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Improvement of Fungal Phytase Production and Its *In-vitro* Application in Ruminant Nutrition

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Abstract

Various fungal genotypes *(Aspergillus niger* NRRL 3135 (AN1), *Aspergillus niger* NRRL 326 (AN26), *Aspergillus terrus* F2-Kh (AT) and *Mucor racemosus* NRRL 3639 (MI)) were studied for their ability to produce phytase and improve the produced enzyme by ethyl-methane sulfonate (EMS) mutagenesis. AN1 showed the highest phytase activity on phytase screening medium supplemented with glucose (PSMG) after 8 days of incubation (reached 1875.40 IU/mL). The phytase activity of AN1 increased with increasing incubation time and the highest value was achieved at 12 days of incubation (2859.33 IU/mL). The exposure of AN1 spore suspension to 200 mM of EMS for different times enhanced the phytase activity and that mutant 20 Mn exhibited the highest phytase activity (reached 4520.5 IU/mL) therefore it was chosen for the next experiment. An *in-vitro* gas production procedure was carried out to evaluate the impact of using various amounts of laboratory produced phytase (PE) compared with commercial phytase (Axtra® PHY) on nutrients availability of ruminant's ration. Six levels (0, 400, 800, 1200, 1600 and 2000 IU phytase^{-1}Kg dry matter) of phytase enzyme were evaluated with tested ration consisted of 40% berseem hay (BH) and 60% concentrate feed mixture (CFM). *In-vitro* dry and organic matter degradability (IVDMD and IVOMD), total gas production (GP), short chain fatty acids (SCFA's) and inorganic phosphorus (P_i) concentration were improved significantly ($P<0.05$) by phytase addition from the two sources of phytase and the highest significant ($P<0.05$) values achieved at the level of 1200 IU. Results suggest that phytase activity was influenced by exposure to EMS mutagen compared to the wild type. Also, the produced enzyme source has the ability to improve the utilization efficiency of phytate diets as evidenced by the significant ($P<0.05$) increase in all tested parameters compared to the commercial source.

Keywords: EMS-mutagenesis, Gas-production, *In-vitro*, Phytase

1 Introduction

About 90% of the world's harvested area is cultivated with grains, legumes and oilseed crops. Thus they consider as the main nutrients source for the animal kingdom. An essential component of these crops is phytic acid (myo-inositol hexakisphosphate), also known as phytate. In forage, two-thirds of phosphorus

(P) is found as organic P (phytate) and only one-third as digestible inorganic P. Phytate is an anhydrous reserve form of phosphate representing more than 80% of the total P in grains and legumes (Alexandra et al 2004). Phytate contains a high phosphate level which causes a high negative charge over a large range of pH therefore it works as an anti-nutritional factor (Singh 2008).

Because of the great importance of phytic acid hydrolysis, a specific class of enzymes hydrolyzing phytic acid (Ins P_6) has been developed (the phytases). Phytase (Ins P_6 phosphohydrolase) is the primary enzyme responsible for Ins P_6 hydrolysis to inositol polyphosphates and inorganic monophosphate (Oh et al 2004). It can be located in bacteria, yeast, fungi, plants and animals. Howeover, the activity of phytase in microorganisms has been commonly located in fungi, especially, *Aspergillus* species. The genus *Aspergillus* (*A. niger* in specific) remains to be prefered for phytase production. This is not only because its generally recognized as safe (GRAS) status, but also because its big secretory possibility and the deep understanding about its growth cultivation (Shivanna and Govindarajulu 2009). Phytase production from fungi could be achieved using several methods and the method selection could depend upon the microorganism and the substrate to be used (Parekh et al 2000). However, the production level of naturally active strains is usually small for trading use. Thus, the success of any production method is reliant on discovering a convenient microorganism and enhancing its productivity if required. Mutagenesis and genetic recombination are the traditional ways that were used for strain enhancement (Punt et al 2002).

Recently, the report "Global Markets for Enzymes in Industrial Applications" by the BCC research (Sep. 2018) announced that the global industrial enzymes market should reach $7.0 billion by 2023 from $5.5 billion in 2018 at a compound annual growth rate (CAGR) of 4.9% for the period 2018-2023. According to this report, the animal feed enzyme industry is estimated to reach its fastest growth rate in growing markets. However, even though phytase addition efficiently enhances nutritional value while declining P waste. The high cost of the phytase production reduces its commercial use, which required the ability to produce the enzyme economically. Therefore, the use of agro-industrial by-products for enzymes production, such as phytase, provids the dual benefit of given an added value to the feed ingredients and prevents P pollution from farm manure. Moreover, the local production of such an important enzyme may encourage our self-reliance and reduce the cost of importation in Egypt.

For all reasons that mentioned above, two experiments were carried out in this study to: 1) screen various fungal strains for their phytase production ability under the optimum fermentation conditions to determine which one is the best producer of extracellular phytase, 2) improve microbial phytase production from the highest producing strain by ethyl-methane sulfonate (EMS) mutagenesis, 3) evaluate the potential use of various concentrations of laboratory produced phytase enzyme (PE) for improving *in-vitro* nutrients degradability of lactating animal ration compared with different levels of commercial phytase enzyme (Axtra® PHY) to decide the most effective level of phytase supplementation.

2 Materials and Methods

2.1 Laboratory microbial phytase production trials

2.1.1 Microbial strains, media and inoculum preparation

Aspergillus niger (syn. A. ficuum) NRRL 3135 (AN1), *Aspergillus niger* NRRL 326 (AN26), *Aspergillus terrus* F2-Kh (AT) (GenBank accession number MH997666) and *Mucor racemosus* NRRL 3639 (MI) were selected for screening their phytase production ability. The above fungal strains were obtained from Applied Microbial Genetics Lab., Genetics and Cytology Dept., National Research Centre, Cairo, Egypt and maintained

on potato dextrose agar (PDA) slants.

For the preparation of active fungal inoculum; fungal strains were incubated for 7 days at 30°C on solid slant medium containing per liter: 3 g $NaNO_3$, 1g K_2HPO_4, 30 g sucrose, 0.01 g $FeSO_4.7H_2O$, 0.5 g $MgSO_4.7H_2O$, 2 g casamino acid, 2 g yeast extract and 15 g agar. After incubation, 5 ml of spore collection solution (1 g Tween 80 and 1 g $CaCO_3$ per liter) was added with scraping the culture surface for 2-3 min. using a sterile inoculating needle under aseptic condition (Kim et al 1999).

2.1.2 Screening for phytase-producing fungi

Submerged fermentation (SmF) was used to investigate the effect of different phytase screening media (PSM) and incubation periods (2, 4 and 8 days) on phytase production by various fungal genotypes (AN1, AN26, AT and MI). The used fermentation media had the following composition (g/l): dextrin, 50; $NaNo_3$, 8.6; KCl, 0.5; KH_2PO_4, 0.04; $FeSO_4.7H_2O$, 0.1; $MgSO_4.7H_2O$, 0.5 and glucose, 52 or sucrose, 25 for PSMG and PSMS, respectively **(**Shieh and Ware 1968). About 100 ml growth media were transferred into 250 ml conical flasks and autoclaved at 121°C for 20 min. After cooling, the media were inoculated with 1% (v/v) of spore suspension and incubated in a rotary shaker (120 rpm) at 30°C for 2, 4 and 8 days. After centrifugation, the supernatant was determined for phytase activity.

A complementary experiment was designed to examine the impact of various incubation times on phytase enzyme production by the most efficient fungal strain at selected medium. Therefore, fungal spores were transferred into 250 ml conical flasks each containing 100 ml of selected medium with wheat bran (10%). Cultures were incubated on a rotary shaker (120 rpm) at 30°C for 2, 4, 6, 8, 10, 12 and 14 days and after centrifugation, the supernatant was evaluated for phytase activity.

2.1.3 Mutagenesis of fungal strain by ethyl-methane sulfonate (EMS)

The fungal spore suspension (5 ml) was transferred to sterile tube containing sodium phosphate buffer (0.1M, pH 7.0) and treated with EMS (200 mM). After different time intervals (20, 40 and 60 minutes), EMS treated spores were washed three times with sterilized sodium thiosulfate buffer (5%), to take off any traces of EMS-mutagen, and centrifuged for 2 min at 13000 rpm (Asia et al 2018).

Obtained mutants were chosen by spreading EMS-treated spores on Petri plates containing 100 ml of phytase screening medium (PSM) supplemented with 2% agar, 0.1% Triton X 100 and heavy metals (250 ppm cobalt or 500 ppm manganese) according to Khattab and Abd-El Salam (2012). Control without mutation (wild type) was also plated, and then all plates were incubated for 2-3 days at 30°C. The selection was determined by the observation of heavy metal resistance, survival percentage was estimated. Mutants were assured for the production of phytase by using the selected medium under SmF with wheat bran (10%) as described before. Cultures were incubated on a rotary shaker at 30°C (120 rpm) for 12 days. After incubation, phytase activity was evaluated according to Gulati et al. (2007).

2.1.4 Enzyme sources

Produced enzyme (PE): Laboratory produced phytase enzyme from the most efficient fungus mutant. Each gram contains 2000 IU of phytase.

Axtra® PHY: A commercial enzyme produced by Danisco Animal Nutrition, UK, and distributed by Multi Vita Co. for Animal Nutrition, Second Industrial, 6 October Governorate, Giza, Egypt. This phytase feed enzyme, is extracted from a *Buttiauxella* species bacterium and is expressed in a *Trichoderma reesei* fungus and including 6000 unit/g of phytase.

2.1.5 Enzyme assay

The phytase activities for PE and Axtra® PHY were estimated according to Gulati et al. (2007). Enzyme activity was expressed in international units (IU). One unit of phytase activity was known as the amount of enzyme that required to release 1µmol of P_i per min under the assay conditions.

2.2. The *in-vitro* trials

2.2.1 Experimental ration and treatments

The tested ration was consisted of 40% berseem hay (BH) and 60% concentrate feed mixture (CFM). The CFM was containing 52.2% yellow corn, 21% wheat bran, 12.5% undecorticated cottonseed meal, 12.5% soybean meal, 0.5% sodium chloride, 0.8% limestone, and 0.5% minerals and vitamins complex. Two sources of phytase enzyme were used (Axtra® PHY and PE) at 6 different levels (0, 400, 800, 1200, 1600 and 2000 IU phytase^{-1}Kg dry matter), samples were incubated for 24 hr.

2.2.2 *In-vitro* gas production technique

A 400 mg of experimental ration consisted of 160 mg BH and 240 mg CFM for each treatment was accurately weighed into 125 ml glass bottles and supplemented with solutions of Axtra® PHY and PE at 6 various levels as described above. Enzyme solutions were added onto the samples immediately before the supplementation of buffer and rumen liquor. Rumen fluid was taken from the rumen of slaughtered steers, fed BH and CFM, and moved directly to the laboratory in a pre-warmed thermos flask. Then it was strained through 4 layers of cheese-cloth, flushed with CO_2, transferred to the McDougall's buffer solution (McDougall 1948) (1:4 v/v), and mixed. Every bottle was incubated with 40 ml of diluted rumen fluid, flushed with CO_2, sealed with stoppers and maintained in the incubator at 39°C for 24 hr. After the end of incubation, contents of each bottle were filtered filter bag 25-micron porosity (ANKOM-USA), and then rumen pH was determined by a pH-meter. Bags residues were oven-dried at 70 °C for 48 hr to determine the *in-vitro* dry and organic matter degradability (IVDMD and IVOMD). The overall volume of gas production (GP) was estimated using Hohenheim Syringe as shown by **Navarro-**Villa et al (2011). Inorganic phosphorus (P_i) concentration was determined according to AOAC (2000). Also, short chain fatty acids (SCFA's) content was calculated as stated by Makkar (2005):

$$SCFA\ (mmol) = 0.0222\ Gas - 0.00425$$

Where: Gas = Gas production at 24 hr incubation (ml/200 mg DM)

2.2.3 Statistical analysis

Collected data were statistically analyzed by SPSS (2008). Two way ANOVA procedure was applied in the second experiment to evaluate the effect of phytase addition from various sources (Axtra®PHY and PE) at different levels (0, 400, 800, 1200, 1600 and 2000 IU phytase^{-1}Kg dry matter) on IVDMD, IVOMD, P_i, GP, SCFA's and pH according to the following model:

$$Y_{ijk} = \mu + S_i + L_j + SL_{ij} + e_{ijk}$$

Where:
Y_{ijk} = any value from the overall population
μ = overall mean.
S_i = effect of the i^{th} different phytase enzyme sources.
L_j = effect of the j^{th} different phytase enzyme levels.
SL_{ij} = effect of the interaction between the i^{th} phytase enzyme sources and the j^{th} phytase enzyme levels.
e_{ijk}= the random error associated with the k^{th} individual receiving the i^{th} phytase enzyme sources and the j^{th} phytase enzyme levels.

3 Results and Discussion

3.1 Laboratory microbial phytase production trials

3.1.1 Screening for phytase-producing fungi

Results in **Fig 1** illustrated the effect of different fermentation media and incubation periods on phytase enzyme production from different fungal genotypes. By using PSMG medium, increasing the incubation period from 2 to 8 days increased the phytase activity of all fungal genotypes. However, AN1 showed the highest phytase activity after 8 days of incubation (recorded 1875.40 IU/mL) compared with the phytase activity from AN26; MI and AT (recorded 404.72; 474.83 and 485.38 IU/mL, respectively). Similar trend was observed with PSMS medium, whereas phytase activity obtained at PSMS medium after 8 days of incubation recorded 1735.4; 556.84; 513.19 and 434.48 IU/mL from AN1; MI; AT and AN26, respectively. It was observed obviously that the highest phytase production was obtained at the PSMG medium compared to the PSMS medium, therefore AN1 and PSMG medium were selected for further work. The effect of different incubation periods on AN1 phytase enzyme production at PSMG medium is presented in **Fig 2**.

Results showed that phytase activity of AN1 at PSMG medium was increasing as the incubation period increased and it reached to the highest value at 12 days of incubation (2859.33 IU/mL), then it was decreased by increasing the incubation time to 14 days (2693.25 IU/mL). These results are in line with previous outcomes mentioned by some other researchers (Ullah and Phillippy 1994; Wodzinski and Ullah 1996; Mullaney et al 2002) that investigated the *Aspergillus niger (ficuum)* NRRL 3135 strain efficiency's for extracellular phytase production compared to other strains as they were impressed with its phytase activity.

3.1.2 Induction of overproducing phytase mutants

Based on the mentioned results, *Aspergillus niger (ficuum)* NRRL 3135 (AN1) was chosen for the further mutation studies because it exhibited the highest phytase activity. Number of the colonies (cfu/ml) and survival percentages following exposure periods of AN1 to ethyl-methane sulfonate (EMS) mutagenesis are given in **Table 1**. The results showed that increasing the exposure time to EMS mutagenesis from 20 to 40 min decreased the survival percentages of AN1 from 59.43% to 35.38%. Moreover, the survival percentages continued to be decreased as the exposure time to EMS mutagenesis increased to 60 min. giving 22.17 %.

Data in **Table 2** represents the phytase activity of mutants obtained from exposure of AN1 to EMS mutagen for 20, 40 and 60 minutes. The results showed that phytase activity was influenced by exposure to EMS mutagen compared to the wild type (WT) at all exposure times. Among different tested mutants, mutant 20 Mn exhibited the highest phytase activity (recorded 4520.5 IU/mL) compared with other mutants at all exposure times (20, 40 and 60 min.).

Phytases are gotten for the most part from genetically improved strains because the wild-type ones produce little protein comparative with commercial request. The enhancement in enzyme yield that can be accomplished by mutating a single gene (once or consistently) is restricted, hence in a strain enhancement program a progression of mutagenic operators is utilized (Parekh et al 2000). Chelius and Wodzinski (1994) built up a strain enhancement plan as ultraviolet radiation was the main mutagen applied to increment phytase creation by *A. niger* NRRL 3135. Shah et al (2009) used a mix of chemical (EMS) and physical mutagen to obtain superior secretory strains of *A. niger* NCIM 563 for phytase creation. Recently, Mehmood et al (2019)

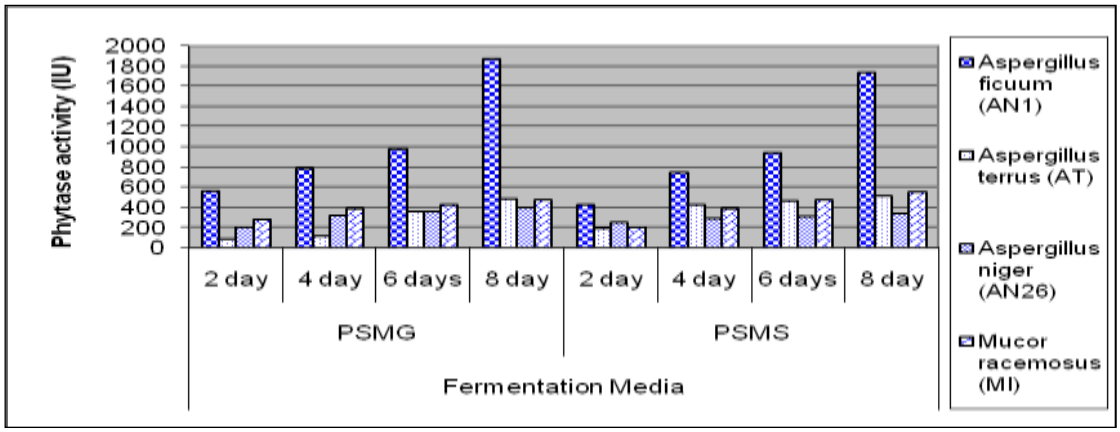

Fig 1. Effect of different fermentation media and incubation periods on phytase production (IU) of different fungal genotypes

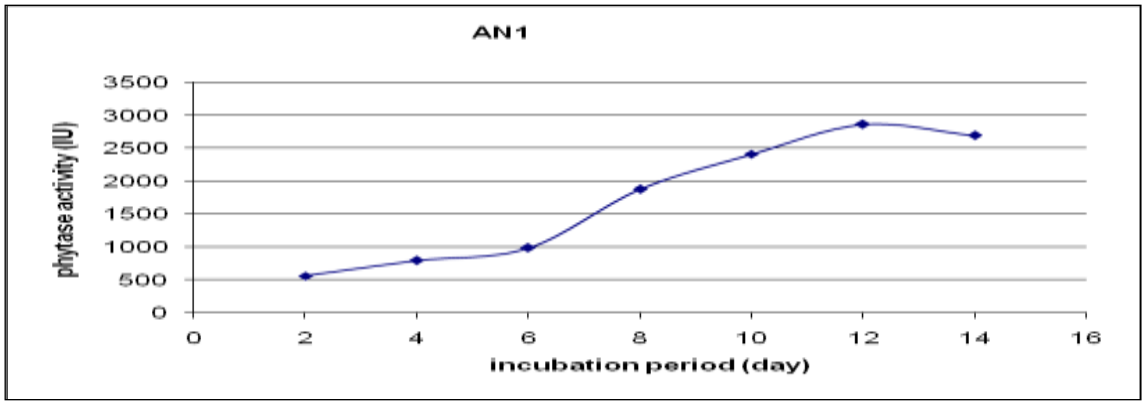

Fig 2. Effect of different incubation periods on phytase production of *Aspergillus ficuum* NRRL 3135 (AN1)

Table 1. Number of the colonies (cfu/ml) and survival percentages following exposure periods of *Aspergillus niger (ficuum)* NRRL 3135 to ethyl-methane sulfonate (EMS) mutagenesis

Treatment (min.)	No. of colonies	Survival %
0	212	100
20	126	59.43
40	75	35.38
60	47	22.17

Table 2. Phytase activity of mutants obtained from exposure of *Aspergillus niger* NRRL 3135 to ethyl-methane sulfonate mutagenesis for 20, 40, and 60 minutes

Mutant No.	Phytase Activity (IU/mL)	% to Wild Type (WT)
Wild Type	2859.3	100
Mutant 20 Mn	4520.5	158.1
Mutant 20 Co	3347.8	117.08
Mutant 40 Mn	3443.1	120.4
Mutant 40 Co	3727.5	130.36
Mutant 60 Mn	3687.8	129.0
Mutant 60 Co	2644.1	92.47

Where: Mn: manganese and Co: cobalt

enhanced *S. thermophile* strain ST20 utilizing chemical and physical mutagens for improved phytase productivity. Authors utilized EMS and gamma rays mutagenesis to improve the phytase activity, they revealed that the mutants created through EMS showed more noteworthy capability of phytase creation when contrasted with the parent strain.

3.2 *In-vitro* trials

3.2.1 Dry matter and organic matter degradability

The effect of phytase supplementation from various sources (Axtra® PHY and PE) and the effect of different phytase enzyme levels (0, 400, 800, 1200, 1600 and 2000 IU phytase^{-1}Kg dry matter) on IVDMD, IVOMD, P_i, GP, SCFA and pH of the experimental ration after 24 hr of incubation are presented in **Table 3**. Dry and organic matter degradability increased significantly ($P<0.05$) with phytase addition from the two sources of phytase. However, phytase supplementation from PE source significantly ($P<0.05$) improved IVDMD and IVOMD values compared to Axtra®PHY source. Increasing the phytase level from both enzyme sources increased ($P<0.05$) the IVDMD and IVOMD and the highest significant ($P<0.05$) value achieved at the level of 1200 IU, then IVDMD and IVOMD values decreased significantly ($P<0.05$) by increasing the level of phytase from 1200 IU to 2000 IU.

Our results are consistent with those of Bravo et al (2003) where they noticed that supplementing 2000 IU of fungal phytase (Nathuphos®, BASF) to the formaldehyde-treated soybean diet slightly increased DM digestibility in sheep. Allam et al (2014) carried out a trials to study the effect of phytase addition on *in-vitro* degradation of feed ingredients. Dry matter and organic matter disappearance were estimated for 4 ingredients that consisted of high phytate P. Five levels (0, 500, 1000, 1500 and 2000 IU/Kg DM) of phytase (Nutrase P 500®) were used. The authors concluded that addition of phytase at 500 IU/Kg DM revealed the highest value of IVDMD and IVOMD. Also, Azzaz et al (2019) used an *in-vitro* batch culture technique to study the impact of xylanase and phytase addition at various levels (0, 1, 2 and 3 g/ kg DM) on rumen characteristics. They found that phytase and xylanase supplementation increased IVDMD and IVOMD.

These positive effects of phytase supplementation on IVDMD and IVOMD may be due to that enzymes were able to digest complicate substrate to simpler compounds which might change the surface structure of substrates, making them more accessible to ruminal microbial degradation and allowing a faster ruminal colonization and fermentation

as suggested by Yang et al (1999) and Colombatto et al (2003). Furthermore, Durand and Komisarczuk (1987) observed that the rumen ecosystem appears to be P dependent for the degradation of cell walls. Therefore, maintaining a stable rumen P_i concentration appears necessary to acheive suitable diet digetion. Moreover, the difference between the two sources of phytase in their effect on IVDMD and IVOMD values may be due to the differences in their composition.

3.2.2 Inorganic phosphorus concentration

Data in **Table 3** indicated an increase ($P<0.05$) in P_i concentration with phytase supplementation after 24 hrs. of incubation. Furthermore, the PE source of phytase had a significant ($P<0.05$) effect on P_i value compared to Axtra®PHY source. Regardless the source of phytase, increasing the level of addition from both enzyme sources significantly ($P<0.05$) improved P_i concentration until it reached its highest value at level of 1200 IU, then decreased ($P<0.05$) by increasing the phytase concentration to 2000 IU.

Our outcomes are in good agreement with previous finding of Shanklin (2001) who found that supplentation of phytase (1000 IU) to the diets of lambs fed with organic P (cottonseed meal diets) resulted in more P_i in the ruminal fluid. Similarly, Bravo et al (2002) illustrated that both phytase addition (2000 IU) and the increases in concentrate feed mixture level to 70 % motivate P solubilization in the rumen of lactating goats. Brask-Pedersen et al (2011) indicated that exogenous phytase can enhance ruminal inositol phosphate degradation and thereby increase P availability and permit a lower allocation of P in the diet.

The increasing of P_i values with phytase supplementation may be explained by the fact that phytases (Ins P_6 phosphohydrolase) are the primary enzymes responsible for the hydrolysis of phytate to inorganic phosphate (Rosenfelder-Kuon et al 2019; Jatuwong et al 2020).

3.2.3 Gas production and SCFA's concentration

Results presented in **Table 3** highlighted that phytase addition enhanced ($P<0.05$) the overall volume of GP and SCFA's concentrations after 24 hrs. of incubation. Phytase supplementation from PE source significantly ($P<0.05$) improved GP and SCFA's concentration compared to Axtra® PHY source. However, increasing the level of supplementation had a significant ($P<0.05$) effect on GP and SCFA's values and the highest values were recorded at a level of 1200 $IU^{-1}Kg$ DM. This result confirms with previous finding of Komisarczuk et al (1987) who stated that reducing Pi concentrations caused a significant reductions in total volatile fatty acids (TVFA's) production, accompanied by a rise in pH value. Sampath et al (1995) reported that when the concentrate feeds was mixed with hay, the total GP and the rate of GP improved. Also, Salem et al (2013) revealed that the addition of enzymes increased SCFA's concentration. Nasser et al (2010) observed that increasing P level improved the cumulative volume of GP. Azzaz et al (2019) found that xylanase and phytase addition at various levels (0, 1, 2 and 3 g^{-1}kg dry matter) increased ruminal NH_3-N and TVFA's concentrations, while it did not affect the volume of total gas production.

3.2.4 Rumen fluid pH

Data in **Table 3** illustrated that phytase supplementation with PE source led to decrease ($P<0.05$) pH values compared to Axtra® PHY source after 24 hr of incubation. However, increasing the level of addition from both enzyme sources significantly ($P<0.05$) decreased pH values until it reached its lowest value at level of 1200 IU, then pH values increased ($P<0.05$) by increasing the level of phytase from 1200 IU to 2000 IU. These results may be due to that phytase supplementation increased the dry and organic matter degradability, the overall volume of GP, SCFA's and P_i concentrations led to an

Table 3. Effect of phytase source and level on *in-vitro* ruminal fermenation characteristics for the experimental raion

Item	Enzyme levels IU	Enzyme sources		Overall mean	SEM
		Commercial (Axtra®PHY)	Produced		
In-vitro dry matter degradability (IVDMD, %)	0	44.07	44.07	44.07^{e}	
	400	43.43	49.73	46.58^{d}	
	800	44.72	51.62	48.17^{c}	
	1200	53.90	54.52	54.20^{a}	
	1600	48.29	51.07	49.68^{b}	
	2000	46.11	47.75	46.93^{cd}	
	Overall mean	46.75^{b}	49.79^{a}		0.66
In-vitro organic matter degradability (IVOMD, %)	0	49.15	49.15	49.15^{e}	
	400	48.17	54.81	51.49^{d}	
	800	50.13	56.70	53.41^{c}	
	1200	57.98	59.94	58.96^{a}	
	1600	53.70	56.15	54.93^{b}	
	2000	51.19	52.83	52.01^{cd}	
	Overall mean	51.72^{b}	54.93^{a}		0.65
Inorganic phosphorus (P_i, µg /ml)	0	228.1	228.1	228.1^{f}	
	400	242.4	245.6	244.0^{c}	
	800	249.5	253.1	251.3^{b}	
	1200	254.7	256.0	255.3^{a}	
	1600	238.3	234.7	236.5^{d}	
	2000	229.4	234.3	231.8^{e}	
	Overall mean	240.4^{b}	242.0^{a}		1.72
Gas production (GP, ml)	0	125.7	125.7	125.7^{e}	
	400	130.0	133.0	131.5^{c}	
	800	131.7	133.0	132.3^{b}	
	1200	135.0	137.3	136.2^{a}	
	1600	129.7	132.0	130.8^{c}	
	2000	127.0	128.0	127.5^{d}	
	Overall mean	129.8^{b}	131.5^{a}		0.62
Short chain fatty acids (SCFA's, mmol)	0	1.39	1.39	1.39^{e}	
	400	1.44	1.47	1.45^{bc}	
	800	1.45	1.47	1.46^{b}	
	1200	1.49	1.51	1.50^{a}	
	1600	1.43	1.46	1.44^{bc}	
	2000	1.40	1.41	1.41^{d}	
	Overall mean	1.43^{b}	1.45^{a}		0.01
pH	0	6.5	6.5	6.5^{a}	
	400	6.2	6.0	6.1^{b}	
	800	6.1	6.0	6.0^{c}	
	1200	5.9	5.8	5.9^{d}	
	1600	6.0	5.9	6.0^{c}	
	2000	6.1	6.0	6.1^{b}	
	Overall mean	6.1^{a}	6.0^{b}		0.02

* Means designated with the same letter in the same column are not significantly different at 0.05 level of probability. SEM: standard error of the means.

improvement in rumen fermentation. Komisarczuk et al (1987) observed that reducing P_i concentrations caused the total VFA's production to decrease and the pH value to rise.

4 Conclusion

It could be concluded that *Aspergillus niger (ficuum)* NRRL 3135 (AN1) exhibited the highest phytase activity at PSMG medium, and the best incubation period was 12 days. Moreover, data showed that phytase activity was influenced by exposure to EMS mutagen compared to the wild type culture at all exposure periods (20, 40 and 60 min.). Among different tested mutants, mutant 20 Mn exhibited the highest phytase activity (reached 4520.5 IU/mL). Furthermore, results suggested that supplementation of phytase enzyme may enhance the utilization efficiency of high concentrate diets as evidenced by the significant ($P<0.05$) increase in IVDMD, IVOMD, GP, SCFA's, and P_i concentration. Phytase supplementation from PE source significantly ($P<0.05$) improved all tested parameters compared to Axtra®PHY source and the optimal enzyme level from both phytase sources was 1200 IU^{-1}Kg ration.

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تحسين إنتاج إنزيم الفايتيز الفطري وتطبيقاته المعملية في تغذية الحيوانات المجترة [20]

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الموجـــــز

تم فحص العديد من السلالات الفطرية (*Aspergillus niger* NRRL 3135 (AN1)، *Aspergillus niger* NRRL 326 (AN26)، *Aspergillus terrus F2-Kh* (AT) و *Mucor racemosus* NRRL 3639 (MI) لدراسة قدرتها على إنتاج انزيم الفايتيز كما تم تحسين انتاجية الانزيم عن طريق التعرض لمطفر إيثيل ميثان سلفونات (EMS). أظهرت السلالة AN1 أعلى نشاط للفايتيز بعد 8 أيام من التحضين (بلغ 1875.40 وحدة دولية/مل). زاد نشاط الانزيم المنتج من السلالة AN1 مع زيادة فترة التحضين وتم تحقيق أعلى قيمة عند 12 يومًا من التحضين على بيئة PSMG (2859.33 وحدة دولية/مل). أدى تعرض الـ AN1 إلى 200 ملي مول من مادة الـ EMS المطفرة لفترات زمنية مختلفة (20 و40 و 60 دقيقة) الى تحسين إنزيم الفايتيز، وأظهرت الطافرة Mn (20) أعلى نشاط للفايتيز (وصل إلى 4520.5 وحدة دولية / مل) ولذلك تم اختيارها من أجل الدراسات اللاحقة. وتم إستخدام تقنية إنتاج الغاز معمليا لتقييم تأثير استخدام مستويات مختلفة من إنزيم الفايتيز المنتج معمليا (PE) مقارنة مع الفايتيز التجاري (Axtra® PHY) على تحلل العناصر الغذائية لعلائق المجترات في المعمل. تم استخدام ستة مستويات (0 ، 400 ، 800 ، 1200 ، 1600 و 2000 وحدة دولية من إنزيم الفايتيز/كجم مادة جافة) من كلا المصدرين لإنزيم الفايتيز، وتكونت العليقة المختبرة من 40٪ دريس برسيم (BH) و 60٪ من مخلوط العلف المركز (CFM). حيث أدت اضافة انزيم الفايتيز من المصدرين الى حدوث تحسن معنوي ($P<0.05$) في كل من معدل تحلل المادة الجافة والمادة العضوية معمليا (IVDMD وIVOMD)، إجمالي إنتاج الغاز (GP)، الأحماض الدهنية قصيرة السلسلة (SCFA) وكذلك تركيز الفوسفور غير العضوي (P_i). ولقد تحققت أعلى قيم معنوية ($P<0.05$) عند مستوى 1200 وحدة دولية من إنزيم الفايتيز/كجم مادة جافة. وجد أن نشاط إنزيم الفايتيز يزداد بعد تعريض السلالة البرية للـ EMS. وتشير النتائج إلى أن مصدر الإنزيم المنتج معمليا من خلال هذه الدراسه لديه الامكانية على تحسين كفاءة الاستفادة من العلائق المرتفعة في محتواها من كل من المركزات والفيتات كما يتضح من الزيادة المعنوية ($P<0.05$) في قيم كل القياسات المختبرة عند مقارنته بمصدر الإنزيم التجاري.