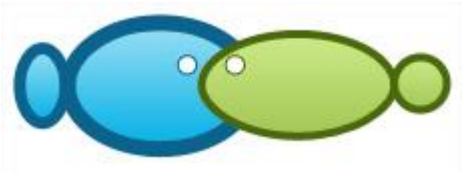

Effect of dietary exogenous protease supplementation on growth performance and flesh quality of Nile tilapia (*Oreochromis niloticus*) reared at different stocking densities

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Abstract. Limited information has been available about the influence of protease supplementation of different stocking densities on the performances of Nile tilapia, *Oreochromis niloticus*. This study evaluated exogenous protease supplementation's performance in improving *O. niloticus*'s growth at different stocking densities. This study's tilapia specimens were stocked at two different densities, 30 and 50 fish m^{-3}, the experiment was performed in triplicate, and the average initial weight was 119.67±4.32 g $fish^{-1}$. The protease was supplemented by top dressing in a commercial diet containing protein content not below 30% crude protein, with 500 ppm, and fed for eight weeks. The results revealed that the growth parameters of two different densities were similar for eight weeks ($p>0.05$). In contrast, feed conversion and protein efficiency ratios significantly improved for the fish reared at a stocking density of 50 fish m^{-3} ($p<0.05$). The amino acid composition in the flesh was not significantly different at $p>0.05$. On the other hand, the highest level of protein content from the metabolomic profiling of isoleucine and valine increased in fish flesh at a stocking density of 50 fish m^{-3} ($p<0.05$). The T/C ratio was likely to increase in fish reared at a density of 50 fish m^{-3}. There were no significant differences in springiness, hardness, and chewiness ($p>0.05$). The histological characteristics of the distal intestine in all experimental groups were similar. Findings indicated that tilapia fed with dietary protease supplementation in a stocking density of 50 fish m^{-3} exhibited a slightly increased growth performance and could significantly improve the feed utilization and flesh quality for Nile tilapia.
Key Words: dietary protease, growth parameters, metabolomic profiling.

Introduction. Tilapia, *Oreochromis niloticus*, is one of the most important species in aquaculture, global tilapia production is expected to increase by 2-4% in 2022 (FAO 2023). Asia is estimated to account for 5.3 million tonnes or 82% of global tilapia production in 2023. The intensive culture systems generate amounts organic matter consisting of uneaten feed, feces, nutrients waste, and living plankton and become major causes of water quality problems in aquaculture ponds and environmental impacts (Chowdhury et al 2013). The waste generated from aquaculture operations has become a major public concern regarding the sustainability a public concern (Martins et al 2010). Intensive culture is characterized by the ability to support extremely high stocking densities, which can produce very high yields on small parcels of land and limited water supply, including environmental control over parameters such as nutrient waste (Timmons et al 2002). Consequently, intensive tank culture in closed culture system offers several advantages over pond and cage culture. Therefore, there is a need to develop culture systems that will increase fish production with efficient waste management in order to limit pollution from fish farms and ensure its sustainability (Dauda et al 2019). Stocking density influences survival, growth, behavior, health, water quality, feeding and production. Increased stocking density can reduce yield because high stocking density tends to result in increased competition among fish for space and access to feed and thus reduced growth (Quiros 1999). Most studies show reduced final weight when stocking density increases (Osofero & Otubusin 2009; Garcia et al 2013; Daudpota

& Kalhoro 2014; Ronald et al 2014; Kapinga et al 2014). Thus, the optimum stocking density is the level where maximum yields are reached. The stocking density is an important indicator that determines the economic viability of the production system (Aksungu & Aksungur 2007).

Currently, the supplementation of diets with exogenous enzymes has been proven to improve nutritional value of diet and decreased the environmental pollution. The dietary exogenous enzymes could improve the growth performance by means of enhancing feed utilization and nutrient digestibility, increasing digestive enzymes activities, improving the histological structure and gut health. Exogenous enzymes may alter substrate availability for specific populations of gut microbes, which enhances nutrients digestibility and synthesis of nutrient substances for improve gut integrity and growth (Lin et al 2007; Jiang et al 2014; Castillo & Gatlin 2015). Previous studies evaluated the beneficial effects of protease supplementation in aquafeed (Li et al 2016; Shi et al 2016; Hassaan et al 2020; Wu et al 2020). Additionally, proteases are a class of enzymes that breakdown proteins into smaller peptides and amino acids (Bedford & Partridge 2010). Supplementation of protease can improve the productive performance of Nile tilapia, spare dietary protein and produce economical diets. Moreover, it can help in improving the water quality of fish via lowering the ammonia and nitrite contents (Saleh et al 2022). Drew et al (2005) observed that diet containing a mixture of rapeseed and pea meals supplementing with a commercial protease improved apparent nutrient digestibility and feed efficiency in rainbow trout (*Oncorhynchus mykiss*). It was suggested that exogenous protease could be supplemented in diet to improve the efficiency of dietary protein utilization in Gibel carp (*Carassius gibelio*) (Liu et al 2018). Consequently, there is a need for further studies to establish the benefits of dietary protease supplementation for performance of tilapia with appropriate stocking densities under a closed culture system.

Material and Method

Experimental fish and diet preparation. Sex reversed *O. niloticus* juveniles were obtained from a commercial farm in Khon Kaen, Thailand. Fish with an initial body weight of 119.67±4.32 g^{-1} were distributed randomly into six tanks (5 m^3) with two different stocking densities at 30 and 50 fish m^{-3} and three replicates per treatment of different stocking density. Fish were reared in a closed culture system and the feeding trial conducted at the Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Thailand. The protease complex obtained from Jefo Nutrition Inc., Saint Hyacinthe, QC (Canada) was supplemented by top dressing in commercial diet (protein content not below 30% CP) with 500 ppm. Water quality parameters (dissolved oxygen, water temperature, pH value and ammonia nitrogen) were monitored and controlled daily. The average temperature was 27±1°C. The pH ranged between 6.8 and 7.8. Dissolved oxygen levels stayed above 6.0 mg L^{-1}. Total ammonia remained below 0.1 mg L^{-1} during the trial.

Growth performance and feed utilization. During the feeding period, fish were fed manually twice daily (08:00 and 17:00) to apparent satiation for eight weeks. Fish were weighed every two weeks (deprived of diet for 12 h prior to weighing) to calculate, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER). The survival rate (SR) was also determined.

WG% = [(final weight (g)-initial weight (g)) / initial weight (g)] × 100
SGR = 100 x [ln final weight (g)-ln initial weight (g)]/time (days)
SR = 100x (final number of fish / initial number of fish)
FCR = feed intake / (final weight (g)-initial weight (g))
PER = wet weight gain (g) / protein intake (g)

Enzyme activity. Five fish from each tank at the end of the experiment were dissected on ice. The whole digestive tract was taken from fish and homogenized (1:2 w/v) with 50 mM Tris-HCl buffer (pH 7.5) on ice water bath, using a tissue homogenizer. The

preparation was centrifuged at 10,000 x g for 15 min, at 4ºC. The floating lipid fraction was removed and the aqueous supernatant was recovered and kept at -20ºC until analysis. The concentration of protein was analyzed by Lowry method (Lowry 1951) and expressed in g dL^{-1}. Total protease activity was determined according to Areekijseree et al (2004), using an azocasein as a substrate. Trypsin and chymotrypsin determination method was that described by Rungruangsak-Torrissen et al (2006). Trypsin activity was determined using a Nα-Benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPNA) as a substrate, chymotrypsin activity was determined using a N-Succinyl-Ala-Ala-Pro-Phe p-nitroanilide as a substrate. Total protease activity was expressed in g dL^{-1}; trypsin and chymotrypsin activities are expressed as produced p-nitroaniline μmol h^{-1} mg^{-1} protein.

Body indices and carcass composition. At the end of the trial, five fish in each tank were chosen randomly to determine viscerosomatic index (VSI), hepatosomatic index (HSI), fillet yield and carcass composition. Carcass composition included crude protein, crude lipid and ash were determined according to AOAC (2005). The amino acid content was performed according to GB/T 18246-2000, the solution was then filtered through a 0.22 μm PTFE filter for amino acid analysis by Reversed-phase high-performance liquid chromatography (RP-HPLC).

Metabolomic profiles. Fish fillets weighing about 100 mg per sample were taken. The metabolite was extracted with methanol and chloroform (1:1). Afterwards, the samples were centrifuged at 18,000 g for 15 mins to separate polar and lipophilic phases. The polar phase was transferred to a new tube and solvents were removed using a speed vacuum concentrator. Then, 600 μL of HPLC water were added to dried sample and vortexed until the sample completely dissolved. The 540 μL of supernatant were mixed with 60 μl of NMR buffer containing 100% D_2O, 1.5 M KH_2PO_4, 2 mM NaN_3 and 1% of 3-trimethysilypropionic acid (TSP). The total volume 560 μL of supernatant was transferred to NMR tube. The NMR spectra were acquired using NMR spectrometer (Bruker, USA).

Texture evaluation. Five tilapia fillets from each replication were used for the instrumental analysis of texture profile analysis (TPA). The fillets were dissected from the dorsal body section, about 1.5 cm above the lateral line. All fillets were cut into cubes of about 2×2×1 cm^3. and stored in ice. The cubes were arranged so that muscle fibers were oriented horizontally, compressed using an aluminum cylindrical probe of 35 mm at speed 2 mm s^{-1}. and a trigger force of 5 g. Two measurements were performed on each fillet and the mean value was used in the data analysis. All raw fillets were investigated for texture properties using a Texture Analyzer (Stable Micro Systems, TA-XT PLUS).

Intestinal histology. The distal intestine was dissected and fixed in 10% phosphate buffered formalin, dehydrated, embedded in paraffin wax and sectioned at 5 μm. All samples were stained with Haematoxylin and Eosin (H&E) and observed using a light microscope. Tissue slides were digitally photographed with a light microscope (Nikon Eclipse Ci) equipped with a CCD camera and NIS-elements D software. Intestine diameter was measured from side-to-side of serosa (SE); villus height was measured from the lowest point between two longitudinal villi to its tip (Peng et al 2013). Villus width and density were also measured.

Statistical analysis. Mean value and standard deviation (SD) were calculated from the results. Two-way analysis of variance (ANOVA) was applied for comparison of the mean values, with $p<0.05$ established as significance level. Statistical analyses were performed using the SPSS version 26.0 software.

Results. The results on growth parameters and feed utilization after fish were fed with protease supplements with different stock densities under a closed culture system are displayed in Table 1. The statistical results of the WG, ADG and SGR were not significantly different ($p>0.05$) among all experimental groups. The lowest level of FI was found in stocking density at 50 fish m^{-3} ($p<0.05$) and FCR and PER value were improved.

The improvement in feed utilization with protease supplementation was comparable between the two stocking densities, improving the FCR from 1.39 to 1.25 and PFR from 2.28 to 2.51 for the 30 and 50 fish m^{-3}, respectively. The overall survival rate within the trial was unaffected by different densities ($p>0.05$).

Table 1

Growth performance and feed utilization of *Oreochromis niloticus* fed with protease supplementation in different densities

Parameters	*Different densities (fish m^{-3})*		*p-value*
	30	*50*	
FI (g $fish^{-1}$)	563.01±9.72[a]	544.14±5.63[b]	0.039
WG (g $fish^{-1}$)	406.00±5.29	437.33±2.96	0.477
SGR (% day^{-1})	1.98±0.04	2.05±0.02	0.368
SR (%)	94.67±1.68	95.33±1.64	0.978
FCR	1.39±0.10 [a]	1.25±.002[b]	0.042
PER	2.28±0.15[b]	2.51±0.04[a]	0.037

Values within the same row with different letters are significantly different ($p<0.05$). Absence of letters indicates no significant difference between treatments. FI: feed intake, WG: Weight gain; SGR: specific growth rate, SR: survival rate; FCR: feed conversion ratio; PER: protein

Specific activities of total protease and trypsin of tilapia were not different ($p>0.05$), while the chymotrypsin was significantly lower in fish reared with a density of 50 fish m^{-3} ($p<0.05$). The T/C ratio was likely to increase in fish were reared with density 50 fish m^{-3} (Table 2).

Table 2

Enzyme activity of *Oreochromis niloticus* fed with protease supplementation in different densities

Parameters ($h^{-1}mg\ protein^{-1}$)	*Different densities (fish m^{-3})*		*p-value*
	30	*50*	
Protease	2.45±0.09	2.18±0.04	0.058
Trypsin	97.62±3.30	107.23±2.04	0.197
Chymotrypsin	4.70±0.84[a]	2.03±0.30[b]	0.009
T:C ratio	26.49±4.49	62.02±8.50	0.090

Values within the same row with different letters are significantly different ($p<0.05$). Absence of letters indicates no significant difference between treatments.

Body indices and flesh composition analysis are summarized in Table 3. The results showed that the fish reared with a density of 50 fish m^{-3} occasioned the highest level of protein content ($p<0.05$), but no significant differences were observed in lipid among dietary treatments (8.63-8.97%) ($p>0.05$).

Table 3

Body indices and flesh composition of *Oreochromis niloticus* fed with protease supplementation in different densities

Parameters	*Different densities (fish m^{-3})*		*p-value*
	30	*50*	
Edible flesh (%)	35.61±0.66	37.56±0.61	0.834
Protein (%)	74.97±1.17[b]	80.35±0.43[a]	0.021
Fat (%)	8.63±0.38	8.97±0.43	0.679
HSI	1.86±0.15	2.23±0.12	0.416
VSI	8.56±0.25	8.21±0.31	0.567

Values within the same row with different letters are significantly different ($p<0.05$). Absence of letters indicates no significant difference between treatments; HSI: hepatosomatic index; VSI: Visceral somatic index.

The percentage of edible flesh, HSI and VSI were unchanged by inclusion protease for fish reared in different stocking densities ($p>0.05$). The effect of dietary protease in different stocking densities on tilapia fillet texture is shown in Table 4. There were no significant differences in TPA among the different samples ($p>0.05$). The results of essential amino acid accumulation are presented in Table 5. There were no significant differences in amino acid levels in the flesh of fish among all trial groups ($p>0.05$).

Table 4

Texture properties of *Oreochromis niloticus* fed with protease supplementation in different densities

Parameters	*Different densities (fish m^{-3})*		*p-value*
	30	*50*	
Cohesiveness	9.42±1.34	7.51±1.40	0.421
Springiness	1.91±0.36	2.15±0.35	0.353
Hardness (gf)	3420.78±132.79	3882.24±533.36	0.225
Chewiness (gf)	60388.01±7305.53	61319.80±10604.04	0.136

Total essential amino acid was showed an improved trend by the addition of protease in fish were reared with density 50 fish m^{-3}. Absolute quantification and grouping of metabolomics of the muscle from tilapia fed with protease supplementation in different stoking densities is shown in Table 6. The representative 1H NMR spectra from muscles is shown in Figure 1. A total of 25 metabolites were found. Tilapia muscle from different groups shared similar spectral profiles. However, only a few differences were observed in the metabolomic profiling of fillet from the experimental group: there were increased concentrations of isoleucine and valine in tilapia reared at a density of 50 fish m^{-3}.

Table 5

Essential amino composition in flesh of *Oreochromis niloticus* fed with protease supplementation in different densities

Parameters (%)	*Different densities (fish m^{-3})*		*p-value*
	30	*50*	
Arginine	4.83±0.05	5.06±0.01	0.251
Histidine	1.77±0.02	1.91±0.01	0.484
Isoleucine	3.29±0.02	3.51±0.01	0.251
Leucine	5.59±0.15	6.04±0.02	0.168
Lysine	6.29±0.13	6.69±0.06	0.551
Methionine	2.12±0.04	2.27±0.01	0.278
Phenylalanine	2.99±0.04	3.24±0.02	0.532
Threonine	3.20±0.08	3.45±0.01	0.075
Tryptophan	2.49±0.04	2.66±0.01	0.312
Valine	3.61±0.02	3.81±0.01	0.485
ΣEAA	36.18	38.64	

Values within the same row with different letters are significantly different ($p<0.05$). Absence of letters indicates no significant difference between treatments; ΣEAA: sum of essential amino acids.

Table 6

Absolute concentrations of metabolites (mM) in muscle from *Oreochromis niloticus* fed with protease supplementation in different stoking densities

Metabolites	*Different densities (fish m^{-3})*		*p-value*
	30	*50*	
Isoleucine	$0.016±0.003^{b}$	$0.217±0.020^{a}$	0.040
Valine	$0.010±0.024^{b}$	$0.047±0.064^{a}$	<0.0001
Lactate	7.213±0.619	5.773±3.513	0.060

Metabolites	*Different densities (fish m^{-3})*		*p-value*
	30	*50*	
Alanine	0.390±0.095	0.320±0.217	0.324
Acetate	0.107±0.015	0.067±0.040	0.250
Dimethylarsinic acid	0.330±0.262 [a]	0.023±0.015 [b]	0.007
Acetone	0.083±0.006	0.067±0.006	1.000
Succinate	0.320±0.046	0.213±0.083	0.465
Isobutyric acid	0.027±0.021	0.030±0.035	0.531
Trimethylamine	0.000±0.000[b]	0.007±0.006 [a]	<0.0001
Phosphocreatine	6.913±0.401	7.101±0.723	0.471
Choline	0.080±0.061	0.107±0.025	0.292
Tuarine	8.250±1.868	6.510±1.626	0.862
Sodium dimethylarsinate	1.143±0.061	1.017±0.115	0.440
Glycine	5.537±0.735	4.823±1.775	0.293
Betaine	0.100±0.020	0.073±0.055	0.233
Glycolic acid	0.350±0.064	0.307±0.064	0.289
Gallic acid	0.170±0.020	0.070±0.111	0.063
Xanthine	0.197±0.015	0.103±0.071	0.089
Hypoxanthine	0.650±0.142	1.173±0.780	0.064
Adenine	0.370±0.260	0.917±0.991	0.129
Hydrochlorothiazide	1.443±0.108 [a]	1.150±0.968 [b]	0.025
Formate	0.460±0.115	0.343±0.142	0.793
Purine	0.130±0.020	0.077±0.061	0.193
Adenosine monophosphate	1.370±0.154	0.993±0.823	0.068

Values within the same row with different letters are significantly different ($p<0.05$). Absence of letters indicates no significant difference between treatments.

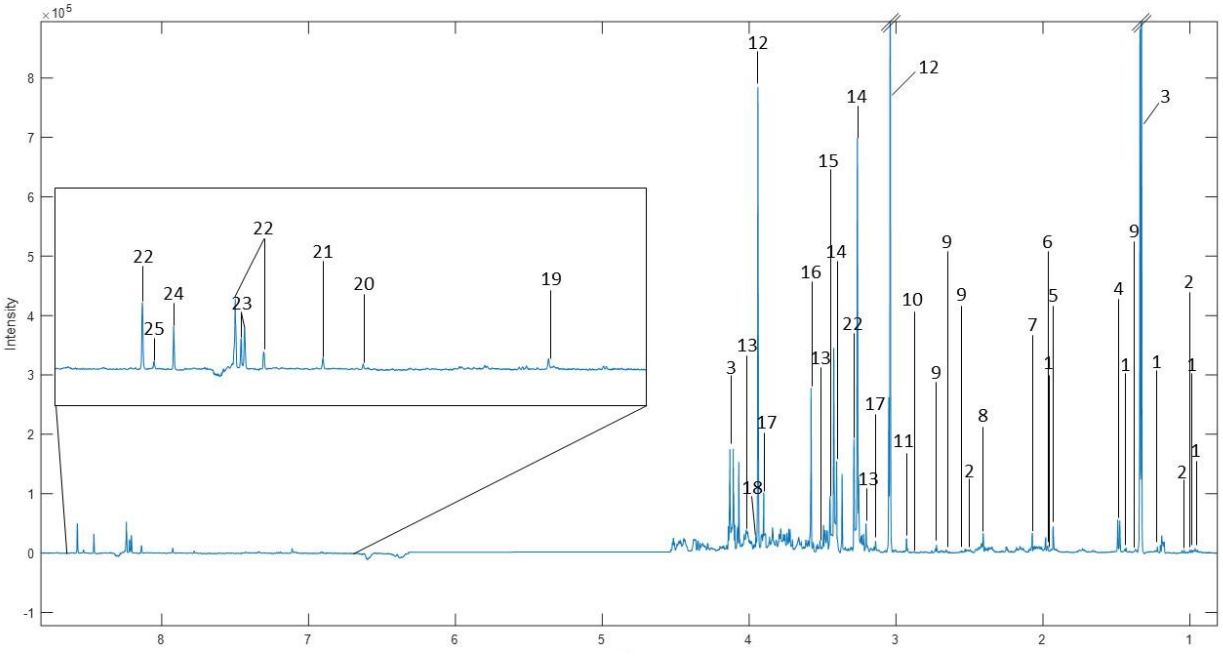

Figure 1. Representative one-dimensional ^{1}H-NMR spectrum of muscle sample obtained from *Oreochromis niloticus* fed with protease supplementation in different stock densities. Keys: (1) Isoleucine (2) Valine (3) Lactate (4) Alanine (5) Acetate (6) Dimethylarsinic acid (7) Acetone (8) Succinate (9) Isobutyric acid (10) Trimethylamine (11) Unknown (12) Phosphocreatine (13) Choline (14) Taurine (15) Sodium dimethylarsinate (16) Glycine (17) Betaine (18) Glycolic acid (19) Gallic acid (20) Unknown (21) Xanthine (22) Unknown (23) Adenine (24) Formate (25) Purine.

The histology of distal intestines is displayed in Figure 2. In distal intestines, the results indicated no remarkable differences in the intestinal villi, lamina propria and muscular layers among treatments. There were no remarkable pathogenic signs observed in distal intestines of trial fish. Supplementing protease in different stoking densities did not lead to a further effect on villi length (390.32±9.09 and 385.64±9.95 µm, respectively).

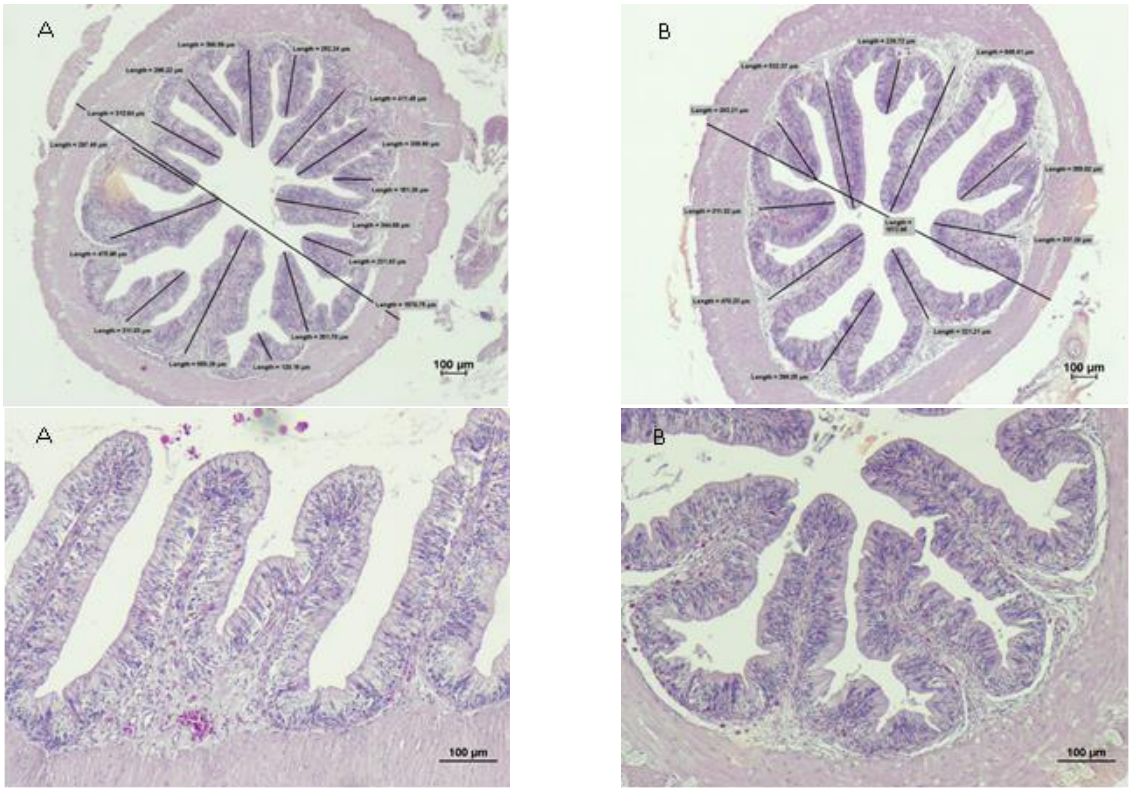

Figure 2. Histological sections of the distal intestine of *Oreochromis niloticus* fed with protease supplementation in different stock densities. A: Stocking density at 30 fish m^{-3}, B: Stocking density at 50 fish m^{-3}; ID= intestinal diameter, VH = villus height; SE = serosa; GCs: goblet cells (scale bar = 100 µm, 10x).

Discussion. Stocking density is one of the important factors in fish growth and productivity of aquaculture systems. Numerous studies have revealed the positive and negative effects of stocking density on growth performance in some fish species (Ridha 2005; Ni et al 2016; Qi et al 2016). With the development of intensive aquaculture systems, the inappropriate stocking densities has an impact on fish rearing. North et al (2006) indicated that high stocking density has caused chronic stress in farmed fish. Growth, survival and yield effects of stocking density on aquaculture is well known for a diversity of species and seem to differently impact production (Mazlum 2007; Garr et al 2011; Zhu et al 2011). Moreover, rearing aquatic animal species at low stocking densities may increase growth performance and survival rate in African catfish, *C. gariepinus* (Naggar et al 2006), in Thai climbing perch, *Anabas testudineus* (Uddin et al 2016), in Amur sturgeon, *Acipenser schrenckii* (Zhu et al 2011), in silver catfish, *Rhamdia quelen* (Menezes et al 2015), in Crayfish, *Astacus leptodactylus* (Mazlum 2007), in *Oreochromis* spp. (Sorphea et al 2010) and in giant freshwater prawn, *Macrobrachium rosenbergii* (Cuvin-Aralar et al 2007). Stocking density is one of the most crucial parameters in aquaculture, as it can significantly impact the growth, health, and overall performance in multiple ways (Garr et al 2011; Mazlum 2007; Zhu et al 2011). High stocking densities in aquaculture can have adverse effects on the growth performance and survival rates (Sorphea et al 2010) but in some cases this effect is either temporary (Garr et al 2011) or absent (Southworth et al 2009). The results of this study also show that there was no significant difference in fish survival regardless of the different level of exogenous

protease supplementation and stocking density involved. Numerous studies have reported that supplementing fish diet with exogenous enzymes can enhance the utilization of dietary protein and amino acids, leading to improved growth performance in various fish species (Farhangi and Carter 2007; Lin et al 2007; Soltan 2009; Shi et al 2016; Hassaan et al 2020; Wu et al 2020). In this present study, the T/C ratio was likely to increase with the increasing in fish were reared with density 50 fish m^{-3}. The phenomenon of improved growth performance with exogenous protease supplementation in fish diet suggests that proteases play a crucial role in supporting a rapid growth. The protease activity ratio of trypsin to chymotrypsin (T/C ratio) is higher during rapid growth period (Rungruangsak-Torrissen et al 2006).

The present data clarified that fish reared at a density of 50 fish m^{-3} occasioned the highest level of protein content ($p<0.05$). This finding contradicts the study of Lin et al (2007), which revealed that when tilapias were fed diets supplemented with an exogenous commercial enzyme complex containing neutral protease, β-glucanase, and xylanase, no significant differences were observed in the whole body's moisture, protein, lipid, and ash content compared to fish fed the control diet. The texture properties of fish are an important quality characteristic that is the most influent on the consumer acceptance (Sigurgisladottir et al 1999). Texture Profile Analysis (TPA) is a well-established method used to assess the textural properties of various fish species (Carbonell et al 2003; Casas et al 2006; Lin et al 2012; Wu et al 2020). In this study, hardness and springiness significantly decreased with increasing stocking density. Hardness represents the internal force and, in most cases, is related to the tensile strength of the sample. The soft texture has an impact on both fish quality and consumer acceptance (Ashton et al 2010). Furthermore, the gumminess is defined as the product of hardness and cohesiveness, and chewiness is defined as the product of gumminess and springiness (hardness × cohesiveness × springiness) (Bourne 2002). Therefore, gumminess and chewiness show the same trends as hardness and springiness in this present study. The results showed that high stocking densities may reduce the texture properties and flesh quality of fillets. Roth et al (2006) reported that fish which have been subjected to acute stress, such as during harvesting or transport, often exhibit a softer texture compared to fish that have been allowed to rest and recover. This phenomenon is likely due to the physical stress placed on the muscle fibrils or connective tissue during the stressful event.

The metabolomics profiling reveals the substrate and products of metabolism, playing a crucial role in cellular functions and their metabolite concentration and being influenced by the ingredients or dietary changes (Brennan et al 2015; Johnson et al 2016). Nuclear Magnetic Resonance (NMR) spectroscopy is a well-established analytical technique that has been widely used for exploring metabolic changes in various biological systems, including fish (Bankefors et al 2011; Kullgren et al 2010; Gribbestad et al 2005), in order to study the response to different diets. It can indicate that metabolomics profiling of fish tissues, such as white muscle, can provide valuable insights into the potential effects of diet on the final flesh quality of fish (Wagner 2015). In the current study on the metabolomic profiling of fillet in an experimental group, there were found increased concentrations of isoleucine and valine in tilapia reared at a density of 50 fish m^{-3}. Amino acids play a fundamental role in the growth and development of fish by serving as the building blocks for protein synthesis (Jasour et al 2017). Apart from protein synthesis, the branched chain amino acids (BCAAs), including leucine, valine and isoleucine, play important roles in the energy production, regulation of gene expression, gut health and immunity (Nie et al 2018). Trimethylamine (TMA) was found in high concentration in tilapia reared at a density of 50 fish m^{-3}. Li et al (2009) identified that trimethylamine is a naturally occurring compound found at high levels in the tissues of marine fish. TMA is produced by the bacterial reduction of trimethylamine N-oxide (TMAO) in the fish's body, as an oxidative stress prevention mechanism. Analyzing the metabolite profile of fish muscle can provide valuable insights into changes in the muscle metabolism in response to various factors, such as diet, environmental conditions, or health status (Wagner 2015).

Conclusions. The findings of the present study showed that FCR and PER was improved of fish reared at a stocking density of 50 fish m^{-3} and resulting in flesh composition with the highest level of protein content. But carcass composition did not show any significant differences in TPA and amino acid composition. The T/C ratio was likely to increase in fish were reared with density 50 fish m^{-3}. The metabolomics analyses showed that fish reared in a stocking density of 50 fish m^{-3} had an increase in muscle isoleucine. Different stock densities in fish fed with protease supplements did not cause histological alterations in distal intestine. Exogenous protease supplementation in tilapia diet enhanced T/C ratio and feed utilization, which is suitable for culture in a closed culture system at a relatively high density of 50 fish m^{-3}.

Conflict of interest. The authors declare that they have no conflict of interest.

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