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The Performance of Quantum Blue Phytase at Different pH Levels Using Wheat Based Diet in an In Vitro Simulation of Poultry Crop

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Abstract

An experiment was carried out to investigate the impact of different dietary pH levels as stimulate the efficacy of Quantum blue phytase in an in vitro simulation of poultry crop. The experiment consisted of two parts; an acid trial and an in vitro experiment.

In the acid trial, four organic acids; propionic acid, formic acid, acidic acid and citric acid were tested separately on two kinds of diet mixtures that made by 4g of barley-based diet or wheat-based diet with 12ml distilled water. Water to feed ratio was 3:1. Each acid was titrated on a diet modal to reach either pH 5.5, 4.5 or 3.5, respectively. The required dosages were noted. Then, modals prepared with corrected pH (5.5, 4.5 and 3.5) were incubated in 40 ℃ water bath for 1 hour. The pH ranges were recorded during incubation at 5min, 10min, 30min and 1hour to observe acids stabilities. As result, the formic acid was applied in the in vitro experiment.

In the in vitro experiment, the treatments were divided into four groups according to the pH values (pH 6.2, 5.5, 4.5 and 3.5). At each group, identical 6 models (include 2 control groups) were prepared by 4 g ground wheat-based diet with 12ml distilled water. The diet models were then corrected the pH by formic acid with 0 µl (pH6.2), 6.5 µl (pH5.5), 43 µl (pH 4.5) and 170 µl (pH3.5), respectively. After that, 4000 µl of 5000 FTU/g Quantum blue phytase was added in each model (except from control groups), mixed well, vortexed and incubated in thermal bath 40 ℃ for 2 hours with shaken every five minutes. The pH measuring was done occasionally during incubation. Samples were collected from each modal during incubation at 10, 30, 60, 90 and 120 min. Then, all the 120 samples were stored in freezer at -21 ℃ and analysed the phosphate concentrates by colorimetric method.

The findings showed that the Quantum blue phytase have a better efficacy at a lower pH condition at pH 3.5 than at higher. The capability of the phytase showed a significant increase at pH 3.5 during 30min and 1hour, and no further increase after 1 hour.

Key words: in vitro, Quantum blue phytase, crop, pH

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1 Introduction

Generally, the cereal, beans and oilseed crops are the main sources of feed for monogastric animals which contain sufficient phosphorus (P) (Elkhalil, et al., 2007). However, phytic acid (Myo-inositol (1,2,3,4,5,6-) hexaphosphoric acid, IP6) (Fig.1) is the main storage form of phosphate in seeds (Raboy, 2003) and accounts for 60- 90% of total phosphorus (Loewus, 2002). The phytate that formed during the ripening period is a salt form of Phytic acid and known for its poor phosphorus availability and anti-nutritive properties in dietary utilization. In feed ingredients, the IP_6 probably exist as mineral-bound complexes involving magnesium, calcium and potassium (Reddy, et al., 1982).

The addition of inorganic P in poultry diet is commonly used to meet the nutrition requirement. However, the inorganic phosphorus is an expensive ingredient in poultry feed industry. In addition, a large amount of P in poultry litter is an environmental concern. Selle and Ravindran (2007) mentioned that because of poultry worldwide consume one million tonnes of phytate-P annually, the improvement on the utilization of phytate-P will contribute to preserving the global P reserves.

Since 1990's, the phytase feed enzyme has been widely available in the poultry industry (Dersjant-Li, et al., 2015). Researchers have revealed that the lower availability of phytate-P in poultry is due to the limited inherent phytase activity (Selle & Ravindran, 2007). It has been reported that phytase addition in birds' diet has a significant effect on plant P utilization and reduction of P excretion (Svihus, et al., 2013). The crop is reported to be the main site of degradation by exogenous phytase (Selle & Ravindran, 2007). Apart from phytase application, the feeding regime such as reducing P-phase feeding, and precision feeding are also used to improve the utilization of feedstuff P (Humer, et al., 2015).

The current study aimed to reveal how the efficiency of exogenous phytase (Quantum blue) changes at different pH conditions. The in vitro experiment simulated a poultry crop using a wheat-based diet. Also, the experiment exhibited how the time and pH interact on the phytase performance. The hypothesis for present study was that the efficacy of exogenous phytase would improve at a lower dietary pH.

2 Literature review

2.1 Phytase

Phytases are commonly occurring in plants, microorganisms and in some animals. They are enzymes that hydrolysis the ester bonds between the phosphate groups and the inositol ring of phytate and enhancing the availability of dietary phosphate (Pirgozliev, et al., 2008). The exogenous microbial phytases are isolated from numerous bacteria, yeast and fungi (Harland & Morris, 1995). Their advantages including high activity and simple production process makes the microbial phytase widely investigated and applied in production (Dvořáková, 1998).

Phytase activity is defined as phytase units (FTU), where one FTU is the amount of phytase that realise 1 μmol inorganic phosphorus/min from 0.00512 mol/L sodium phytate at pH 5.5 and at a temperature of 37 °C (Selle & Ravindran, 2007). The phytase efficiency is affected by several factors including pH stability, temperature stability and moisture content (Humer, et al., 2015).

Except for exogenous supplementation, poultry have other possible sources of phytase. The endogenous phytase produced by the small intestine mucosa, microbial phytase mainly in the large intestine and intrinsic plant phytase from some feedstuff are important sources (Selle & Ravindran, 2007). However, some previous research (Humer, et al., 2015) reported that the intestinal mucosal phytases in monogastric organisms are incapable of hydrolysing sufficient amounts of phytate-bound P. In comparison to the endogenous mucosa phytases, microfloral phytases are more capable of hydrolyzing phytate-P. In a research from Kerr et al. (2000), microfloral phytases are considered to have some influences in further hydrolyzing the undigested phytate-P. The plant phytases are reported less effective at a low pH and more susceptible during feed processing by proteolytic digestion and thermal destruction (Noraini, 2007).

The benefits of exogenous phytase on poultry diet have been reported by many authors. Besides their significant effect on P utilization and reduction of p pollution (Svihus, et al., 2013), they improve feed intake and feed efficiency for chicken (Lü, et al., 2009). Moreover, the weight gain was observed significantly to increase in the broiler fed phytase diet (Pirgozliev, et al., 2008). The phytase also positively affected the availability of minerals such as calcium, magnesium, potassium, zinc and amino acids, especially threonine (Rutherfurd, et al., 2012). Dersjant-Li, et al. (2015) stated that the phytase can reduce the antinutritional effect of phytate and improve the availability of calcium, amino acids and energy. In addition, Warden and Schaible (1962) show that the phytase addition enhances bone mineralisation in broiler chicks.

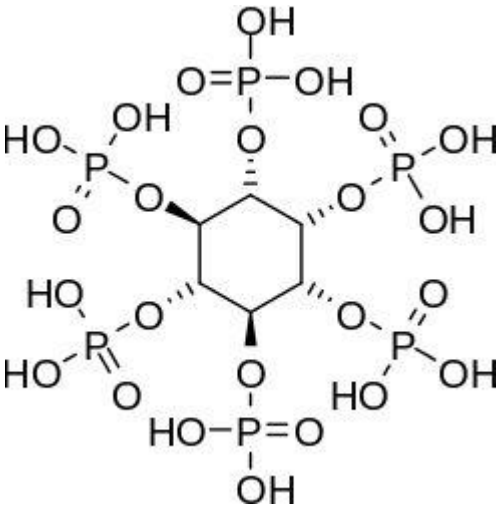

Figure 1. Structure of phytic acid (myo-inositol, 1,2,3,4,5,6- hexaphosphoric acid IP6).

2.2 Interaction of crop and exogenous phytase

Based on previous finding, it is likely that phytate activity mainly takes place in the fore-stomach (crop, proventriculus, gizzard) where the pH is more conducive to phytate activity (Selle & Ravindran, 2007). Same study also pointed out that the crop is the most likely primary site of degradation by exogenous phytase. However, when

analysing the active site for phytase, the type of bacteria the phytase is derived from should be considered. For example, *E.coli* derived phytase is more active in the small intestine than phytase derived from *P.lycii* (Selle & Ravindran, 2007; Onyango, et al., 2005).

The soaking and retention time of exogenous phytase in crop is considered as a limiting factor for enzyme utilization (Svihus, et al., 2011). However, the situation of diet in a crop is various regarding pH level, moisture content and retention time. The retention time in the crop is around 50min, but the time varies greatly (Dänicke, et al., 1999). A study from Svihus, et al. (2013) reported that the retention time decreases with age due to increased feed intake. In addition, some previous finding (Svihus, et al., 2011) also suggested that the content of crop is highly moistened up to 50%, within 90 min.

The intermittent feeding has considerable benefits on increase phytase efficiency through improve crop utilization. The crop is mainly a storage organ of birds when intermittent feeding and have no digestion activity (Jackson & Duke, 1995). And studies have shown that during intermittent feeding, the crop contents have significantly increased compared with those of the ad libitum broilers (Amponsem, et al., 1991). In addition, experiments from Svihus *et al*. (2010) expressed that the intermittent feeding broilers, eat a large amount of diet in a short time and use their anterior digestive tract as storage organ, while the ad libitum fed broilers do not have a habit to use the crop that much. Moreover, Barash *et al.* (1993) found that the intermittent feeding can increase the weight content and feed holding capacity of both crop and gizzard of chicks.

A previous research (Svihus, et al., 2010) reported when retention was stimulated through intermittent feeding, a considerable IP6 degradation was observed in the crop. A similar finding said that the feed content retention in the crop can be increased by intermittent feeding and structural components in the diet (Svihus, et al., 2011). In conclusion, it is assumed that the intermittent feeding and structural components contribute to lengthening the retention time of feed contents in the crop that have a

proper acidic condition, thus, improve the utilization of both enzyme and feeds (Svihus, et al., 2013).

2.3 The feed pH

It is suggested that the pH in the crop will be similar to the diet enters in the crop (Svihus, et al., 2011). The same study also mentioned that the diet pH of monogastric animals usually reported to varying between 5.5 and 6.5.

3 Material and method

3.1 Diets and diet compositions

Diets used in the current experiment were produced at Centre of Feed Technology (ForTek), at the University of Life Science (NMBU), Aas, Norway. The detailed compositions of barley-based diet were given in table 1, wheat-based diet in table 2 (Sacranie, et al., 2017).

In the acids assay conducted before the in vitro experiment, both two diets were used. Whereas, at the *in vitro* digestion experiment, only the wheat-based diet was applied.

3.2 Acids and acid trials

Four acids were tested; the propionic acid (SIGMA-ALDRICH, Germany), formic acid (Merck, Finland), acidic acid (Merck, Germany) and citric acid (E.Merck, Germany).

There were two types of diet mixtures, made by barley-based diet and wheat-based diet, respectively. Each modal was made by the mixture of 4 g of the corresponding type of feed and 12ml distilled water. Water to feed ratio (3:1) was chosen based on Lyberg et al. (2006).

The acid tests were applied to identify the required dosage of acids and their stabilities. Each acid was titrated on a diet modal to reach either pH 5.5, pH 4.5 or pH 3.5, respectively. The required dosages were then noted. After that, made models with the three levels of corrected pH and incubated them in a water bath at 40℃ for 1 hour. This was for observing the stabilities of acids. The pH readings were recorded after 5min, 10min, 30min and 1 hour during incubation. A VWR pH-meter (Radnor, Pennsylvania, US) was used.

3.3 The in vitro experiments

3.3.1 The Agency

The Quantum Blue Phytase (5000 FTU/kg) provided by AB Vista, Rajamäki, Finland was applied in the in vitro experiment. The general information of the phytase is listed in table1. While, the 4% TCA solution was used as a stop agent. The solution was prepared by solid Trichloroacetic acid (TCA, Applichem) dissolved in proper amount of distilled water.

Table 1. The phytase information

Producer	AB Vista
Commercial name	Quantum® Blue
Donor organism	E. coli
Formulation	Liquid

3.3.2 The experimental procedure

In the in vitro experiment, the feed models were divided into four groups according to the four pH values; 6.20 (original), 5.50, 4.50, and 3.50. At each group, identical 4 samples of ground wheat-based diet (4g) were weighted into a 20ml glass bottle with screw caps. The feed models were then mixed well with 12ml distilled water. After that, the feed mixtures have corrected the pH by adding formic acid 0 µl (pH 6.20), 6.5 µl (pH 5.50), 43 µl (pH 4.50) and 170 µl (pH 3.50), respectively. Then 4000 µl of 5000 FTU/g Quantum blue was added to each bottle, mixed well, vortexed and incubated in the thermal bath (TECHEN, UK) at 40 ℃ for 2 hours with shaken every

five minutes. The step of the procedure simulated digestion in poultry crop. The pH measuring (by pH meter, WTW, Germany) was done occasionally during incubation to ensure there are no wide swings from the corrected pH levels.

3.3.3 Control groups

For each of the four experimental groups, there were made two diet models in control group. Thus, there were 8 control models in total. The same method and solutions were applied on the control group diets, except for Quantum blue phytase were not added.

3.3.4 Sample collection

Samples were collected from each model during incubation at 10, 30, 60, 90 and 120 min. When time was up, 0.5 g of sample was weighted into 1ml microcentrifuge tube that already contained 0.5ml stop agent inside (4 % TCA solution). Those taken at the same time were following equilibration for within one minute. The resulting suspensions were then centrifuged (centrifuge, Hettich, Germany) at 3000rpm for 5min. After that, they were placed in a freezer at -21 ℃. There were 120 samples in total.

Table 2. Composition and nutrient content of barley-based diet

Ingredients	Diet 1 (g/kg)
Barley	660
Fish meal	90
Soy bean meal	184
Soy oil	30
Lime stone	10
Mono calcium phosphate	10
DL-Methionine	2
L-Threonine	1
Salt	2.5
Mineral	
Premix.	1.5
Vitamins	
A	0.5
ADKB	1
D_3	0.8
E	0.5
Choline chloride	1.2
Titanium dioxide	0.5

Table 3. Composition and nutrient content of the wheat-basal diet (g/kg unless otherwise stated)

Ingredient	wheat-based
Fishmeal	
Barley	
Wheat	529.5
Soybean meal, 440 g of CP/kg	200.0
Rapeseed meal, 380 g of CP/kg	80.0
Rice bran	60.0
Oat hulls or cellulose powder	50.0
Soy oil	40.0
Limestone	14.0
Salt	1.8
Sodium bicarbonate	2.6
Mineral premix	1.3
Vitamin premix	2.6
L-lysine, 780 g/kg	3.0
DL-methionine, 990 g/kg	2.0
L-threonine, 985 g/kg	2.0
Titanium dioxide	5.0
Enzyme premix [1]	5.0
Choline chloride	1.2
Nutrient content	
Calculated AMEn (MJ/kg)	11.8
Analysed crude protein	188
Analysed starch	345
Calculated lysine	12
Calculated methionine	9
Calculated threonine	6
Calculated calcium	7.1
Analysed P	4.4
Analysed IP6	12.5
Calculated non-phytate P	1.6

[1]Xylanase (Econase XT25 (32 000 XBU/kg) was used in all diets.

3.3.5 Analysis of phosphate concentrate

The analysis of phosphate concentration was done by a colorimetric method after all the 120 samples were collected. Firstly, ten times diluted supernatants were prepared. From each sample, 100 µl supernatants were taken and diluted by 900 µl distilled water into a 1ml microcentrifuge tube. Then, from the diluted modal, 100 µl sample were taken and mixed with 240 µl colour reagent into the hole of a microtiter plate. After all the samples were processed, the plate was covered and the sample shaked in

a rotary shaker for 5 minutes, followed by placing it into an incubator (Termaks, Bergen, Norway) at 37 ℃ for 1 hour. The phosphate concentration was then quantified by using a multi-mode microplate reader (Spectra Max M2e).

A colour reagent was made fresh each day. There were two solutions used to make the colour reagent. Solution A was prepared by dissolving ascorbic acid solution (4.5818g Ascorbic acid and 45ml distilled water) with 2.7ml of concentrated sulphuric acid and made the volume to 100ml. Solution B was made from 2.5g of ammonium molybdate dissolved in 40 ml distilled water and made up the volume to 50 ml by volumetric flasks. The colour reagent was the mixture of Solution A, solution B and distilled water in a ratio of 5:1:10. To do this, always add solution B to solution A and add the water at last.

Phosphate standards were made from the 7.2 mM stock and reaction buffer. All weights were recorded to calculate the amount of phosphate correctly in a single standard; the details were listed in table3.

For the agent tween 20 and reaction buffer preparation, after 5g of Tween was completely dissolved in 40ml of distilled water, filled the volume to 50ml, which was Tween 20 solution. Whereas, the reaction buffer was made by 3.75g of glycine dissolved in 1800ml distilled water and then added 0.2ml of 10% Tween. The pH was adjusted to 3 with 6M HCI and the volume filled to 2000ml.

Potassium phosphate stock (7.2mM) preparation. Dry KH_2PO_4 in a vacuum oven at 100-103 ºC for two hours. Subsequently, 0.976 g of them were dissolved in 1L reaction buffer and match the pH to 3 with 6M HCI or 4M NaOH.

Table 4. The concentrations of phosphate standards

Standard	Volume of buffer (ml)	Volume of 7.2mM phosphate stock(ml)	Concentration standard (mM)
STD 0	20	0.00	0
STD 1	19.95	0.05	0.018
STD 2	19.80	0.20	0.072
STD 3	19.60	0.40	0.144
STD 4	19.40	0.60	0.216
STD 5	19.00	1.00	0.36
STD 6	18.00	2.00	0.72
STD 7	16.00	4.00	1.44

3.3.6 The calculation

The data measured from microplate reader is the phosphate concentrate that expressed in mmol/L. The unit was changed to mg/g using the phosphate molecular mass 94.9714 g/mol. The phosphate concentrate based on feed was then calculated in below.

$-PO_4$ concentrate based on dry feed = $\frac{phosphate\ concentrate\ in\ each\ sample}{dry\ feed\ content\ in\ each\ sample}$ (mg/g)

Phosphate concentrate in each sample =measured phosphate concentrates (mg/g) * weight of liquid in each sample (g)

Weight of liquid in each sample= total weight of mixture in each tube – weight of feed content in each sample

Weight of feed content in sample = weight of sample added in tube (~ 0.5g) × concentrate of test mixture (in bottle)

Concentrate of diet mixture = $\frac{feed\ weight\ (\sim 4g)}{feed\ weight + 12ml\ H_2O + (4.8\ g\ phytase + acid\ weight)}$

3.4 Statistical analysis

The experimental data were analysed by the supervisor Birger Svihus using SAS (SAS institute, 2006). The significance level was $p < 0.05$. The square root of mean square error in the analysis of variance was used as a measure of random variation (Root MSE).

4 Result

4.1 Result from acid trial

In accordance with the result from acid trial, the formic acid was applied to adjust the diets pH for the in vitro experiment. Table 5 show that the amount of formic acid used to match the pH 5.5 was minimum 3 µl at the wheat-based diet (4g feed meal with 12ml distilled water), which was rather lower than those corresponding levels of other acids. In addition, the amounts of formic acid on pH 4.5 and 3.5 were clearly lower than those of any other acids on the same situation. Generally, the stabilities of acidic

conditions of all the diets showed limited changes throughout the trial, especially at the diets added propionic acid. The stabilities of diets with formic acid at pH 5.5 were found almost no changes within 1 hour on both wheat-based and barley-based diet. However, at the diets with formic acid with pH 4.5 and 3.5, the acidities slightly weakened during the time flows (on both two diets).

Table 5. The required dosages of four acids (pure form) and their pH changes within one hour on barley-based diet and wheat-based diet.

Target pH	Acid	Acid dosage/ μl	Calibrated pH	pH after 5min	pH after 10min	pH after 30min	pH after 1hour
Barley-based diet (4g)							
5.5	Propionic	15	5.50	--	5.50	5.521	5.65
4.5	Propionic	100	4.51	4.52	4.56	4.43	4.52
3.5	Propionic	1100	3.54	3.56	3.58	3.65	3.53
5.5	Formic	5.5	5.49	5.56	5.52	5.56	5.55
4.5	Formic	20	4.54	4.63	4.62	5.04	4.79
3.5	Formic	70	3.51	3.69	3.95	4.03	4.01
5.5	Acidic	40	5.57	5.62	5.66	5.55	5.42
4.5	Acidic	280	4.51	--	4.46	4.50	4.62
3.5	Acidic	2000	3.51	3.52	3.54	3.42	3.45
5.5	Citric	40	5.52	5.52	5.33	5.46	5.45
4.5	Citric	200	4.59	4.77	4.56	4.97	5.08
3.5	Citric	1400	3.58	3.64	3.65	3.67	3.76
Wheat-based diet (4g)							
5.5	Propionic	18	5.52	--	5.59	5.65	5.63
4.5	Propionic	140	4.54	4.64	4.67	4.75	4.73
3.5	Propionic	1200	3.52	--	3.42	3.60	3.72
5.5	Formic	3	5.51	5.55	5.56	5.66	5.55
4.5	Formic	20	4.47	4.58	4.65	4.65	4.71
3.5	Formic	70	3.57	3.86	3.98	3.93	3.99
5.5	Acidic	50	5.55	--	5.52	5.45	5.45
4.5	Acidic	280	4.57	--	4.52	4.58	4.65
3.5	Acidic	2020	3.51	3.73	3.88	3.77	3.81
5.5	Citric	70	5.57	5.56	5.62	5.34	5.56
4.5	Citric	360	4.56	--	4.53	4.83	4.85
3.5	Citric	1100	3.58	3.76	3.86	3.97	3.96

--means no data.

4.2 Result from in vitro experiment

From table 6, the released amounts of net phosphate (phosphate that excreted by exogenous phytase) were remarkable at 10min, which 0.31 mg/g (ph6.2) ,0.40 mg/g (ph5.5), 0.58 mg/g (ph4.5) and 0.62 mg/g (ph3.5), respectively. At pH 6.2, 5.5 and 4.5, the released amounts of net phosphate at 10min and corresponding 30min were not significant ($p>0.05$). Only the amounts at pH 3.5 (10min and 30min) were significant ($p<0.05$) (Table 6). Considering the released net phosphate at starting 0min was zero, it revealed that there was fast reaction between 0-10min in the current experiment.

An improvement in phytase efficiency as the diet acidity was getting lower from pH 5.5 to 4.5 and 3.5, was observed. From table 6, the amount of liberated net phosphate showed an increasing trend during the pH 5.5 reduce to 4.5 and 3.5. However, the differences of released net phosphate between pH 6.2 and 5.5 were not significant ($p>0.05$). The maximum release of net phosphate occurred at pH 3.5, with 0.76 mg/g on average. When examining the interactions, the effect of time, pH and time & pH on the values of net phosphate were significant, $p = 0.0004$, $p < 0.0001$ and $p = 0.0347$, respectively.

There was a significant increase in phytase capability during 30min and 1hour at the lower acidic condition of pH 3.5. As present in table 6, the liberated net phosphate was significantly greater ($p < 0.05$) at 30 min (0.88 mg/g) and 1 hour (0.83 mg/g) than at 10 min (0.62 mg/g). However, the amount of net phosphate at pH 3.5 expressed no further enhances after 1 hour. Similarly, the values of net phosphate at pH 4.5 increased from 10 min to 30 min but not significant ($p>0.05$), and no significant differences found in this acidic level.

Table 6. The degradation of phosphate from digestion experiment

pH	Time /min	Degradation of gross phosphate mg/g	Degradation of net phosphate mg/g	Control value mg/g
6.2	10	4.88^{J}	0.31^{F}	1.45
6.2	30	6.21^{HIJ}	0.37^{F}	2.17
6.2	60	6.87^{FGHI}	0.37^{F}	2.78
6.2	90	7.25^{EFGHI}	0.35^{F}	3.35
6.2	120	7.15^{FGHI}	0.34^{F}	3.39
5.5	10	5.70^{JI}	0.40^{F}	1.29
5.5	30	6.64^{HIG}	0.40^{F}	2.23
5.5	60	7.96^{CDEFGH}	0.44^{EF}	3.08
5.5	90	8.54^{CDEF}	0.44^{EF}	3.70
5.5	120	8.08^{CDEFG}	0.36^{F}	4.18
4.5	10	7.64^{DEFGH}	0.58^{DE}	1.32
4.5	30	9.09^{BCD}	0.64^{CD}	2.03
4.5	60	9.04^{BCDE}	0.59^{CDE}	2.58
4.5	90	10.37^{AB}	0.67^{CD}	3.06
4.5	120	9.54^{ABC}	0.59^{CDE}	3.06
3.5	10	8.04^{CDEFGH}	0.62^{CD}	1.24
3.5	30	11.29^{A}	0.88^{A}	1.59
3.5	60	10.92^{A}	0.83^{AB}	1.80
3.5	90	10.41^{AB}	0.75^{ABC}	2.22
3.5	120	10.31^{AB}	0.70^{BCD}	2.59
Mean effect of time				
	10	6.57^{C}	0.48^{C}	
	30	8.31^{B}	0.57^{A}	
	60	8.70^{AB}	0.56^{AB}	
	90	9.14^{A}	0.55^{AB}	
	120	8.77^{AB}	0.50^{BC}	
Root MSE		0.750605	0.06829	
Mean effect of pH				
6.2		6.47^{D}	0.35^{D}	
5.5		7.38^{C}	0.41^{C}	
4.5		9.14^{B}	0.61^{B}	
3.5		10.19^{A}	0.76^{A}	
P-values				
time		< 0.0001	0.0004	
pH		< 0.0001	< 0.0001	
Time&pH		0.043	0.0347	
Root MSE (time&pH)		0.750605	0.06829	

5 Discussion

The reason for choosing formic acid was their significantly smaller required amounts on all the target pH levels. In addition, their stable acidic changes within 2 hours was taken into consideration.

The result from in vitro experiment expressed that the Quantum blue efficiency at pH 3.5 was better than at higher pH levels (pH 4.5, 5.5 and 6.2). This is similar with previous finding (Menezes-Blackburn, et al., 2015) that the 80% of the optimal activity of Quantum blue was in a pH range 3.5-5.0. Same study also recorded that the Quantum Blue exhibited a similar phytate-degrading capability at pH 3.0 and pH 5.5 (Menezes-Blackburn, et al., 2015). The phytase efficiency in current study had a growth along with the pH range getting lower, which is in accordance with our hypothesis that in a lower dietary pH, the Quantum blue has a better performance.

Some organic acids that added in poultry diet have significant effects. A research from Liem, et al. (2008) suggested that the application of citric acid and malic acid to p-deficient diet of broiler chicks significantly enhanced the retention of P and phytate-p. This observation corresponds with Woyengo, et al. (2010) who reported that the addition of citric acid to a phytase- supplemented diet further increased ($p< 0.05$) p digestibility to 51.5%. This is likely due to those acids changed the pH of gastrointestinal tract to a pH that more optimum for phytase efficiency (Liem, et al., 2008). Besides, Boling et al. (2000) suggested that citric acid promoted phytate p utilization by competitively chelating Ca, reducing the formation of insoluble Ca-phytate complexes.

For further enhance the performance, it may possible that apply intermittent feeding in combination with low pH diet. It was reported by Svihus, et al. (2013) that intermittent feeding broiler chickens improved the feed efficiency but did not improve the efficiency of dietary phytase. However, the present study show that a lower pH condition promotes phytase efficacy.

6 Conclusion

The current experiment revealed that the Quantum blue had a better efficacy at a lower acidity condition at pH 3.5 compared to at pH 4.5, 5.5 and 6.2. There was a significant increase in the phytase capability at 30min and 1h during pH 3.5, which observation is needed in later research to get a more detailed understanding of their interactional factors.

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