Tolerance properties and growth performance assessment of *Yarrowia lipolytica* lipase in broilers

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ABSTRACT

Lipases have attracted more interest for several biotechnological applications due to their unique properties, such as security, high stability at low pH values and substrate specificity. Nevertheless, no systematic studies on its effectiveness in corn-soybean when fed to broilers have been published. In this study, *in vitro* and *in vivo* assessment of a lipase from the yeast *Yarrowia lipolytica* for feed purpose was performed. Results showed that *Y. lipolytica* lipase (YLL) displayed the highest stability at low pH values when compared with other lipases from different sources. YLL stabilized with skimmed milk powder and starch maintained 55% and 48% of its initial activity after 2 h of incubation at pH 6 with bile salt and proteases, respectively. In addition, using broilers fed as substrate, YLL formulations displayed a higher hydrolysis degree after 6 h reaction in the presence of bile salt and digestive enzymes. Moreover, *in vivo* effect of YLL on growth performance in broilers was studied. From 1 to 42 d., it appears from this study that there is a significant (*p* < 0.05) effect for YLL to improve feed conversion ratio. Consequently, YLL has a great potential for using as a feed supplement in the poultry industry.

1. Introduction

Exogenous enzymes, acting as an efficient feed supplement, have got increasing concern by the poultry and livestock industry and become widely acceptable (Beauchemin et al. 2003; Sarica et al. 2005). Lipases (E.C.3.1.1.3) are well known as versatile biocatalysts for various industrial fields including oleochemical, textile, detergent, biodegradable polymers, food additive, biodiesel and so on (Yan et al. 2011; Zinjarde 2014; Yan et al. 2017). However, the use of lipases as inducer of oil and fat digestion in the feed industry has been relatively ignored. Oils and fats represent minor components in animal growth through energy supply and storage, as well as for providing necessary fatty acids. Young animals with immature digestive capability for full absorption of lipids would particularly benefit from feed lipases (Schmid and Verger 1998; Fickers, Fudalej, Le Dall, et al. 2005; Fickers, Fudalej, Nicaud, et al. 2005). Therefore, there is an increasing trend in the feed industry to develop lipases as feed additives.

Lipases, as many other enzymes, cannot stand the effect of high temperature, extreme pH, high ionic strength and organic solvents. Consequently, due to some deficiencies including inactivation by gastric acidity and physiological concentration of bile salts in the small intestine, and degradation by digestive tract proteases, most of lipases cannot be applied to animal feed (Moreau et al. 1988; Zentler-Munro et al. 1992). Therefore, in order to improve the stability and utilization, there remains a need for improved enzyme preparations, which can maintain their activity under poor conditions in the stomach and small intestine due to a good feed additive lipase candidate that is resistant to low pH, bile salts and proteases. According to previous research, *Yarrowia lipolytica* lipase (YLL) formulated with gum arabic and milk powder seemed to have great potential for use as a therapeutic tool for patients with pancreatic insufficiency due to its stability under physiological conditions pertaining to the human gastrointestinal tract (Turki et al. 2010).

In this report, we have mainly focused on the following three aims: (1) to determine the residual activities of YLL, *Rhizopus oryzae* lipase (ROL), *Candida rugosa* lipase (CRL) and *Thermoascus lanuginosus* lipase (TLL) after 2 h of incubation with variation in pH values by measuring with an olive oil-polyvinyl alcohol (PVA) emulsion substrate, (2) to study the effect of bile salts and proteases on YLL stability and hydrolysis degree by comparing with two YLL formulations at pH 6 and (3) to measure the effect of YLL on growth performance of broiler chickens.
2. Materials and methods

2.1. Lipases

YLL was overproduced by a modified Y. lipolytica strain in a high-efficiency fermentation process of a 3-L fermenter. The cell-free enzyme supernatant was obtained by centrifugation at 12,000 × rpm for 10 min at 4°C. The crude enzyme preparation was subjected to 0.25 nm filterable membrane to remove any residual cell. The specific activity of the YLL supernatant was 18,000 U/ml. Samples of 0.5-L were taken and stored at 4°C. For formulation A, a 0.5-L sample was supplemented with 10% (m/v) skimmed milk powder, whereas for formulation B, a 0.5-L sample was supplemented with 10% (m/v) starch. These two formulations were spray-dried in a spray dryer (JOYN-8000 T) with inlet and outlet temperatures of 90°C and 60°C, respectively, at a flow rate of 0.5 L/h (Alloue et al. 2007). The final specific activities of the two formulations were 115,000 and 93,200 U/g, respectively.

The CRL and ROL supernatant used in the present study were collected by centrifuging to remove deposition after flask shaking fermentation. Their final specific activities were 720 and 750 U/ml under the optimum conditions, respectively. The TLL was obtained by a fermentation process of the 3-L fermenter. The post processing was similar to that of the YLL.

2.2. Reagents and digestive enzymes and chemicals

Pepsin (Sigma P6887), chymotrypsin (Sigma C4129), was also obtained from Sigma-Aldrich. All other chemicals were from Sinopharm and of analytical grade.

2.3. Influence of pH on four fungal lipases’ stability

To study the stability of four fungal lipases in acidic conditions, 1 ml of the enzyme solutions were incubated for 2 h at room temperature in various buffers with different pH ranging from 2 to 9, and the residual activity was measured. The following 50 mM buffers systems were used: critic buffer, pH 2–6; Tris-HCl buffer, pH 7–8; glycine buffer, pH 9 (Adamczak and Bednarski 2004). The percentage of the residual activity was calculated by the ratio between apparent activity and optimum activity.

2.4. Comparative study of the in vitro effects of YLL and two formulations on stability and hydrolysis degree

To evaluate stability of YLL under the digestion environment, pepsin, chymotrypsin and bile salts were added to the reaction solution comprising olive oil emulsion and Tris-HCl buffer with pH adjusted to 6. The final concentration of pepsin and chymotrypsin was 3.2 mg/ml and 4 mM, respectively. Control groups were free of pepsin and chymotrypsin. After 2 h of incubation, the residual activity was determined. The reaction condition was set at its optimum used in this study.

To study the effect of enzymes on hydrolysis degree based on corn-soybean feed containing 3.2% of soybean oil, we prepared a reaction system mimicking the digestive environment of chicken which contains bile salt, pepsin and chymotrypsin. Composition of the broiler feed used for lipolytic assay is given in Table 1. In each assay, 5.0 g of the corn-soybean feed containing triacylglyceride (TAG) (16 mg) was mixed with water (3 ml) and Tris-HCl buffer (pH 8.0, 5 ml). Subsequently, 0.5 ml free YLL supernatant liquid (9000 U) was added into the reaction system. Then, 0.08 g (9200 U) and 0.1 g (9320 U) of the formula prepared with skimmed milk and starch were added, simultaneously. When the experiments were performed with enzyme preparations in the water shake for 6 h, separation and quantitative analysis of free fatty acid (FFA) were carried out using Agilent 7890A/5975C gas chromatography and mass spectrometry (GC-MS) equipped with capillary (HP-INNOWAX, 30 m × 0.5 μm × 0.25 μm). Analysis of fatty acid methyl esters (FAMEs) extracted from the reaction product is a well-established procedure (Metherel et al. 2009).

Hydrolysis degree (%) of the TAGs in the diet was calculated by the following formula:

\[
\text{Hydrolysis degree (％)} = \left( \frac{\text{Total fatty acids in the diet, mg}}{\text{Free fatty acids from hydrolysis of TAGs, mg}} \right) \times 100
\]

The experimental protocols are as follows. Briefly, after the reaction, the solution was mixed with 10 ml Folch reagent (chloroform:methanol, 2:1, v/v); then, layers were separated using centrifugation for 10 min at 10,000 g. The chloroform layer was removed to exclude the contained precipitated proteins by adding 2 ml NaCl saturated solution, and it was dried in a Nitrogen Concentration (Thermolyne 62700) with heating at 37°C. According to previous studies, formation of FAMEs by esterification can be accomplished by several methods (Aparicio and Aparicio-Ruiz 2000). Here, 10 mg dried lipid samples were derivatized by 1% sulphurous-methanol solution at 70°C for 30 min. After cooling, FAMEs were extracted by adding 4 ml n-hexane. Then, 200 μl upper liquid was mixed with equal volume of internal standard for GC-MS analysis.

The GC-MS program was set as follows: The oven temperature was initially set at 150°C for 2 min, then increased from 150°C to 250°C at the rate of 10°C/min and maintained at 250°C for 10 min. The temperatures of the injector and connector were set at 250°C and 260°C, respectively. The MS program was set as follows: The time of solvent delay was 3 min with He flow rate of 1 ml/min. The temperatures of MS source and MS quad were set at 230°C and 150°C, respectively. The range of detectable molecular weight was between 30 and 500 (Yan et al. 2011).

Table 1. Ingredient and nutrient composition of broiler diets.

| Item                        | Starter (d 1–17) | Grower (d 18–42) |
|-----------------------------|-----------------|------------------|
| Ingredient, %               |                 |                  |
| Soybean meal (46% CP)       | 32.3            | 28.2             |
| Maize                       | 51.2            | 58.6             |
| Soy oil                     | 5               | 3.2              |
| Wheat                       | 1.5             | 5                |
| Vitamin-mineral premix      | 5               | 5                |
| Calculated nutrient analysis|                 |                  |
| AMEn (kcal/kg)              | 2965            | 3.88             |
| CP (%)                      | 21.1            | 19.7             |
| Lys (%)                     | 1.198           | 1.097            |
| Met (%)                     | 0.492           | 0.472            |
| Thr (%)                     | 0.781           | 0.755            |
| Ca (%)                      | 0.79            | 0.78             |
| Na (%)                      | 0.34            | 0.34             |
| P (%)                       | 0.57            | 0.55             |
2.5. Lipase activities

Lipase activity was determined by the titrimetric method using an olive oil emulsion. Olive oil (5%) was emulsified in distilled water containing 2% (w/v) of PVA in a homogenizer for 10 min. Then 1 ml enzyme solution, pure or diluted, depending on the quantity of lipase, was added to 5 ml of substrate emulsion and 4 ml of 50 mM Tris-HCl buffer, pH 8.0. Samples were incubated for 10 min at 40°C. The reaction was stopped by adding 15 ml acetone–ethanol mixture (1:1 [v/v]). Enzyme activity was determined by titration of the fatty acid released with 50 mM NaOH. Phenolphthalein was used as an indicator. One activity unit of lipase was defined as the amount of enzyme which released 1 µmol of fatty acid per minute under assay conditions (Destain et al. 2005; Yan et al. 2007).

2.6. In vivo effects of YLL supernatant on growth performance in broilers

In total, 500 one-day-old Arbor Acres 264 chickens were obtained from a commercial hatchery. The birds were weighed at d 1 and distributed into litter-floor pens of identical size. The dietary treatments were control diet without feed additives and 2 additional diets with 4 or 6 U/g of YLL, with 4 replicates of 22 birds each. The feeding program consisted of a starter diet, which was fed until d 17, and a grower diet, which was fed from d 18 until 42, and was based on soybean, maize and wheat, as shown in Table 1. All broilers received ad libitum access to food and water. The animals were not subjected to several other antibiotics as growth promoters. The lighting program was as follows: d 1–4, 24L:0D; d 5–7, 23L:1D; d 8–35, 22L:2D and d 36–42, 24L:0D. The room temperature was initially set at 34°C and was accordingly decreased to reach 18°C by d 42, which was the last day of the experiment. The environment in the house and the lighting program were commanded by sensors and timers, respectively.

Date on the BW and feed consumption were measured and used to calculate the performance at 17 and 42 d of age (BW gain, feed intake and feed conversion ratio (FCR)). The FCR was calculated by using this formula (Ahmad et al. 2012):

\[
\text{FCR} = \frac{\text{feed intake (total feed offered} - \text{total feed residue})\,(\text{g})}{\text{BW gain (total final wt} - \text{total initial wt + total mortality wt})\,(\text{g})}
\]

2.7. Statistical analysis

Data were analysed by ANOVA with the statistical package SPSS 20.0 for Windows (IBM Corp., Somers, NY), with the results expressed as mean ± standard deviation (SD). A Student’s t-test was also carried out to assess the significance differences between two groups. The significance level was set at \( P < 0.05 \). These analyses were performed within each production phase with 10 observations for each dietary treatment.

3. Results and discussion

3.1. Effect of pH on lipases stability

A good feed additive lipase candidate must be resistant to low pH values (Aloulou et al. 2015). In the gizzard stomach, the pH is very acidic (pH 2–4). However, post-prandial, the acidity is quite neutralized and higher gastric pH values of 4 can be reached.

The effect of pH on the stability of lipases is shown in Figure 1. Similar to previous reports (Aloulou, Puccinelli, et al. 2007; Aloulou, Rodriguez, et al. 2007; Fickers et al. 2011), YL lipase was stable between pH 3.5 and 9.0, but the enzyme inactivation occurred at extreme pH conditions. Our results showed that all four lipases deactivated under extreme gastric acidity. However, at higher pH (4–5), highest lipase activity was observed for YLL; 52% and 75% of its maximum activity were maintained at pH 4 and 5, respectively (Figure 1). By contrast, at pH 4, significant degradation of the other three lipases was observed after 2 h of incubation. Although CRL showed the best stability among them, merely 22% and 39% of the initial activity was maintained at pH 4 and 5, respectively. It is obviously demonstrated that YLL was more stable in quite neutral and acidic conditions. The results show that YLL was suggested as a potential candidate in an application of the feed industry for stability in low pH (pH 4 or 5) by comparison with ROL, TLL and CRL. Therefore, YLL can fulﬁl this requirement due to its stability when acting on dietary TAGs at low pH values.

3.2. Effect of bile salts and proteases on YLL stability and hydrolysis degree based on corn-soybean feed mimicking environment of chicken

In this study, residual activity of the culture supernatant was compared to that of the supernatant formulated with skimmed milk and starch by spray-drying after 2 h of incubation with and without bile salts and proteases at pH 6. For both free YLL and two formulations, no significant differences in residual activity after incubation without bile salts and proteases were observed (Figure 2). However, significant degradation of lipase was
observed when free YLL was incubated in the bile salts and proteases. Under these conditions, approx. 70% of the initial activity was lost after 2 h of incubation. By contrast, in the presence of skimmed milk powder and starch as additives, higher resistance of lipase to bile salts and proteases was obviously observed. Of the initial activity, 58% and 48% were present after 2 h of bile salts’ and proteases’ treatment of formulations with skimmed milk powder and starch at pH 6, respectively.

Without additives, it was impossible for lipases to maintain high activity after 2 h of incubation at lower pH values. The fact was due to the water content which disorganized the protein conformation. As previously described (Suzuki et al. 1997; Dokie et al. 1998; Millqvist-Fureby et al. 1999), the powder formulated with milk powder showed enzyme-stabilizing abilities, mainly due to the readily attainable amorphous form. In addition, best stability was observed when the water activity ($a_w$) was stable at 0.4. When $a_w$ was increased to 0.75, enzymatic activity decreased. The stability of enzymatic activity per gram of dry matter was correlated to the water activity of dry powders (Lamikanra and Watson 2007). As previously demonstrated for YLL formulations using skimmed milk as additive, some compounds of the milk reduced the surface energy and decreased the oil/water interfacial energy and allowed the enzyme to adsorb to TAGs even in the presence of high concentration of bile salts (Fickers et al. 2006).

Therefore, in the presence of starch and skimmed milk as additives, better stability of the lipolytic activity could be maintained at a pH range of 4–7, suggesting that the enzyme would not be affected substantially by changes in pH that occur throughout the digestive process in the stomach and small intestine of broilers. Although the formulation with skimmed milk was the first formulation most often used during lipase downstream processing and showed enzyme-stabilizing abilities in several studies, starch presents the advantage of being an attractive additive owing to the low cost.

In vitro hydrolysis degree of FFAs of free YLL was compared with formulations with bile salts and proteases after 6 h of reaction at pH 6 (Figure 3). Our results showed formulations A and B exhibited higher hydrolytic activity, with most of the FFA reaching values above 60% and 56%, respectively. However, for free YLL, merely less than 40% of hydrolysis degree was observed due to degradation by digestive proteases and bile acids’ inhibitory effect.

3.3. Effect of the YLL feed additive on growth performance in the broiler chickens

The effects of supplementation of YLL on growth performance variables in broiler chickens fed corn-soybean diets were investigated. No significance mortalities were recorded between the groups (Table 2). The performance data for the broilers on days 17 and 42 of age are summarized in Table 2. From 1 to 17 d, and 18 to 42 d, mortality, feed intake, BW gain and FCR were not significantly influenced by the feed additive. Interestingly, in the overall (1–42 d) periods, it showed a significant ($P < .05$) effect on the FCR. Particularly, the FCR showed an improvement related to the increase of lipase dose in the diet.

The lack of responses on growth performance to exogenous enzyme has been reported in many studies in which broilers were fed corn-soybean diets (Kocher et al. 2003; Olukosi et al. 2007; West et al. 2007). However, it does not necessarily mean that enzyme products fail to work on their specific substrates (Cowieson and Adeola 2005). A previous study suggested that the effects of enzymes on growth performance are undetectable under these experimental designs (Meng et al. 2005). However, these imperceptible differences will bring much economic benefits when used in the industrial process.

4. Conclusion

As a suitable and warm place without bile salts and proteases, crop provides optimum environment for enzymatic degradation in the fat hydrolysis process. YLL may increase hydrolysis degree of fat in feed significantly when dietary was swallowed into crop. As the first internal organ in the digestive system, the...
Table 2. Effect of the YLL feed additive on growth performance in the broilers.

| Item                | Control         | 4 U/g | 6 U/g | Significance |
|---------------------|-----------------|-------|-------|--------------|
| Mortality (%)       |                 |       |       |              |
| 0–17 d              | 1.25 ± 2.5      | 3.75 ± 4.8 | 2.50 ± 2.9 | NS           |
| 18–42 d             | 5.00 ± 4.7      | 3.75 ± 4.8 | 3.75 ± 4.8 | NS           |
| 0–42 d              | 7.50 ± 6.6      | 6.25 ± 2.5 | 3.75 ± 4.8 | NS           |
| Feed intake (g)     |                 |       |       |              |
| 0–17 d              | 696.8 ± 25.4    | 705.8 ± 35.7 | 690.4 ± 15.6 | NS           |
| 18–42 d             | 4351.5 ± 65.2   | 4232.3 ± 88.1 | 4278.2 ± 61.7 | NS           |
| 0–42 d              | 5048.2 ± 75.4   | 4954.0 ± 119.1 | 4968.7 ± 70.8 | NS           |
| BW gain (g)         |                 |       |       |              |
| 0–17 d              | 489.2 ± 26.6    | 489.1 ± 16.6 | 480.6 ± 28.9 | NS           |
| 18–42 d             | 2584.9 ± 159.2  | 2608.2 ± 76.5 | 2635.3 ± 77.5 | NS           |
| 0–42 d              | 3062.1 ± 122.7  | 3094.7 ± 71.4 | 3150.6 ± 66.7 | NS           |
| FCR                 |                 |       |       |              |
| 0–17 d              | 1.427 ± 0.018   | 1.419 ± 0.020 | 1.440 ± 0.047 | NS           |
| 18–42 d             | 1.688 ± 0.088   | 1.636 ± 0.072 | 1.628 ± 0.052 | NS           |
| 0–42 d              | 1.650 ± 0.048\* | 1.591 ± 0.009\* | 1.577 ± 0.014\* | <0.05        |

Note: Results are reported as mean ± SD, and means represent four replicates per treatment. NS, no significance.

Ahmad H, Tian J, Wang J, Khan MA, Wang Y, Zhang L, Wang T. 2012. Effects of dietary sodium selenite and selenium yeast on antioxidant enzyme activities and oxidative stability of chicken breast meat. J Agr Food Chem. 60:7111–7120.

Alloue WAM, Destain J, Amighi K, Thonart P. 2007. Storage of Yarrowia lipolytica lipase after spray-drying in the presence of additives. Process Biochem. 42:1357–1361.

Aloulou A, Puccinelli D, De Caro A, Leblond Y, Carrière F. 2007. Comparative study on two fungal lipases from Thermomyces lanuginosus and Yarrowia lipolytica shows the combined effects of detergents and pH on lipase adsorption and activity. BBA-Mol Cell Biol. 1771:1446–1456.

Aloulou A, Rodriguez JA, Puccinelli D, Mouz N, Leclaire J, Leblond Y, Carrière F. 2007. Purification and biochemical characterization of the LIP2 lipase from Yarrowia lipolytica. BBA-Mol Cell Biol. 1771:228–237.

Aloulou A, Schué M, Puccinelli D, Milano S, Delchambre C, Leblond Y, Laugier R, Carrière F. 2015. Yarrowia lipolytica lipase 2 is stable and highly active in test meals and increases fat absorption in an animal model of pancreatic exocrine insufficiency. GastroenteroL. 149:1910–1919.e5.

Aparicio R, Aparicio-Ruíz R. 2000. Authentication of vegetable oils by chromatographic techniques. J Chromatogr A. 881:93–104.

Beauchemin KA, Colomboatto D, Morgavi DP, Yang WZ. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by Ruminants.12. J Anim Sci. 81:E37–E47.

Cowieison AJ, Adeola O. 2005. Carbohydrates, protease, and phythe have an additive beneficial effect in nutritionally marginal diets for broiler chicks. Poultry Sci. 84:1860–1867.

Destain J, Fickers P, Weekers F, Moreau B, Thonart P. 2005. Utilization of methylolate in production of microbial lipase. In: Twenty-sixth symposium on biotechnology for fuels and chemicals. Totowa (NJ): Humana Press; p. 269–277.

Dokic P, Jakovlijevic J, Dokic-Baucal L. 1998. Molecular characteristics of maltodextrins and rheological behaviour of diluted and concentrated solutions. Colloid Surface A: Physicochem Eng Aspects. 141:435–440.

Fickers P, Fudalej F, Le Dall MT, Casaregola S, Gaillardin C, Thonart P, Nicaud JM. 2005. Identification and characterisation of LIP7 and LIP8 genes encoding two extracellular triacylglycerol lipases in the yeast Yarrowia lipolytica. Fungal Genet Biol. 42:264–274.

Fickers P, Fudalej F, Nicaud J-M, Destain J, Thonart P. 2005. Selection of new over-producing derivatives for the improvement of extracellular lipase production by the non-conventional yeast Yarrowia lipolytica. J Biotechnol. 115:379–386.

Fickers P, Marty A, Nicaud JM. 2011. The lipases from Yarrowia lipolytica: genetics, production, regulation, biochemical characterization and biotechnological applications. Biotechnol Adv. 29:632–644.

Fickers P, Ongena M, Destain J, Weekers F, Thonart P. 2006. Production and downstream processing of an extracellular lipase from the yeast Yarrowia lipolytica. Enzyme Microb Tech. 38:756–759.

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References

Adamczak M, Bednarski W. 2004. Enhanced activity of intracellular lipases from Rhizomucor miehei and Yarrowia lipolytica by immobilization on biomass support particles. Process Biochem. 39:1347–1361.

majority of FFA hydrolysis of fats may carry out in crop. This stage take the most important part of the hydrolysis process in the digestion of fat in broilers.

In this study, in vitro and in vivo assessment of the effect of Yarrowia lipolytica lipase on stability, hydrolysis and growth performance was done. It could be concluded that YLL supernatant displayed the best stability under low pH values among four lipases derived from different fungi. Comparing YLL with the two formulations spayed drying with skimmed milk powder and starch, the later showed better stability under low pH with proteases and bile salt. Especially, formulation A displayed better performance when compared with formulation B. After 6 h of reaction with corn-soybean diets as substrate under the same condition, formulation A displayed the highest hydrolysis degree.

Furthermore, the in vivo effect of Yarrowia lipolytica lipase on growth performance of broilers was studied. YLL significantly reduced FCR throughout the overall period. It is noteworthy that in cases of significant differences, the effects of enzyme additives on body weight gain, feed intake and FCR are often inconsistent. Therefore, due to complexity of comprehensive function of coenzyme and other compounds, further research is required to confirm the effects of feed enzyme preparations.
Kocher A, Choc M, Ross G, Broz J, Chung TK. 2003. Effects of enzyme combinations on apparent metabolizable energy of corn-soybean meal-based diets in broilers. J Appl Poultry Res. 12:275–283.

Lamikanra O, Watson MA. 2007. Mild heat and calcium treatment effects on fresh-cut cantaloupe melon during storage. Food Chem. 102:1383–1388.

Meng X, Slominski BA, Nyachoti CM, Campbell LD, Guenter W. 2005. Degradation of cell wall polysaccharides by combinations of carbohydrase enzymes and their effect on nutrient utilization and broiler chicken performance. Poult Sci. 84:37–47.

Metherel AH, Armstrong JM, Patterson AC, Stark KD. 2009. Assessment of blood measures of n-3 polyunsaturated fatty acids with acute fish oil supplementation and washout in men and women. Prostag Leukot Ess Fatty Acids. 81:23–29.

Millqvist-Fureby A, Malmsten M, Bergenståhl B. 1999. Spray-drying of trypsin – surface characterisation and activity preservation. Int J Pharmaceut. 188:243–253.

Moreau J, Bouisson M, Saint-Marc-Girardin MF, Pignal F, Bommelaer G, Ribet A. 1988. Comparison of fungal lipase and pancreatic lipase in exocrine pancreatic insufficiency in man. Study of their in vitro properties and intraduodenal bioavailability. Gastroenterol Clin Biol. 12:787–792.

Olukosi OA, Cowieson AJ, Adeola O. 2007. Age-related influence of a cocktail of xylanase, amylase, and protease or phytase individually or in combination in broilers. Poult Sci. 86:77–86.

Sarica S, Ciftci A, Demir E, Kilinc K, Yildirim Y. 2005. Use of an antibiotic growth promoter and two herbal natural feed additives with and without exogenous enzymes in wheat based broiler diets. S Afr J Anim Sci. 35:61–72.

Schmid RD, Verger R. 1998. Lipases: interfacial enzymes with attractive applications. Angew Chem Int Edit. 37:1608–1633.

Suzuki T, Imamura K, Yamamoto K, Satoh T, Okazaki M. 1997. Thermal stabilization of freeze-dried enzymes by sugars. J Chem Eng Jpn. 30:609–613.

Turki S, Mrabet G, Jabloun Z, Destain J, Thonart P, Kallel H. 2010. A highly stable Yarrowia lipolytica lipase formulation for the treatment of pancreatic exocrine insufficiency. Biotechnol Appl Bioc. 57:139–149.

West ML, Corzo A, Dozier WA, Blair ME, Kidd MT. 2007. Assessment of dietary Rovabio excel in practical United States broiler diets. J Appl Poultry Res. 16:313–321.

Yan J, Liu S, Hu J, Gui X, Wang G, Yan Y. 2011. Enzymatic enrichment of polyunsaturated fatty acids using novel lipase preparations modified by combination of immobilization and fish oil treatment. Bioresource Technol. 102:7154–7158.

Yan J, Yan Y, Madzak C, Han B. 2017. Harnessing biodiesel-producing microbes: from genetic engineering of lipase to metabolic engineering of fatty acid biosynthetic pathway. Crit Rev Biotechnol. 37:26–36.

Yan J, Yang J, Xu L, Yan Y. 2007. Gene cloning, overexpression and characterization of a novel organic solvent tolerant and thermostable lipase from Galactomyces geotrichum Y05. J Mol Catal B-Enzym. 49:28–35.

Zentler-Munro PL, Assoufi BA, Balasubramanian K, Cornell S, Benoliel D, Northfield TC, Hodson ME. 1992. Therapeutic potential and clinical efficacy of acid-resistant fungal lipase in the treatment of pancreatic steatorrhea due to cystic fibrosis. Pancreas. 7:311–319.

Zinjarde SS. 2014. Food-related applications of Yarrowia lipolytica. Food Chem. 152:1–10.