reduced disease incidence, and reduced economic loss (Alagawany et al., 2018). However, various herbs and spices in broiler production provide inconsistent effects. Hence, this study was conducted to investigate the effects of oregano powder as phytobiotic feed additives on the growth performance, cell-mediated immunity, and economic traits of broiler chickens.

MATERIALS AND METHODS

Experimental birds, diets, and management

The study was carried out following the standard rearing of farm animals as stipulated in the good animal husbandry practices of the Philippines concerning animal farming, health, and welfare (Ampode and Asimpen, 2021). A week before the arrival of the experimental birds, the brooding pen was constructed in an elevated type made of bamboo slats, lumber, and nipa. It was cleaned thoroughly and disinfected. A total of 60 day-old Cobb broiler chicks were purchased from a reliable Agrivet supply in Kidapawan Public Market, Kidapawan North Cotabato, and housed at Tecson residence at Purok Miracle, Barangay, Poblacion, Kabacan North Cotabato. During the brooding period, artificial light was provided for twenty-four (24) hours day and night for fourteen (14) days. After brooding, the experimental birds were randomly distributed into four treatments and replicated three times, having five birds in every replication.

All broilers were raised in a wire-floored pen measuring 1 square foot per bird, fed *ad libitum,* and individual waterer and feeding trough were provided each pen. The feeding trial lasted for 42 days with two feeding periods, the starter and the finisher phase. The broiler chickens were given a starter ration from 15 to 28 days and gradually shifted to finisher ration from 29 to 42 days of age. The formulated diets meet or exceed the nutrients requirements based on the Philippine Recommends for poultry and Livestock Feed Formulation (PCAARRD, 2000). The experimental diet was incorporated with oregano powder at 0% (T_1), 1% (T_2), 3% (T_3), and 5% (T_4) of the basal diet (Table 1).

Collection and preparation of oregano powder

Fresh oregano leaves were collected at Maltana National High School botanical garden, Tampakan South Cotabato. The oregano leaves were washed thoroughly with tap water, sundried for three days until the leaves turned dark brown, and then pulverized using an electric blender to become oregano powder. The oregano powder was subjected to the proximate analysis (AOAC, 2016), and the result of the chemical analysis were used in formulating rations.

Data collection

The initial weight (g/bird) was taken on the 15th day-old chicks (right after brooding) and recorded at the start of the study. The final weight was determined at the end of the study using an electronic weighing scale with a capacity of 10,000 grams and a division of 0.1 gram. The bi-weekly body gain weight (BWG) was measured to monitor its gain in weight. The feed intake was determined by offering weighted amounts of feed, and weighing the feed refused every morning. The feed conversion ratio (FCR) was determined by calculating the total amount consumed over the broilers' final body weight gain (Eladia and Ampode, 2021).

Table 1: Composition and chemical analysis of starter and finisher ration.

Ingredients	Starter (15-28days)				Finisher (29-42days)			
	T1	T2	T3	T4	T1	T2	T3	T4
Ground yellow corn	50.25	50.35	50.00	50.10	52.10	52.50	51.50	50.00
Rice Bran D_1*	11.05	11.10	10.00	9.15	12.30	12.85	12.30	12.00
Soybean Meal	32.05	30.55	29.60	29.05	28.75	27.20	27.00	27.00
Fish Meal, 60%	1.90	2.35	2.85	1.80	1.90	1.75	1.45	1.50
Copra Meal	1.10	1.00	1.00	1.05	1.20	1.00	1.00	1.00
Oregano Powder	0.00	1.00	3.00	5.00	0.00	1.00	3.00	5.00
Dicalcium phosphate	1.10	1.10	1.00	1.20	1.15	1.20	1.15	1.10
Limestone	0.80	0.80	0.80	0.90	0.80	0.70	0.80	0.64
Lysine HCL	0.30	0.30	0.30	0.30	0.35	0.35	0.35	0.35
D-L Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L Treonine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.05
L tryptopan	0.10	0.00	0.10	0.10	0.10	0.10	0.10	0.11
Vit. Premix	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vegetable oil	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Chemical analysis (%DM)[3]								
Crude Protein	21.39	21.17	21.54	21.50	19.49	19.83	19.42	19.13
Crude Fiber	5.30	5.37	5.83	5.47	5.55	6.20	6.25	6.53
Moisture	10.58	10.71	11.24	13.09	10.90	11.25	11.81	11.54
Ash	7.94	6.78	6.74	7.28	6.80	6.75	6.85	6.61
Calculated analysis								
ME,Kcal/Kg	2949.74	2949.68	2949.18	2949.4	3050.16	3050.06	3050.19	3050.01
Available Phosphorous	0.39	0.44	0.41	0.44	0.41	0.41	0.43	0.41
Calcium,%	0.74	0.87	0.85	0.87	0.83	0.81	0.82	0.80
Lysine,%	1.18	1.01	1.21	1.18	1.09	1.1	1.66	1.12
Methionine,%	0.41	0.43	0.42	0.41	0.38	0.4	1.2	0.37
Met + Cyst,%	0.73	0.76	0.73	0.73	0.69	0.69	0.89	0.69
Treonine,%	0.86	0.86	0.82	0.86	0.70	0.80	1.00	0.8
Tryptophan,%	0.35	0.28	0.45	0.28	0.33	0.33	0.33	0.31

* D1 is a category of rice bran which has fine quality/texture. Vit. A. 12000000 iu, Vit, D3 2000000 iu, Vit. E 1500 mg, Vit. K3 2000 mg, Vit. C 1000 mg, Vit B12 2000 mg, Vit. B2 4000 mg, Vit B6 3000 mg, Vit. B12 25000 mg, Folic acid 700 mg, Iron 25000 mg, Lysine 2500 mg, Panthothenic acid 15000 mg, Niacin 25000 mg, DL-Methionine 8500 mg, Magnesium 250000 mg, Zinc 50000 mg, Antioxidant 1000 mg, Manganese 10000 mg, Biotin 10000 mg, Copper 2000 mg, Iodine 500 mg, Cobalt 5 mg, Selenium 500 mg, Probiotics 50000 mg.

Cell-mediated immunity

The immune organ indices were evaluated after the 42 days of the feeding trial. The experimental broiler chickens fasted for twelve (12) hours. After fasting, birds are individually weighed, and birds with the nearest to the mean weight per replication were selected for slaughtering to determine the immune response by weighing the lymphoid organs (bursa of Fabricius and spleen). The broiler chickens were slaughtered following the rules and regulations on humane handling in slaughtering animals for food (DA, 2008). Following an actual visual appraisal, the bursa of Fabricius and spleen were immediately removed and individually weighed (g). The Bursa index was calculated by getting the bursa weight over total body weight. The spleen index was calculated by dividing the spleen weight by the body weight following the formula of Latif et al. (2014) and Dumaup and Ampode (2020), as shown below.

$$\text{Spleen Index} = \frac{\text{Spleen Weight}}{\text{Body Weight}} x\ 100$$

$$\text{Bursa Index} = \frac{\text{Bursa of Fabricius Weight}}{\text{Body Weight}} x\ 100$$

Cost and return analysis

The return above feed and chick cost was determined by subtracting the cost of chicks and feeds from bird sales at the end of the study (Eladia and Ampode, 2021).

Statistical analysis

The data collected were subjected to a One-way Analysis of Variance (ANOVA) test using the Statistical Package of Social Science software of version 17. The results with significant differences are further compared using Tukey's Honest Significant Difference (HSD), and the differences were statistically assessed at the $p<0.05$.

RESULTS AND DISCUSSION

Growth performance

The oregano powder had a chemical composition percentage of 8.31% crude protein (CP), 4.97% crude fiber (CF), and 13.31% ash (Table 2). In the initial weight, no significant difference was observed. However, after the feeding trial, it was observed that final weight, body weight gain, average daily gain, feed intake, and feed conversion ratio were significantly affected ($p<0.05$) by the inclusion of oregano powder into the diet (Table 3). The final body weight of birds fed with oregano powder is heavier in T_4 (5% OP) than birds in T_1 (0% OP), T_2 (1% OP), and T_3 (3% OP). The same trend was observed in the body weight gain that birds in T_4 (5% OP) obtained higher body weight gain, followed by birds in T_3 (3% OP) and T_1 (0% OP). Similarly, on the average daily gain, the higher value was observed in T_4 (5% OP), followed by T_3 (3%) and T_1 (0%), and the lowest weight gain was observed in T_2 (1% OP). The voluntary feed intake of Broiler chickens fed with 5% OP (T_4) obtained a higher feed intake, and the lowest was observed in T_2 (1% OP). Also, the feed conversion ratio revealed that birds in T_4 fed with 5% OP showed better FCR than T_1 (0% OP), T_2 (1% OP), and T_3 (3% OP).

Table 2: Proximate analysis of oregano powder.

Nutrient	OP (%)
Crude protein	8.31
Crude fiber	4.97
Ash	13.31

Analyzed following the methods described by the AOAC (2016).

Table 3: Effect of oregano powder on the growth performance of broiler chicken.

Parameters (days)	Treatments					
	T1	T2	T3	T4		
	0%	1%	3%	5%	CV	p-value
Initial weight (g)	166.13±57.78	181.67±13.97	189.33±46.15	190.07±21.88	21.22	0.865^{ns}
Final weight. (g)						
15-28	788.27±33.32^{b}	790.07±20.72^{b}	843.60±19.73^{b}	959.67±33.40^{a}	3.36	0.000**
29-42	1258.20±43.14^{c}	1258.80±11.47^{c}	1368.33±28.65^{b}	1546.33±27.28^{a}	2.18	0.000**
Body weight gain (g)						
15-28	622.13±90.57^{b}	608.40±8.32^{b}	654.27±33.50^{ab}	769.60±13.26^{a}	7.71	0.014*
29-42	469.93±72.65^{b}	468.73±10.66^{b}	524.73±10.62^{ab}	586.67±10.21^{a}	6.85	0.014*
15-42	1092.07±25.26^{c}	1077.13±2.61^{c}	1179.00±23.97^{b}	1356.27±13.57^{a}	1.99	0.000**
Average daily gain (g)						
15-28	44.44±6.47^{b}	43.46±0.59^{b}	46.73±2.39^{ab}	54.97±0.95^{a}	7.71	0.014*
29-42	33.57±5.19^{b}	33.48±0.76^{b}	37.48±0.76^{ab}	41.90±0.73^{a}	6.85	0.014*
15-42	39.00±0.90^{c}	38.47±0.09^{c}	42.11±0.85^{b}	64.68±28.38^{a}	30.46	0.151*
Voluntary feed intake (g)						
15-28	956.80±22.33^{c}	917.40±24.49^{c}	1028.93±17.84^{b}	1107.87±7.63^{a}	1.96	0.000**
29-42	1046.33±.26.50^{ab}	1023.93±18.85^{b}	1043.80±8.18^{ab}	1086.00±12.42^{a}	1.53	0.017*
15-42	2003.13±31.32^{c}	1941.33±23.22^{d}	2072.73±9.82^{b}	2193.87±15.95^{a}	1.01	0.000**
Feed conversion ratio						
15-28	1.56±0.27	1.51±0.05	1.58±0.10	1.44±0.02	8.98	0.676^{ns}
29-42	2.26±0.30	2.18±0.05	1.99±0.05	1.85±0.05	7.19	0.050^{ns}
15-42	1.83±0.07^{b}	1.80±0.02^{b}	1.76±0.04^{b}	1.62±0.02^{a}	2.44	0.001*

^{abcd} Means±SD with a different superscript in the same row differ significantly: * ($p<0.05$); ** ($p<0.01$).

Cell-mediated immunity

The lymphoid organs (spleen and bursa of Fabricius) are the immune indicators, and both were not significantly affected ($p>0.05$) by the inclusion of oregano powder in the diet (Table 4). Numerically, the heaviest bursa weight was observed in birds fed with 5% OP, and the lowest weight was observed in T_2 (1% OP). The same trend was observed in the spleen weight that broiler chickens fed with 5% OP (T_4) got the heaviest weight, and the lowest weight was observed in birds fed with 0% OP (T_1) and 3% OP (T_3). Numerical values revealed that birds fed with oregano powder have higher immunity than birds without oregano powder in the diet. However, statistical analysis revealed no significant differences in the spleen and bursa indices.

Return above feed and chick cost

As shown in Table 5, broiler chickens fed with 5% OP (T_4) obtained the highest final weight with 1546.92g/bird, followed by T_3 (1368.33 g/bird), T_2 (1258.80g/bird), and T_1 got the lowest final weight of 1258.20g/bird. With the same price per kilo (Php130/kg), T_4 revealed a potential asset for the higher market with Php201.10 gross income per chicken compared to the control with Php163.56/ chicken. With this result, T_4 showed the highest return above feed and chick cost amounting to Php106.50/ chicken compared to the control with Php74.46/ chicken.

Incorporating phytobiotic plants into poultry diets as a growth promoter, antimicrobial, and antioxidant is gaining popularity worldwide. In the present study, broiler chickens fed with oregano powder exhibited a significant increase in the final weight, bi-weekly and average daily gain in weight, voluntary feed intake, and feed conversion ratio. These findings are supported by Ri et al. (2017) and Roofchaee et al. (2011), who reported that oregano powder supplementation significantly increases the average daily

Table 4: Effect of oregano powder on the lymphoid organs and cell-mediated immunity of broiler chicken

Parameters	Treatments					
(days)	T1 0%	T2 1%	T3 3%	T4 5%	CV	p-value
Bursa weight (g)	1.33±0.58	1.67±1.15	1.33±0.58	2.00±0.00	20.92	0.627^{ns}
Spleen weight (g)	1.67±0.58	1.00±0.00	1.33±0.58	2.00±0.00	27.44	0.077^{ns}
Bursa Index	0.11±0.04	0.13±0.09	0.10±0.04	0.13±0.00	9.09	0.801^{ns}
Spleen Index	0.13±0.04	0.08±0.00	0.10±0.04	0.03±0.00	25.00	0.215^{ns}

Table 5: Return above feed and chick cost of broiler chickens fed with graded levels of oregano powder.

Particulars	Treatments			
	T1	T2	T3	T4
	0%	1%	3%	5%
Final live weight kg	1258.20	1258.80	1368.33	1546.92
Price/kg in live weight (PhP)	130.00	130.00	130.00	130.00
Gross return/head (PhP)	163.56	163.54	177.88	201.10
Cost of DOC/head (Php)	31.00	31.00	31.00	31.00
Feed Consumption (kg/head)				
a. Chick Booster Mash (kg)	0.33	0.33	0.33	0.33
b. Starter ration (kg)	0.956	0.917	1.028	1.107
c. Finisher ration (kg)	1.046	1.023	1.043	1.086
Price/kg of Feed (kg)				
a. CBM (kg)	32.00	32.00	32.00	32.00
b. Starter ration (kg)	23.27	24.27	25.03	24.06
c. Finisher ration (kg)	24.18	25.5	25.09	24.32
Total Feed Cost (Php)				
a. Chick Booster Mash	10.56	10.56	10.56	10.56
b. Starter ration (kg)	22.25	22.26	25.73	26.63
c. Finisher ration (kg)	25.29	26.09	26.17.	26.41
Total Cost (Php)	89.10	89.91	93.46	94.60
RAFCC*	74.46	73.63	84.42	106.50

All costs were shown in PhP (Philippine peso); 1 USD= 50.30 PhP. * RAFCC: Return above feed and chick cost.

gain in weight and feed intake of broiler chickens. In contrast, several authors reported that oregano supplementation had no effects on the body weight gains and voluntary feed intake of broiler chickens (Karimi et al., 2010; Avila-Ramos et al., 2012; Fukayama et al., 2005). Moreover, the feed conversion ratio (FCR) in the present study indicates that broiler chickens fed with 5% oregano powder are efficient ($p<0.05$) in converting feeds into the desired output than the other treatments. The significant improvement of the FCR is supported by Ri et al. (2017), Roofchaee et al. (2011), and Alp et al. (2012), but in contrast to the investigation of Parvizi et al. (2020), that the supplementation of oregano powder did not significantly influence the FCR of broiler chickens.

The potential improvement in the growth performance of broiler chickens fed with oregano powder may be due to the modulated health status of birds, as evidenced by the improved systemic antioxidative capacity (Ri et al., 2017). Also, evidence reported that spices, herbs, and various plant extracts have appetite and digestion stimulating properties (Chun et al., 2005; Hernandez et al., 2004), which indicates that phytobiotic compounds may specifically enhance digestive enzymes and nutrient absorption (Windisch et al., 2008). Furthermore, the positive effect of oregano powder as phytobiotic feed additives in broiler diets might be due to the antimicrobial and antioxidant content of the plants (Chun et al., 2005).

In the present study, the dietary treatments did not significantly affect the cell-mediated immunity of broiler chickens. The relationship between spleen and bursa size and immune system provides solid evidence for the validity of their correlation. This implies that the bigger the size of the spleen and the bursa of Fabricius represented a strong immune system. Although the immune organ indices were not significantly affected, the weight of the spleen and bursa of Fabricius of broiler chickens fed with oregano powder is numerically higher than the control. This investigation concurs with the previous reports of (Basmacioğlu Malayoğlu et al., 2010; Alp et al., 2012; Hashemipour et al., 2013; Ri et al., 2017) but in contrast to the report of (Alagawany et al., 2018) that 240mg/kg of oregano supplementation had a significant effect on the immunity of broilers and can protect the chickens from *C. perfringens* infections. Morever, Alagawany et al. (2018) reported that oregano supplementation might be used as natural antibiotics and drugs due to the absence of side effects and residues.

The inconsistent results of the oregano powder supplementation on broiler chickens' growth performance and immunity might be due to the concentration of oregano powder incorporated in the diet and the variety of the oregano plants used in the experiment. In terms of the total expenses, broiler chickens fed with 5% OP (T_4) were reduced by 5.81 % of the total inputs. Additionally, the market shows that the income generated for this study would increase as high as 30.86% of the return above feed and chick cost. Thus, the utilization of oregano powder in poultry diets seems efficient in broiler production.

CONCLUSIONS AND RECOMMENDATIONS

The result indicates that the inclusion of oregano powder in poultry diets significantly improved the growth performance of broiler chickens. Moreover, the body weight, average daily gain, and feed conversion ratio of broiler chickens fed with 5% oregano powder showed a higher value than those fed diets without oregano powder. In cell-mediated immunity, no significant difference was observed. Still, numerical data showed that bursa and spleen weight is heavier than the lymphoid organs of broiler chickens without oregano powder in the diet. Moreover, the total expenses of broiler chickens fed with 5% oregano powder were reduced by 5.81% of the total inputs, and the income generated increases as high as 30.86% of the return above feed and chick cost. In conclusion, 5% oregano powder can be incorporated without the adverse effect on growth performance and immunity. However, more research into oregano powder as a phytobiotic feed additive in some poultry and livestock species is needed to establish the impact on carcass yield, meat quality, nutritional digestibility, lipid metabolism, and blood metabolites.

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NOVELTY STATEMENT

Several studies used oregano powder as feed supplements to determine its effect on broiler chickens' growth performance and meat quality. However, the economic traits and cell-mediated immunity were not considered. Therefore, the present study contributed scientific information in raising broilers fed with oregano powder as phytobiotic feed additives and assessed its effect on cell-

mediated immunity and economic benefits.

AUTHOR'S CONTRIBUTION

The authors contributed equally as co-first authors of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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