p-ISSN 1410-5659 e-ISSN 2621-5144
Published by Fakultas Peternakan UNPAD
Unpad Press

Jurnal Ilmu Ternak Universitas Padjadjaran, Desember 2022, 22(2):79-87
DOI: 10.24198/jit.v22i2.40047
Available online at http://jurnal.unpad.ac.id/jurnalilmuternak

Effect of Dietary Citric Acid and Microbial Phytase on the Growth Performance of Broiler Chickens

Michael S. Paras[1], Listya Purnamasari[2], Joseph F. dela Cruz[1,a]
[1]*Department of Veterinary Basic Sciences, College of Veterinary Medicine, University of the Philippines Los Baños, Philippines, 4031*
[2]*Department of Animal Husbandry, University of Jember, Jl Kalimantan 37, Jember, 68121*
[a] *email:* jfdelacruz@up.edu.ph

Abstract
Feed additives are one of the most essential parts of broiler production. Feed additives such as citric acid and microbial phytase are usually used separately despite the theory that they can work together. In this study, 320 broiler Cobb were reared and then fed four different treatments. 10 birds were reared per pen and there were 8 replications per treatment. The birds were reared using normal broiler management and health management protocols. The growth parameters and mortalities were recorded after each growth period. Production cost and the market price during the duration of the experiment were used to assess profitability. Out of all the treatments, the combination of citric acid and microbial phytase gave the highest body weight, weight gain, second-lowest feed intake, best feed conversion ratio, and the highest profitability. Results indicated that there is a synergistic interaction with the combination of 3% citric acid and 500 U/kg microbial phytases. The concurrent supplementation produced a significant increase in the growth parameters and profitability.
Keywords: broiler, microbial phytase, citric acid, growth parameters, profitability

Efek Penambahan Asam Sitrat dan Fitase Mikroba dalam Ransum terhadap Pertumbuhan Ayam Broiler

Abstrak
Pakan aditif adalah salah satu bagian terpenting dari produksi ayam pedaging. Pakan aditif seperti asam sitrat dan fitase mikroba biasanya digunakan secara terpisah meskipun ada teori yang menyatakan bahwa mereka dapat digunakan bersama. Dalam penelitian ini, 320 ekor ayam broiler dipelihara dan diberi pakan empat perlakuan berbeda dengan 8 ulangan dengan 10 ekor ayam per kandang. Ayam broiler Cobb dipelihara dengan manajemen ayam pedaging normal dan protokol manajemen kesehatan. Parameter pertumbuhan dan kematian dicatat setelah setiap periode pertumbuhan. Biaya produksi dan harga pasar selama percobaan digunakan untuk menilai profitabilitas. Dari semua perlakuan, kombinasi asam sitrat dan fitase mikroba memberikan bobot badan tertinggi, pertambahan bobot badan, konsumsi pakan terendah kedua, rasio konversi pakan terbaik, dan profitabilitas tertinggi. Hasil penelitian menunjukkan bahwa terdapat interaksi sinergis dengan kombinasi asam sitrat 3% dan fitase mikroba 500 U/kg. Suplementasi bersamaan menghasilkan peningkatan yang signifikan dalam parameter pertumbuhan dan profitabilitas.
Kata kunci: broiler, fitase mikroba, asam sitrat, performa produksi, profitabilitas

Introduction

Global food consumption is increasing as the population continuously increases and this led to higher demands for animal protein (Van Huis, 2013). Moreover, the inclusion of the ban on antibiotics in the European Commission Regulation (No.1831/2003) stopped the use of antibiotics as growth promoters due to their dangers. Consequently, this created a search for alternative growth promoters to maintain and improve broiler production. Feed additives are a wide array of substances that improves the performance of broilers. It can be categorized as antioxidants, enzymes, probiotics, prebiotics, synbiotics, antimicrobials, organic acids, coccidiostats, mycotoxin binders, immunomodulators, hen egg antibodies, hormones, beta-agonist, methane inhibitors, defaunating agents, essential oil, and herbal feed additives (Singh, 2015). Organic acids have been used as preservatives for decades, but it is also one of the alternatives that produced promising results as a growth promoter (Khan and Iqbal, 2015).

Organic acids are used as sanitizing agents to prevent bacterial infection in animals. In addition, organic acids such as citric acid have been reported to increase the feed conversion

ratio in broiler chickens due to their ability to increase the solubility of feeds and modify gastrointestinal pH (Nourmohammadi et al., 2012). Although organic acids are well known for their antimicrobial properties, they also enhance the performance of birds in ways such as the increase in digestion and absorption of nutrients, improvement in crypt-villus structure, and stimulation of secretions of the digestive tract in various ways (Al-Kassi and Mohssen, 2009). One of the most commonly available organic acids is citric acid.

Enzymes are also important to feed additives that greatly increase feed efficiency. During the 1970s, there have been studies about the use of phytases in improving phosphorus availability from plant-based feeding materials (Ravindran, 2013). It was another decade before its quantity became commercially sustainable for use. That the result of groundbreaking innovations in the biotechnology field made the production of enzymes cheap enough to be commercially distributed (Ravindran, 2013). One of these enzymes is the microbial phytase which is now widely available on the market.

According to Boling et al. (2000), citric acid and microbial phytase are hypothesized to have some, if not additive, synergistic effects on the utilization of phosphorus in broilers. Microbial phytase has been observed to have higher enzymatic activity in the range of pH 2.5 – 5.5 (Tran et al., 2011). Concurrent supplementation of microbial phytase and citric acid is theorized to increase the digestibility of minerals attached to phytic acid (Nourmohammadi et al., 2011; Karami et al., 2020). Adding increasing levels of Phytase to diets, particularly at high doses, improved tibia bone ash and P contents (Farhadi et al, 2017) Because of the increasing demand for food sources due to the rapid increase in the global population, increase efficiency in the production of food such as broiler are needed to address the increasing need. A wide array of feed additives is being used to improve efficiency (Hofmann et al., 2022). The addition of 3% citric acid and 500 U/kg of microbial phytase and their combination showed great promise in improving growth parameters and profitability in the quails (Hezaveh et al., 2020).

This study hypothesized that citric acid and microbial phytase supplementation would increase the efficiency of broiler chicken and the combination of these two additives would act synergistically to improve growth performance, nutrient utilization, bone mineralization, and gut health in the broiler chicken. This study aimed to investigate the synergistic effect of concurrent supplementation of citric acid and microbial phytase in a conventional production broiler production system.

Materials and Methods

All procedures performed on domestic chickens were approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine, University of the Philippines Los Baños (UPLB).

Feeding Management

The study employed four corn-soybean meal-based diets. Diets of the booster, broiler starter, and broiler finisher were formulated according to the feeds available in the market. All feeds that were used in this study have their respective inclusion rates of the enzyme as per the recommendation of the manufacturer. With T1 as the control group, the study employed the following corn-soybean meal-based diets: T1 = basal diet without citric acid and enzyme supplementation; T2 = basal diet with 3% citric acid; T3 = basal diet with 500 U/kg of microbial phytase; T4 = basal diet with 3% citric acid and 500 U/kg of microbial phytase.

All the broiler birds were subjected to the same practices. Ad libitum feeding was done using feeders, and a steady supply of water was provided. The birds were fed booster, starter, and finisher diets were prepared by citric acid and microbial phytase as top dressing of the basal diet at day 0 to 10, day 11 to 21, and day 22 to 35, respectively as shown in Table 1. The systems followed are normal farm broiler management and health management protocols.

Table 1. Nutrient composition (%) of broiler basal diets used in the experiment

Item	Basal booster	Basal starter	Basal finisher
Ingredients (%)			
Corn	52.2	55.9	61.7
Soybean meal	34.1	31.6	27.2
Wheat bran	2.7	2.5	2.1
Corn gluten meal	4.5	3.8	2.9
Limestone	1.1	1.0	1.0
Salt	0.25	0.25	0.25
Dicalcium phosphate	1.3	1.25	1.1
L-Lysine	0.1	0.1	0.1
DL-Methionine	0.25	0.2	0.15
Premix	1.0	1.0	1.0
Nutrient composition			
ME (kcal/kg)	3052	3104	3154
CP (%)	23.1	21.3	19.4
Lysine (%)	1.22	1.1	1.0

Experimental Design

A total of three hundred twenty (320) vaccinated Cobb day-old chicks from a commercial hatchery were used in the study. The birds' growth parameters were measured in groups of ten birds that were randomly distributed to 32 pens (2x1 m^2) with feeder and drinker for 35 days. The four treatments were randomly distributed to 32 pens following a completely randomized design (CRD) with eight replications.

Data collection

The initial chick weight was determined by weighing 10 chicks and recording the average of their weight. The growth performance was monitored per period while the survivability was monitored daily throughout the experimental period. The live weight gain was determined by subtracting the final weight by the initial chick weight per replicate per treatment (gram). The feed intake was collected by dividing the total feed consumed by the number of birds per replicate per treatment (gram). The weight gain was determined by subtracting the final weight by the initial chick weight per replicate per treatment (gram), and feed conversion ratio (FCR) were computed at the end of each growth period.

All the measurements were recorded on a pen basis and by using high-precision electronic scales. The number of birds was recorded all throughout the experiment and any dead birds were counted to determine the mortality rate of each treatment The profitability was assessed using the income over feed and chick cost (IOFCC). The cost of chick and feed consumed were recorded with the price being the average price during the duration of the experiment. In the same manner, the final weight, and the market price during the duration of the experiment were also recorded.

Data were analyzed using the General Linear Model with $P < 0.05$. The collected data were subjected to Analysis of Variance (ANOVA). The means of the treatment groups were compared using Tukey's Honest Significant Difference Test.

Results and Discussion

The average body weights, feed intake, body weight gain, feed conversion ratio, and mortalities for each growth period of the birds fed with different combinations of basal diets with citric and microbial phytase are shown in Table 2. The average initial chick weight of the different treatments ranged from 40.3- 40.4 g, and analysis showed that the initial body weights were homogenous. The initial weight of each treatment did significantly differ from one another ($P \leq 0.05$). On days 10^{th}, 21^{st}, and 35^{th}, the average total body weight of the different treatments showed that the T2, T3, and T4 were significantly heavier than the birds of the control group (T1) ($P \leq 0.05$). On the other hand, T3 and T4 were significantly heavier than the birds of T2, and T4 was significantly heavier than the birds of T3 ($P \leq 0.05$).

Feed additives such as citric acid and Microbial phytase increase the efficiency of broiler production. It results in to increase in weight gain and a decrease in feed conversion ratio. Citric acid and microbial phytase are usually used separately even if their properties are theoretically compatible in combination. In

this study, the most efficient treatment was the combination of 3% citric acid and 500 U/kg microbial phytases. Ceylan et al., (2012) reported that broilers with low phosphorus concentration due to a lack of phytase supplementation had reduced feed consumption and poorer growth performance which was consistent with the results of this experiment. According to Boling et al. (2000), citric acid and microbial phytase have some, if not additive, synergistic effect on the utilization of phosphorus in broilers that leads to increased growth performance.

The results for the supplementation of citric acid coincide with Chowdhury et al. (2009) and Khooshechin et al. (2015), where there is an increase in weight gain and feed intake. The addition of citric acid decreased the pH of the broiler feed and improved the immune status of the birds. Moreover, the increased growth response observed from the citric acid supplementation in this study is also in agreement with the results reported by Abdel-Fattah et al. (2008), where the addition of organic acids such as dietary citric acid, acetic acid, or lactic acid improved the live BW of broilers in contrast to those without supplementation. On the other hand, Nezhad et al. (2007) reported that supplementation of citric acid had no significant effect on feed intake. The increased performance may be attributed to the citric acid's effect on increasing phosphorus availability (Khooshechin et al., 2015). Afsharmanesh et al. (2005), reported that the inclusion of 0.5% citric acid increased the body weight gain, feed intake, and feed conversion ratio which is consistent with the finding of this research.

Table 2. Average Body Weight, Feed Intake, Body Weight Gain, Feed Conversion Ratio, and Mortalities from All Stages of Production

Production Stage (Days)	T1	T2	T3	T4
Average Body Weight (g)				
0	40.4±0.11	40.3±0.11	40.3±0.23	40.3±0.15
10	296.0±5.60^{a}	306.5±4.47^{b}	311.8±5.11^{b}	312.1±8.20^{b}
21	909.8±3.96^{a}	935.4±8.51^{b}	940.6±11.65^{b}	947.5±11.43^{b}
35	1688.4±14.98^{a}	1727.6±17.31^{b}	1775.3±22.31^{c}	1804.5±31.83^{c}
Average Feed Intake (g)				
0-10	303.1±11.43	304.6±5.00	302.7±5.89	304.3±9.99
10-20	971.8±11.11	982.1±6.90	975.5±13.93	974.1±10.53
21-35	1973.9±12.81^{a}	1962.1±15.70^{ab}	1940.6±28.52^{b}	1942.4±24.69^{b}
0-35	3250.3±16.12^{a}	3248.0±12.99^{ab}	3217.6±31.96^{c}	3219.8±21.57^{bc}
Weight Gain (g)				
0-10	255.6±5.57^{a}	266.2±4.54^{b}	271.5±5.19^{b}	271.83±8.07^{b}
10-20	613.8±7.91^{a}	628.9±9.22^{ab}	628.9±14.92^{ab}	635.38±13.36^{b}
21-35	778.6±16.01^{a}	792.3±19.19^{a}	834.6±31.66^{b}	857.0±39.33^{b}
0-35	1684.0±14.97a	1687.3±17.33b	1735.0±22.45c	1764.2±31.9c
Feed Convertion Ratio				
0	1.19±0.05^{a}	1.14±0.02^{ab}	1.11±0.03^{b}	1.12±0.04^{b}
10	1.58±0.02^{a}	1.56±0.02^{ab}	1.55±0.05^{ab}	1.53±0.03^{b}
21	2.54±0.06^{a}	2.48±0.05^{a}	2.33±0.07^{b}	2.27±0.11^{b}
35	1.97±0.02^{a}	1.93±0.02^{b}	1.85±0.02^{c}	1.83±0.04^{c}
Mortalities (%)				
0-10	0	0	0	0
10-20	0	0	0	0
21-35	0	0	0	0
0-35	0	0	0	0

$^{a\ b\ c}$column means with different superscripts significantly difference (P<0.05)

The average weight gain for each growth period of the birds fed with different combinations of basal diets with citric and microbial phytase are shown in Table 1. On days 0 to 10, the average weight gain of the birds fed with different treatments observed that T2, T3, and T4 have significantly higher weight gain than the birds of T1 ($P \leq 0.05$). From day 11th to 21st, T4 have significantly higher weight gain than the birds of T1 ($P \leq 0.05$). On days 21 to 35, T3 and T4 have significantly higher weight gain than the birds

of T1 and T2 ($P \leq 0.05$). On the whole operation from day 0 to day 35, T2, T3, and T4 have significantly higher weight gain than the birds of T1, while T3 and T4 have significantly higher weight gain than the birds of T2 ($P \leq 0.05$).

The average feed consumption for each growth period of the birds fed with different combinations of basal diets with citric and microbial phytase are shown in Table 1. There were no significant differences on days 0 and 10. However, on day 21, the average feed consumption of the different treatments demonstrated that T3 and T4 have significantly lower feed consumption than T1, and T3 has significantly lower feed intake than T2 ($P \leq 0.05$). On the 35th, T3 and T4 have significantly lower feed consumption than T1, while T3 has significantly lower feed intake than T2 ($P \leq 0.05$).

Feed consumptions were lower with treatments containing enzymes and even lower for the combined organic acid and enzyme. This can be attributed to the ability of organic acid to increase digestion and absorption of nutrients, improvement in crypt-villus structure, and stimulation of secretions of the digestive tract (Omogbenigun et al., 2003). One of the commonly used organic acids in the poultry industry is citric acid (Al-Kassi and Mohssen, 2009). Moreover, the microbial phytase further supported the results of the current 10 experiments since it stimulates the utilization of bound phosphorus present in phytic acids of basal diets (Oatway et al., 2001). Chowdhury et al. (2009), reported that supplementation of citric acid increased the feed intake of broilers which is in line with the results of this experiment. Dos Santos et al. (2013), and Gautier et al. (2018), both reported an increase in feed intake for phytase supplementation which is consistent with the findings of this experiment.

The average feed conversion ratio for each growth period of the birds fed with different combinations of basal diets with citric and microbial phytase are shown in Table 1. On day 10, the average FCR of the different treatments was analyzed and T4 has significantly better FCR than the birds of T1($P \leq 0.05$). On the 21st day, T3 and T4 have significantly better FCR than the birds of T1 and 2 ($P \leq 0.05$). On the other on day 35, T2, T3, and T4 have significantly better FCR than the birds of T1, while T3 and T4 have significantly better FCR than the birds of T2 ($P \leq 0.05$).

The feed conversion ratio of the treatments containing microbial phytase was better. Microbial phytase is known to help in increasing mineral availability in feeds (Nourmohammadi et al., 2011). The FCR of the concurrent supplementation of citric acid and microbial phytase were even higher since citric acid's pH is in the range of 3-5 (Waseem et al., 2016), which increases the enzymatic activity of microbial phytase which is higher at the range of pH 2.5 – 5.5 (Tran et al., 2011). Moreover, concurrent supplementation of microbial phytase and citric acid is theorized to increase the digestibility of minerals attached to phytic acid (Nourmohammadi et al., 2011). The concurrent supplementation of the organic acid and enzyme increases the availability of nutrients for the birds' use. The improvement in the growth performance such as body weight is consistent with the results of the works published by Santos et al. (2008) that observed better performance with the addition of phytase. The addition of phytase led to increased utilization of nutrients, protein, minerals, and energy in the poultry diet. In contrast to the results of the experiment, Gautier et al., (2018) reported that there was no increase in gain to feed ratio due to phytase supplementation. Dos Santos et al. (2013), reported results that are more in line with the result of this experiment, that there is an improved gain to feed ratio with the inclusion of phytase, the difference of which may be attributed to management practices, environmental conditions, bird genetics, and feed ingredients.

The results of increased body weight coincide with the results of phytase supplementation by Gautier et al. (2018), phytase supplementation increased the body weight of broiler birds but there was no increase in feed efficiency. The result that phytase supplementation increased BW gain of birds, it must also be noted that those results were observed regardless of dietary mineral concentration, which is due to the ability of this exogenous phytase in the liberation of nutrients and minerals from phytate.

Among all treatments, the basal diet with the combination of citric acid and microbial phytase has shown a consistently good weight gain, feed intake, survivability, and feed conversion ratio. Along with the growth parameters, it also showed the highest

profitability. The concurrent supplementation is an effective combination that increases the production performance and profitability of broilers. This is consistent with the findings of Nezhad et al. (2011), where there was a significant interaction between citric acid and microbial phytase that increase the digestibility of Calcium and Phosphorus. This can be attributed to the ability of citric acid to increase the utilization of P (Brenes et al., 2003), while microbial phytase increased the digestibility of P (Nezhad et al., 2011). Moreover, Deepa et al. (2011) and Nourmohammadi et al. (2010), reported that concurrent supplementation of citric acid and microbial phytase increases body weight gain, and feed conversion ratio which is consistent with the current experiment. In contrast, Woyengo et al. (2010) reported that the addition of citric acid to phytase-supplemented diets did not affect body weight gain, but the researchers also mentioned that the addition of citric acid to low-P and Ca broiler diets can improve nutrient digestibility and retention. The difference in findings may be attributed to the concentration of no phytate phosphorus present in the basal diet. Snow et al. (2004), reported that there is improved growth performance and bone mineralization in concurrent supplementation of citric acid and microbial phytase on low phosphorus broiler diets.

The number of birds from the start was the same until the end (Table 2) of the experiment. Since there were no recorded mortalities, all treatments have 100% survivability. The profitability of Philippine Peso (PhP) (Table 3) was computed afterward, and it was shown that the income over feed and chick cost of T4 is the highest with PhP 34.995 followed by PhP 33.958 (T3), PhP 30.694 (T2), and PhP 28.956 (T1).

Table 3. Income Over Feed Cost

Parameters	T1	T2	T3	T4
Chick cost	27.00	27.00	27.00	27.00
Feed intake cost	62.23	63.24	63.31	64.32
Average Live Weight	1688.38	1727.63	1775.25	1804.05
Live Weight	70.00	70.00	70.00	70.00
Price	118.19	120.93	124.27	126.32
IOFCC	28.96	30.69	33.96	35.00

IOFC = Income Over Feed Cost; $^{a\,b\,c}$column means with different superscripts significantly difference ($P<0.05$)

In a farm setting with a higher scale of operation, 100% survivability is nearly impossible to achieve. It only implies that the treatments are not associated with the survivability of the broiler birds. Survivability still depends on proper management. However, in the study of Waldroup et al. (1999) and Vieira et al. (2008), results showed that birds that had a deficiency in phosphorus and lack of phytase supplementation resulted to higher mortality which is different from the results of the experiment that suggest that there is no association between survivability and the treatments.

In this study, there is high income over feed and chick cost in the combination. The additional cost from microbial phytase and citric acid is negligible in comparison to the profit gained from its addition. In the setting of conventional farming, this study has a high potential to help backyard broiler farmers increase their profit.

Conclusion

In conclusion, the combination of 500U/kg Microbial Phytase and 3% citric acid had shown consistent results in its growth parameters, survivability, and profitability. Based on this result, the citric acid-microbial phytase treatment has yielded more than just the individual supplementation of citric acid or microbial phytase. There was a synergistic interaction between the citric acid and microbial phytase that led to the increase in body weight gain, feed intake, feed conversion ratio, and profitability.

References

Abdel-Fattah, S. A., EI-Sanhoury, M. H., EI-Mednay, N. M., & Abdul-Azeem, F. (2008). Thyroid activity of broiler chicks fed supplemental organic acids. *International Journal of Poultry Science*, 7(3): 215–222.

http://dx.doi.org/10.3923/ijps.2008.215.222

Afsharmanesh, M., & Pourreza, J. (2005). Effects of calcium, citric acid, ascorbic acid, vitamin D3 on the efficacy of microbial phytase in broiler starters fed wheat-based diets. *International Journal of Poultry Science*, 4(6): 418-424. https://dx.doi.org/10.3923/ijps.2005.418.424

Al-Kassi, A. G., & Mohssen, M. A. 2009. Comparative Study Between Single Organic Acid Effect on Broiler Performance. *Pakistan Journal of Nutrition*, 8(6): 896-899. Doi:10.3923/pjn.2009.896.899. https://dx.doi.org/10.3923/pjn.2009.896.899

Boling, S. D., Webel, D. M., Mavromichalis, I., Parsons, C. M., & Baker, D. H. (2000). The effects of citric acid on phytate-phosphorus utilization in young chicks and pigs. *Journal of Animal Science*, 78(3): 682-689. 13 https://doi.org/10.2527/2000.783682x

Boling, S. D., Douglas, M. W., Johnson, M. L., Wang, X., Parsons, C. M., Koelkebeck, K. W., & Zimmerman, R. A. (2000). The effects of dietary available phosphorus levels and phytase on performance of young and older laying hens. *Poultry Science*, 79(2): 224–230. https://doi.org/10.1093/ps/79.2.224

Brenes, A, Viveros, A., Arija, I., Centeno, C., Pizarro, M., & Bravo, C. (2003). The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. *Animal Feed Science and Technology*, 110(1): 201-219. https://doi.org/10.1016/S0377-8401(03)00207-4

Ceylan, N, Cangir, S., Corduk, M., Grigorov, A., & Adabi, S. H. G. (2012). The effects of phytase supplementation and dietary phosphorus level on performance and on tibia ash and phosphorus contents in broilers fed maize-soya-based diets. *Journal of Animal and Feed Science*, 21: 696–704 https://doi.org/10.22358/jafs/66142/2012

Chowdhury, R., Islam, K. M. S., Khan, M. J., Karim, M. R., Haque, M. N., Khatun, M., & Pesti, G. M. (2009). Effect of citric acid, avilamycin and their combination on the performance, tibia ash and immune status of broiler chicks. *Poultry Science*, 88(8): 1616-1622. https://doi.org/10.3382/ps.2009-00119

Deepa, C., Jeyanthi, G. P., & Chandrasekaran, D. (2011). Effect of Phytase and Citric Acid Supplementation on the Growth Performance, Phosphorus, Calcium and Nitrogen Retention on Broiler Chicks Fed with Low Level of Available Phosphorus. *Asian Journal of Poultry Science*, 5(1): 28-34 http://dx.doi.org/10.3923/ajpsaj.2011.28.34

Dos Santos, T. T., Srinongkote, S., Bedford, M. R., & Walk, C. L. (2013). Effect of high phytase inclusion rates on performance of broilers fed diets not severely limited in available phosphorus. *Asian-Australian Journal of Animal Science*, 26(2): 227–232. https://doi.org/10.5713%2Fajas.2012.12445

European Commission. 2003. REGULATION (EC) No 1831/2003 of the European Parliament and of the Council. Retrieved August 18, 2019, from https://eur-lex.europa.eu/LexUriServ/LexUriServ.do ?uri=OJ: L:2003:268:0029:0043: EN:PDF

Farhadi, D., Karimi, A., Sadeghi, Gh, Rostamzadeh, J., & Bedford, M. R. (2017). Effects of a high dose of microbial phytase and Myo-inositol supplementation on growth performance, tibia mineralization, nutrient digestibility, litter moisture content, and foot problems in broiler chickens fed phosphorus-deficient diets. *Poultry Science* 96: 3664-3675

Gautier, A.E., Walk, C. L., & Dilger, R. N. (2018). Effects of a high level of phytase on broiler performance, bone ash, phosphorus utilization, and phytate dephosphorylation to inositol. *Poultry Science*, 97(1): 211–218 https://doi.org/10.3382/ps/pex291

Hezaveh, M. S. S., Ghazemi, H. A., Hajkhodadi, I., & Moradi M. H. (2020) Single and combined effects of phytase and citric acid on growth performance, nutrient digestibility, bone characteristics, intestinal morphology, and blood components in meat-type quails fed low-phosphorous diets. *Animal Feed Science and Technology* 269 https://doi.org/10.1016/j.anifeedsci.2020.114677

Hofmann, P., Krieg, J., Francesch, M., Feuerstein, D., & Rodehutscord, M. 2022. Effects of added phytase on growth performance, carcass traits, and tibia ash of broiler chickens fed diets with reduced amino acid, crude protein, and phosphorus concentration. *Journal of Applied Poultry Research* 31(3) https://doi.org/10.1016/j.japr.2022.100258

Karami, M., Karimi, A., Sadelghi, A., Zentek, J., & Goodarzi, B. (2020). Effects of phytase and benzoic acid supplementation on growth performance, nutrient digestibility, tibia mineralization and serum traits in male broiler chickens. *Livestock Science* 242 https://doi.org/10.1016/j.livsci.2020.104258

Khan, S., & Iqbal, J. (2015). Recent advances in the role of organic acids in poultry nutrition. *Journal of Applied Animal Research*. 44(1): 359-369. http://dx.doi.org/10.1080/09712119.2015.1079527

Khooshechin, F, Hosseini, S. M., & Nourmohammadi, R. (2015). Effect of Dietary Acidification in Broiler Chickens: Growth Performance and Nutrients Ileal Digestibility. *Italian Journal of Animal Science*, 14(3). 3885, http://dx.doi.org/10.4081/ijas.2015.3885

Mirza, M. W., Rehman, Z. U., & Mukhtar, N. (2016). Use of Organic Acids as Potential Feed Additives in Poultry Production. *Journal of World's Poultry Research*, 6(3): 105-116

Nezhad, Y. E., Gale-Kandi, J. G., Farahvash, T., & Yenageh, A. R. (2011). Effect of combination of citric acid and microbial phytase on digestibility of calcium, phosphorous and mineralization parameters of tibia bone in broilers. *African Journal of Biotechnology*, 10(66): 15089-15093 http://dx.doi.org/10.5897/AJB10.2553

Nezhad, Y. E., Shivazad, M., Nazeeradl, M., & Babak, M. M. S. (2007). Influence of citric acid and microbial phytase on performance and phytate utilization in broiler chicks fed a corn-soybean meal diet. *Journal of Veterinary Research*, 61(4): 407–413.

Nourmohammadi, R, Hosseini, S. M., & Farhangfar, H. (2010). Influence of citric acid and microbial phytase on growth performance and carcass characteristics of broiler chickens. *American Journal of Animal and Veterinary Sciences*, 5(4): 282-288. http://dx.doi.org/10.3844/ajavsp.2010.282.288

Nourmohammadi, R, Hosseini, S. M., & Farhangfar, H. (2011). Effect of citric acid and microbial phytase on serum enzyme activities and plasma minerals retention in broiler chicks. *African Journal of Biotechnology*, 10(62): 13640- 3650. http://dx.doi.org/10.5897/AJB11.1744

Nourmohammadi, R, S Hosseini, S. M., Farhangfar, H, & Bashtani, M. (2012). Effect of citric acid and microbial phytase enzyme on ileal digestibility of some nutrients in broiler chicks fed corn-soybean meal diets. *Italian Journal of Animal Science*, 11(1): 36-40. http://dx.doi.org/10.4081/2326

Oatway, L., Vasanthan, T., & Helm, J. H. (2001). Phytic acid. *Food Reviews International*, 17(4): 419–431. https://doi.org/10.1081/FRI-100108531

Omogbenigun, F. O., Nyachoti, C. M., & Slominski, B. A. (2003). The effects of supplementing microbial phytase and organic acids to a corn-soybean based diet fed to early weaned pigs. *Journal of Animal Science*, 81(7): 1806-1813 https://doi.org/10.2527/2003.8171806x

Santos, F. R., Hruby, M., Pierson, E. E. M., Remus, J. C., & Sakomura, N. K. (2008). Effect of phytase supplementation in diets on nutrient digestibility and performance in broiler chicks. *Journal of Applied Poultry Research*, 17(2): 191–201. https://doi.org/10.3382/japr.2007-00028

Singh, P. (2015). *Animal Feed Additive*. Vikas Surya Plaza, CU Block, L.S.C. Market, Pitam Pura, New Delhi: New India Publishing Agency.

Snow, J. L., Baker, D. H., & Parsons, C. M. (2004). Phytase, citric acid, and 1α-hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a corn-soybean meal diet. *Poultry Science*, 83(7): 1187–1192. https://doi.org/10.1093/ps/83.7.1187

Tran, T. T., Hatti-Kaul, R., Dalsgaard, S., & Yu, S. (2011). A simple and fast kinetic assay for phytases using phytic acid–protein complex as substrate. *Analytical*

Biochemistry, 410: 177–184. https://doi.org/10.1016/j.ab.2010.10.034

Van Huis, A. (2013). Assuring Food Security. *Annual Review of Entomology*, 58, 563-583. https://doi.org/10.1146/annurev-ento-120811-153704

Vieira, S. L., Anschau, D. L., Stefanello, Serafini, N. C., Kindlein, L., Cowieson, A. J., & Sorbara, J. O. B. 2015. Phosphorus equivalency of a Citrobracter braakii phytase in broilers. *Journal of Applied Poultry Research*, 24: 335–342. https://doi.org/10.3382/japr/pfv033

Waldroup, P. (1999). Nutritional approaches to reducing phosphorus excretion by poultry. *Poultry Science*, 78: 683–691. https://doi.org/10.1093/ps/78.5.683

Woyengo, T. A., Slominski, B. A., & Jones, R. O. (2010). Growth performance and nutrient utilization of broiler chickens fed diets supplemented with phytase alone or in combination with citric acid and multi carbohydrase. *Poultry Science*, 89(10): 2221-2229 https://doi.org/10.3382/ps.2010-00832