Research article

Growth Performance and Feed Utilization of Common Lowland Frog (*Rana rugulosa* Wiegmann) Fed with Supplementation by Bromelain Extracted from Pineapple Feed

RUNGKAN KLAHAN*
Faculty of Agricultural Technology, Phetchaburi Rajabhat University, Phetchaburi, Thailand
Email: rukiirun@windowslive.com

BANYAT SIRITHANAWONG
Faculty of Agricultural Technology, Phetchaburi Rajabhat University, Phetchaburi, Thailand

Received 15 December 2014 Accepted 7 April 2015 (*Corresponding Author)

Abstract This study aimed to determine the effect of bromelain extracted from the crown of pineapple supplemented in diet on growth performance and feed utilization of common lowland frog. Diets were isonitrogenous at 35% protein and 3,000 $KcalKg^{-1}$ supplemented at 0, 0.25, 0.5 and 1 mL bromelain extraction g^{-1} of feed. Frog with an initial weight of 0.25 ± 0.1 g were fed on feed trial in triplicate groups for 120 days. The results showed that the enzyme extracts did not effect growth performance but enhancement the survival rate with all levels ($P<0.05$) especially at 1 mL/g feed had the highest feed utilization and protein efficiency ratio (PER). The percentage of edible flesh of 0, 0.5 and 1 mL/g feed group were higher than the 0.25 mL/g feed group. These results demonstrated that bromelain extracted supplementation of all levels improved survival rate and feed utilization and PER especially at 1 mL/g feed.

Keywords common lowland frog, bromelain, pineapple, growth performance, feed utilization

INTRODUCTION

In 2011 the total area of pineapple cultivation in Thailand is around 104,571 hectare (646,000 rai). Prachuapkhirikhan has the largest share at 47% of the total pineapple cultivated areas of the country; other major growing provinces are Chonburi, Rayong, Trat, Chacheongsao, Kanchanaburi, Phetchaburi and Ratchaburi. Thailand is the world's number one exporter of canned pineapple. In 2011, Thailand exported canned pineapple totaling 641,167 tons (or 36,430 FCLs; 1 FCL = 17.6 tons), with the total value of 669.4 million dollars. Thailand's main export markets are United States, EU and Russia. The waste from pineapple (peel, core, stem and crown) generally is about for 50% of the whole fruit that causing a serious environmental problem (Ketnawa et al., 2012). The utilization of waste as feed additive in aquatic animal feed is the alternative way for handling the great deal of waste problem.

Bromelain is a mixture of proteolytic enzyme, belong to a group of protein digesting enzyme derived from the fruit or stem of pineapple (*Ananas comosus* L.) (Bhattacharyya, 2008; Bala et al., 2012). Bromelain is the most effectiveness in reduction of inflammatory and decreasing swelling as a natural anti – inflammatory enzyme. It's a mixture of different thiol endopeptidase and other components like phosphatase, peroxidases, glucosidases, glycoproteins, cellulose, carbohydratase and several protease inhibitors (Bhattacharyya, 2008). Bromelain is not very specific in action but preferentially cleaves glycol, anayl and leucyl bonds. The bromelain actions on protein predigestion, diarrhea, digestive aid, anti thrombotic, oedema treatment and osteoarthritis and promotes the absorption of antibiotic drug. (Corzo et al., 2012). It is contains, among other components, various closely related proteinases, anti - edematous, anti - inflammatory,

antithrombotic and fibrinolytic activities. Moreover, due to its safety and lack of undesired side effects, bromelain has earned growing acceptance and compliance among patients as a phytotherapeutical drug. The pharmacological properties depend on the proteolytic activity only. Bromelain contains several distinct cysteine proteinases that have similar but distinct amino acid sequences, as well as differences in proteolytic specificity and sensitivity to inactivation (Hale et al., 2005). From the mentioned properties, the bromelain from pineapple should be used as feed additive in frog feed. Because of the gastrointestinal tract of tadpole and juvenile frog are similar to fish larvae that required feed all the time that causes digestive aid and dead in final. In addition, at the disappearence of the tail stage the frog does not eat feed experiences mortality also. This problem is often found in aquatic animal larvae. The digestion depend on digestive enzyme such as protease, amylase and lipase especially protease because the frog is carnivorous and has the high protein requirement (Duellman and Trueb, 1994). From the properties of bromelain and the problem on digestion of frog, bromelain should be used as feed additive in frog feed for improving the digestion and depressing the digestive aid and the mortality.

OBJECTIVE

The purpose of this study was to investigated the ability of diet supplemented with bromelian extraction from pineapple on feed utilization, growth performance, percentage of edible flesh and survival rate of common lowland frog.

METHODOLOGY

Experimental Frogs

Frogs at 10 days old (initial mean weight 0.25 ± 0.10 g), produced in Petchaburi Rajabhat University aquaculture program were randomly stocked into 1 m^2 cement tank at a density of 30 frog per tank. Frogs were acclimated for 1 week with control feed (pellet feed without bromelain supplementation) before start the experiment.

Raw Material and Crude Extract Preparation

The pineapple (*Ananus comosus* L.) (“Batavia”) was collected from plantation in the Ratchaburi province, Thailand. The fruit was washed and air dried. The crown (Fig. 1) was separated and chopped to small pieces. This study used the crown because from the previous study it was found that the bromelain activity (from waste: peel and crown) was highest in crown. Small pieces of crown were blended with cold distilled water at a 1:1 ratio for 5 min and the blended liquid was filtered through a sieve (250 μm) and then centrifuged at 10,000 x g at 4 ^{o}C for 20 minnutes (Ketnawa et al., 2012). The supernatant (crude extract) will be collected to mix with feed.

Experimental Diets

Frogs were fed with floating pellet diet (35% protein, 3000 Kcalkg^{-1} energy). Proximate composition of the experimental diets is determined by analysis (Kjeldahl method for crude protein, Soxhlet method for lipid, Detergent method for crude fiber, Oven drying method for moisture and determination of ash by muffle furnace) (AOAC, 2000). Bromelain extraction was used at 0, 0.25, 0.5 and 1 mg^{-1} diet as a feed supplement. In the preparation of experimental diet, bromelain were mixed with a diet and control diet was also mixed with 1 mL water for 1 g feed. These pelleted diets were coated with fish oil at 1% and then incubated at 37 ^{o}C for 1 hour then dried in the hot air oven at 90 ^{o}C for 2 hours. The dry pellets were placed in covered plastic bag and stored at room temperature.

A B

Fig. 1 Pineapple whole fruit (A) and crown proportion (B)

Experimental Procedure

Each of the four experimental diets was randomly assigned to triplicate groups of frog and all the groups were fed with the prepared diet at 5% body weight twice daily for 120 days. Also, water was changed for every two days for 100% throughout the study.

Analytical Method

During experiment, the mortality was recorded daily and frog in each tank were monthly counted and weighed individually. Growth rate were monitored to determine the final weight, weighed gain, Specific Growth Rate (SGR), average daily gain, survival rate, feed intake, Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) were calculated according to Castell and Tiews (1980). At the start of experiment, 50 frogs randomly were dried for the determination of body proximate composition. At the end of the feeding trial, 10 frogs from each group (n = 30 frogs/group) were analyzed for final whole body proximate composition. Proximate composition of body was analyzed following the AOAC (2000) method.

Statistical Analysis

In the experiment, all data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. A significance level of $P<0.05$ was used.

RESULTS AND DISCUSSION

Growth Performance

The result of the growth trial showed that all groups were not significantly different ($P>0.05$) (Table 1). The mean final weight, weigh gain, ADG and SGR of different diets ranged between 84.94 – 98.62 g/f, 84.60 – 98.37 g/f, 0.91 – 1.11 g/f/day and 2.54 – 2.79 %, respectively. In the contrary, survival rate in all groups fed with supplemented bromelain feed showed significantly the highest survival rate ($P<0.05$). Bromelain is contains, among other components, various closely related anti - edematous, anti - inflammatory, antithrombotic and fibrinolytic activities that promote the great health effected on the high survival rate. The growth rate was similar with among group because frog is carnivorous which the density effected on the growth rate. The amount of frog after fed with supplement bromelain feed for 120 days has the high density proportion when compared with no supplement bromelain feed group so make the growth rate not outstanding.

Table1 Growth performance of frog fed with supplementation diet by bromelain extracted at 120 days

Growth performance	Bromelain supplementation (mL/g feed)			
	0	0.25	0.5	1
Initial weight (g/f)	0.24 ± 0.02	0.25 ± 0.01	0.25 ± 0.01	0.26 ± 0.00
Final weight (g/f)	98.62 ± 3.71	84.94 ± 2.55	89.92 ± 5.76	87.23 ± 6.75
Weight gain (g/f)	98.37 ± 3.73	84.70 ± 2.54	89.66 ± 5.76	86.79 ± 6.74
Average daily gain (g/f/day)	1.11 ± 0.13	1.11 ± 0.39	1.09 ± 0.25	0.91 ± 0.07
Specific growth rate (%/day)	2.79 ± 0.19	2.74 ± 0.03	2.72 ± 0.28	2.54 ± 0.08
Survival rate (%)	75.00 ± 2.36^{b}	85.55 ± 5.09^{a}	92.22 ± 1.92^{a}	91.11 ± 7.69^{a}

^{a,b,c} *Means within a row with common superscript are significantly different (P<0.05). n = 30*

Feed Utilization

The result of feed utilization of frog fed with supplementation with bromelain at different level for 120 days showed that diet containing 0.25 – 1 mL of bromelain per 1 g feed were significantly higher (P<0.05) than controlled group especially at 1 mL of bromelain per 1 g feed group resulted in the highest feed utilization i.e. the best FCR, FCE and PER and the worst feed intake (Table 2). Bromelain is a mixture of proteolytic enzymes, belonging to a group of protein digesting enzyme (Bhattacharyya, 2008 and Bala et al., 2012). It's a mixture of different thiol endopeptidase and other components like phosphatase, peroxidases, glucosidases, glycoproteins, cellulose, carbohydratase and several protease inhibitors (Bhattacharyya, 2008 Maurer, 2001). On the other hand, the highest caseinolytic activity at a pH 5.5 - 8 (Ketnawa et al., 2012; Corzo et al., 2012 and Bala et al., 2012) these cause make it has high digestibility efficiency. In addition, bromelain has been successfully used as a digestive enzyme following pancreatectomy, in case of exocrine pancreas insufficiency and the advantage is it has activity in the stomach as well as the small intestine which also shown to be an adequate replacement of pepsin and trypsin in case of deficiency (Bhattacharyya, 2008).

Table 2 Feed utilization of frog fed with supplementation diet by bromelain extracted at 120 days

Feed utilization	Bromelain supplementation (mL/g feed)			
	0	0.25	0.5	1
Feed intake (g/f/day)	1.56 ± 0.02^{a}	1.05 ± 0.15^{bc}	1.17 ± 0.24^{ab}	0.65 ± 0.00^{c}
Feed conversion ratio	1.64 ± 0.52^{a}	1.18 ± 0.14^{b}	1.47 ± 0.43^{ab}	0.80 ± 0.09^{c}
Feed conversion efficiency (%)	74.43 ± 8.03^{b}	85.42 ± 9.81^{b}	81.59 ± 12.01^{b}	133.99 ± 0.21^{a}
Protein efficiency ratio	1.84 ± 0.51^{b}	1.73 ± 1.23^{b}	2.04 ± 0.55^{b}	3.60 ± 0.38^{a}

^{a,b,c} *Means within a row with common superscript are significantly different (P<0.05). n = 30*

Carcass Composition

The effect of different bromelain supplementation level on carcass composition showed in Table 3. The results showed that there was no significant difference on percentage of bone and skin, viscera and fat and hepatosomatic index (HSI) (P>0.05) but it effected on percentage of edible flesh (P<0.05) that showed the highest on control group, 0.5 and 1 ml/g feed and has the lowest in 0.25 mL/g feed group. The composition of the amino acid (AA) mixture that was significantly influence the composition of the fish free amino acid (FAA) pool. However, when fish are fed a feed supplemented bromelain, a faster absorption of FAA may lead to transient AA imbalances and consequently to decreased protein utilization if crystalline AA are used to supplement dietary protein.

Table 3 Carcass composition of frog fed with supplementation diet by bromelain extracted at 120 days

Carcass composition (%)	Bromelain supplementation (mL/g feed)			
	0	0.25	0.5	1
Edible flesh	31.58 ± 1.34[a]	27.63 ± 1.69[b]	28.72 ± 0.54[ab]	30.21 ± 0.48[ab]
Bone and skin	41.71 ± 0.37	43.36 ± 1.46	44.30 ± 1.97	41.10 ± 2.07
Viscera and fat	28.84 ± 2.55	29.01 ± 1.81	28.77 ± 2.05	31.49 ± 0.14
Hepatosomatic index	6.04 ± 0.12	6.38 ± 0.10	5.59 ± 1.06	6.01 ± 0.82

[a,b,c] *Means within a row with common superscript are significantly different (P<0.05). n = 30*

CONCLUSION

Bromelain extracted from pineapple could be used as frog's survival rate promoter. The supplementation bromelain all levels improved survival rate, feed utilization and PER especially at 1 mL/g feed. It can be concluded that bromelain extracted from pineapple's crown is useful for aquaculture and environment to get rid of the waste from agriculture.

ACKNOWLEDGEMENTS

This research was supported by the grant of Phetchaburi Rajabhat University.

REFERENCES

A.O.A.C. 2000. Official methods of analysis. Virginia Association of Official Analytical Chemist, Inc.

Bala, M., Ismail, N.A., Mel, M., Jami, M.S., Salleh, H.M. and Amid, A. 2012. Bromelain production: Current trends and perspective. Archives Des Sciences, 65, 369-399.

Bhattacharyya, B.K. 2008. Bromelain: An overview. Natural Production Radiance, 7, 359-363.

Castell, J.D. and Tiews, K. 1980. Report on the EIFAC, IUNS and ICES. Working group on the standardization of methodology in fish nutrition research, Hamburg, Federal Republic of Germany. 21-23 March, EIFAC Technical Paper, 26 p.

Corzo, C.A., Krzysztof, N.W. and Jorge, W.C. 2012. Pineapple fruit bromelain affinity to different protein substrates. Food Chemistry, 133, 631-635.

Duellman E. and L. Trueb. 1994. Biology of amphibians. The Johns Hopkins University press, London.

Hale, L.P., Paula, K.G., Chau, T.T. and Cindy, L.J. 2005. Proteinase activity and stability of natural bromelain preparations. International Immunophamacology, 5, 783-793.

Ketnawa, S., Phanuphong, C. and Saroat, R. 2012. Pineapple wastes: A potential source for bromelain extraction. Food and Bioproducts Processing, 90, 385-391.