ISSN 1682-8356
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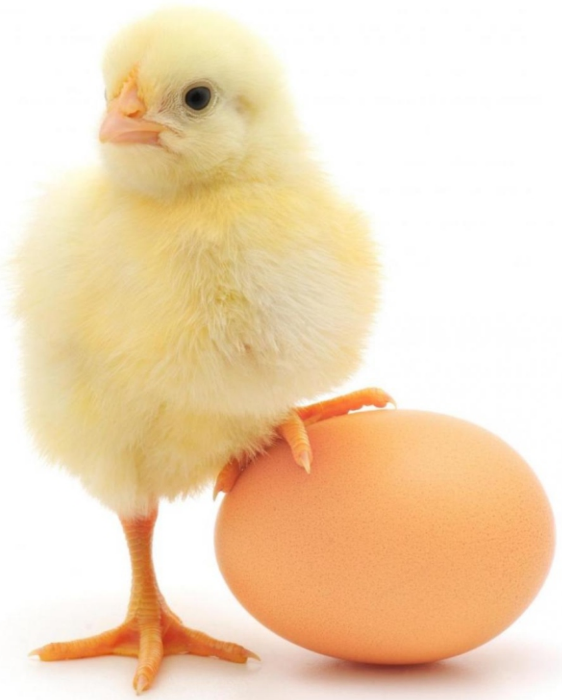

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2019.648.655

Research Article

Effect of Supplemental Multi-Enzymes in the Diet of Meat-Type Ducks on Production Performance, Carcass Yields and Gastrointestinal Morphology

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Abstract

Objective: The effect of mixed enzymes supplementation in the diet of meat-type ducks were determined on the production performance, carcass yield and gastrointestinal morphology from 1-45 days of age. **Materials and Methods:** A total of 384 ducks were divided into 4 treatments and each treatment consisted of 6 replicates of 16 ducks each. A completely randomized design with a 2×2 factorial pattern was used with 2 main effects: (1) Nutrients levels (D) (conventional diet, CD and low protein and energy diet, LD) and (2) Multi-enzyme supplementation (E) (non-supplemented, NS and supplemented, S). **Results:** In terms of nutrient levels, reduction of protein and energy in diet (LD) significantly depressed the average body weight (BW, $p<0.05$) and average daily gain (ADG, $p<0.05$) during the starter period. Moreover, the feed conversion ratio (FCR) of the LD groups was poor ($p<0.05$) throughout the experimental period. Supplementation with multi-enzymes (S) had no significant effect on production performance but reduced body fat accumulation (subcutaneous and abdominal fat, $p<0.05$). There was an interaction effect between D and E ($p<0.05$) on the villus height/crypt depth ratio (V/C ratio) of the duodenum, that is, the supplementation of multi-enzymes in the LD group decreased the V/C ratio. **Conclusion:** Throughout the study, low dietary protein and energy contents did not depress the growth rate since the ducks could compensate by increasing their feed intake to achieve a maximal growth rate, though consequently, the FCR was poor. Supplementation with multi-enzymes did not improve production performance but fat accumulation was reduced. The effects of supplemental multi-enzymes on gut morphology should be a future focus.

Key words: Multi-enzymes, production performance, carcass yields, gut morphology, meat-type ducks

Citation: Chudapun Puangkhum, Choawit Rakangthong, Theerawit Poeikhampha, Kanokporn Puangphong and Chaiyapoom Bunchasak, 2019. Effect of supplemental multi-enzymes in the diet of meat-type ducks on production performance, carcass yields and gastrointestinal morphology. Int. J. Poult. Sci., 18: 648-655.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In general, feed is the main cost in commercial animal production. However, many feedstuffs such as corn, wheat and soybean meal consist of non-starch polysaccharides (NSP) which disturb dietary carbohydrate and protein utilization, while monogastric animals do not have autozymes to digest them. Thus, supplementation using exogenous enzymes is widely used to improve the digestibility of NSP and protein. Water-insoluble NSP is considered practically indigestable and only soluble NSPs are partially digested by birds, while NSPs increase digesta viscosity, reduce the digestibility of nutrients and depress growth performance[1,2].

Xylanase is an enzyme of hydrolyzed NSP (including soluble and insoluble arabinoxylans) that releases the encapsulated nutrients from the cell walls to reduce digesta viscosity and improve poultry growth performance[3]. In addition, proteases increase protein digestibility through hydrolysis of stored and structural proteins and disrupt interactions of proteins with starch and fiber in the diets[4,5]. Supplementing protease in corn-soybean diets could potentially degrade proteins and some anti-nutritional factors (lectin and trypsin-inhibitor) in inadequately processed soybean meal[6], thereby improving poultry nutrient digestibility and growth performance. Therefore, the combination of xylanase and protease improved the growth performance, energy and nutrient availability and reduced nutrient excretion in broiler chickens[7,8], although Barekatain *et al.*[9] reported that the combination of xylanase and protease did not exhibit any significant synergy regarding the growth performance of the birds or nutrient utilization.

Although studies of enzyme supplementations have been widely conducted in chickens (broiler chickens and laying hens), such information regarding ducks is rather limited. Therefore, this study investigated the effects of adding multi-enzymes on growth performance, carcass yield and gut morphology responses of meat-type ducks fed a corn-soybean diet.

MATERIAL AND METHODS

Animal and managements: A total of 384 Grimaud meat-type ducks (192 male and 192 female) were randomly distributed to 24 pens. The ducks were divided into four groups and each group consisted of six replications of 16 ducks (8 male and 8 female) each. The experimental period was divided into two phases: starter (1-14 days) and grower (15-45 days) periods.

The ducks were kept in an evaporative cooling house system. The management and vaccination were provided according to commercial practice throughout the experimental period. Ducks received continuous light for 24 h daily, feed and water were provided *ad libitum*. Two hanging feeders/pen and an automatic drinker with six nipples/pen were used.

Experimental diets: The study was divided into two phases (starter: 1-14 days and grower: 15-45 days). Two main effects were investigated: 1) nutrient density (D: protein and energy contents) and 2) multi-enzyme supplemented (E: xylanase+β-glucanase+protease).

The multi-enzymes are referred to as the combination of xylanase+β-glucanase (0.01% diet) and protease (0.02% diet).The multi-enzymes were added according to the recommendation of each product as follows:

- **Conventionaldiet (CD):** Without the supplementation
- **Conventionaldiet (CD):** With multi-enzyme supplementation
- **Low nutrient diet (LD):** without the supplementation
- **Low nutrient diet (LD):** With multi-enzyme supplementation

Therefore, the protein and energy densities in the diets throughout the experimental period (starter and grower period) were calculated as follows:

- **Conventional diet (CD):** According to minimum nutrients requirement of Grimaud strain.

$$\text{Starter period} = 20.0\%\ \text{CP and } 2{,}900\ \text{ME kcal kg}^{-1}$$

$$\text{Grower period} = 18.0\%\ \text{CP and } 3{,}050\ \text{ME kcal kg}^{-1}$$

- **Low nutrient diet (LD):** Densities of protein and energy in CD diet were defined according to the matrix values of the enzymes.

$$\text{Starter period} = 19.20\%\ \text{CP and } 2{,}800\ \text{ME kcal kg}^{-1}$$

$$\text{Grower period} = 17.28\%\ \text{CP and } 2{,}950\ \text{ME kcal kg}^{-1}$$

For the basal diet, broken rice and soybean meal were used as the main source of energy and protein, respectively. All nutrient requirements were formulated according to recommendation for the Grimaud strain (Table 1 and 2).

Table 1: Experimental diets

	Starter (1-14 days)		Grower (15-45 days)	
Ingredient (%)	CD	LD	CD	LD
Broken rice	49.71	54.56	53.60	58.19
Rice bran oil	3.38	1.00	4.84	2.51
Soybean meal 48%	30.36	27.86	25.62	23.36
Rice bran	12.00	12.00	12.00	12.00
L-threonine	0.01	0.02	-	-
DL-methionine	0.24	0.24	0.14	0.14
Mono-dicalcium phosphate (22% phosphorus)	1.60	1.60	1.38	1.38
Calcium carbonate	1.72	1.74	1.44	1.45
Salt	0.42	0.42	0.42	0.43
Toxin binder	0.05	0.05	0.05	0.05
Premix[1]	0.50	0.50	0.50	0.50

[1] Premix (per kg) consists of; Vitamin A: 1,000,000 IU, Vitamin D3: 200,000 IU, Vitamin E: 2,500 IU, Vitamin B1: 50 mg, Vitamin B2: 500 mg, Pantothenic acid: 800 mg, Nicotinic acid: 4,000 mg, Vitamin B6: 300 mg, Folic acid: 100 mg, Vitamin B12: 1.5 mg, Biotin: 10 mg, Vitamin K3: 125 mg, Choline: 20,000 mg, $CuSO_4{\cdot}5H_2O$: 1,200 mg, $FeSO_4{\cdot}H_2O$: 4,500 mg, MnO: 7,000 mg, $ZnSO_4{\cdot}H_2O$: 3,700, $Ca(IO_3)_2$: 100 mg and $Na_2SeO_3{\cdot}5H_2O$: 15 mg

Table 2: Nutrients densities in t experimental diets

	Starter (1-14 days)		Grower (15-45 days)	
Nutrient by calculation	CD	LD	CD	LD
Metabolisable energy (kcal Kg^{-1})	2,900.00	2,800.00	3,050.00	2,950.00
Crude protein (%)	20.00	19.20	18.00	17.28
Methionine (%)	0.57	0.56	0.45	0.44
Methionine+cysteine (%)	0.85	0.83	0.70	0.68
Lysine (%)	1.06	1.00	0.94	0.89
Threonine (%)	0.75	0.73	0.66	0.64
Tryptophan (%)	0.25	0.24	0.22	0.21
Fiber (%)	3.76	3.66	3.51	3.42
Fat (%)	4.64	2.29	6.04	3.74
Calcium (%)	1.06	1.06	0.90	0.90
Total phosphorus (%)	0.83	0.83	0.76	0.75
Available phosphorus (%)	0.45	0.45	0.40	0.40
Sodium (%)	0.18	0.18	0.18	0.18

Production performance: The body weight was determined weekly (without feed deprivation). Average body weight (BW), average daily gain (ADG) and feed intake (FI) were calculated from pen data. The feed conversion ratio (FCR) was calculated by dividing the total feed consumption per pen by the total body weight of the surviving ducks in each phase. Mortality was checked twice daily.

Carcass yields: At the end of the study (45 days), four ducks/replication (two male and two female) were used for assessment of carcass yield following asphyxiation, scalding, plucking and eviscerating. The abdominal fat pad (including mesentery fat), breast meat (including pectoralis major and pectoralis minor), wing, leg meat (including thigh and drumstick) and subcutaneous fat were manually weighed. The fat index (breast meat weight: subcutaneous fat weight ratio) and body fat accumulation (abdominal fat+subcutaneous fat) were determined.

Small intestinal morphology: At the end of the study, one duck/replication (in total 24 ducks) were used for evaluation of the villus height, crypt depth and villus height/crypt depth ratio in each segment of the small intestine. The middle sections of the duodenum, jejunum and ileum tissue were collected. Consequently, the intestinal mucosa samples were embedded in paraffin; histological sections were obtained from tissue blocks cut perpendicular to the mucosal surface and stained with hematoxylin and eosin. Intestinal morphology was evaluated using a computer-assisted image-analysis system (Biowizard, Thaitec, Thailand).

Statistical analysis: A completely randomized design with a 2×2 factorial pattern was used with 2 main effects: (1) Nutrient density diets (CD and LD) described as D and (2) Multi-enzyme supplementation (non-supplemented, NS; supplemented, S) described as E. Statistical significance was tested at the $p<0.05$ level. Significant differences among treatments were tested using Duncan's multiple range test[10].

RESULTS AND DISCUSSION

Production performance: The effects of adding multi-enzymes in the diet on the production performance of meat-type ducks from 1-14 days of age are presented in Table 3. During the starter period (1-14 days of age), there was no interaction effect between D and E ($p>0.05$) on the BW, ADG, FI and FCR. Reduction of the nutrient content (D) resulted in a significantly poorer growth rate and FCR, while multi-enzymes (E) did not significantly affect any parameters of productive performance. Results for the grower period (15-45 days of age) and overall period (1-45 days of age) are shown in Table 4 and 5. There were no interactions between D and E ($p>0.05$) for any of the observations. A reduction in the nutrient content (D) resulted in a significantly poorer FCR, while there were no significant effects of supplemental multi-enzymes (E) on productive performance.

Low nutrient consumption generally reduces the productive performance of meat-type ducks[11,12]. In this study, both the growth rate and FCR of ducks fed the LD diet were significantly poorer than that of CD during the starter period, while only FCR was poorer for the grower or overall period. During the starter period (1-14 days of age), feed intake was not significantly affected by the reduction of protein (-4% of

Table 3: Effect of supplemental multi-enzymes in diet on growth performance of meat-type ducks during 1-14 days of age

		Diet (D)			p-value			
Items	Enzyme (E)	CD	LD	Average	D	E	D×E	SEM
BW (g)	NS	733.62	708.07	720.84				
	S	727.83	700.77	714.30	<0.05	0.50	0.94	5.30
	Average	730.72^{a}	704.41^{b}	717.57				
ADG (g day^{-1})	NS	48.88	47.05	47.96				
	S	48.47	46.53	47.50	<0.05	0.51	0.94	0.38
	Average	48.67^{a}	46.79^{b}	47.73				
FI (g)	NS	791.87	785.56	788.71				
	S	785.66	778.75	782.21	0.52	0.53	0.98	4.83
	Average	788.77	782.15	785.46				
FCR	NS	1.08	1.11	1.09				
	S	1.08	1.11	1.10	<0.05	0.94	0.94	0.01
	Average	1.07^{b}	1.11^{a}	1.09				

NS: Non-supplemented, S: Supplemented, ^{a,b}Means within the same row without the same superscript letter are significant different ($p<0.05$)

Table 4: Effect of supplemental multi-enzymes in diet on growth performance of meat-type ducks during 15-45 days of age

		Diet (D)			p-value			
Items	Enzyme (E)	CD	LD	Average	D	E	D×E	SEM
BW (g)	NS	2,804.53	2,769.75	2,787.14				
	S	2,782.00	2,775.53	2,778.76	0.48	0.77	0.62	13.51
	Average	2,793.27	2,772.64	2,782.95				
ADG (g day^{-1})	NS	90.47	89.35	89.91				
	S	89.74	89.53	89.64	0.48	0.77	0.62	0.44
	Average	90.11	89.44	89.77				
FI (g)	NS	5,664.05	5,739.69	5,701.87				
	S	5,548.14	5,682.96	5,615.55	0.21	0.30	0.72	40.87
	Average	5,606.10	5,711.32	5,658.71				
FCR	NS	2.02	2.07	2.05				
	S	2.00	2.05	2.02	<0.05	0.26	0.97	0.01
	Average	2.01^{b}	2.06^{a}	2.03				

NS: Non-supplemented, S: Supplemented, ^{a,b}Means within the same row without the same superscript letter are significant different ($p<0.05$)

Table 5: Effect of supplemental multi-enzymes in diet on growth performance of meat-type ducks during 1-45 days of age

		Diet (D)			p-value			
Items	Enzyme (E)	PC	NC	Average	D	E	D×E	SEM
BW (g)	NS	3,538.15	3,477.82	3,507.98				
	S	3,509.83	3,476.30	3,493.06	0.18	0.67	0.70	16.74
	Average	3,523.99	3,477.06	3,500.52				
ADG (g day^{-1})	NS	77.53	76.19	76.86				
	S	76.90	76.16	76.53	0.18	0.67	0.70	0.37
	Average	77.22	76.17	76.70				
FI (g)	NS	6,455.92	6,525.25	6,490.58				
	S	6,333.81	6,461.71	6,397.76	0.28	0.30	0.74	43.51
	Average	6,394.86	6,493.48	6,444.17				
FCR	NS	1.82	1.88	1.85				
	S	1.80	1.86	1.83	<0.05	0.20	0.95	0.01
	Average	1.81^{b}	1.87^{a}	1.84				

NS: Non-supplemented, S: Supplemented, ^{a,b}Means within the same row without the same superscript letter are significant different($p<0.05$)

total protein) and energy (-100 ME kcal kg^{-1}). Due to the physical and physiological limitations of a young animal[13], the ducklings were not able to increase their feed intake to make up for the deficit in their nutrient requirements. Consequently, both the growth rate and feed utilization (FCR) were negative. However, the ducks could increase their feed consumption to achieve a maximal growth rate during the grower period, while the FCR was still poor.

Supplementation with multi-enzymes improves the activities of endogenous digestible enzymes[14]. Engberg *et al.*[15] and Kang *et al.*[16] reported that supplementation with xylanase or multi-enzymes (xylanase, beta-glucanase, mannanase and cellulase) increased the activity of endogenous digestive enzymes in duck, with a consequent improvement in BW of 6-8% and in the FCR of 4-9%. However, in the current study, the supplementation with multi-enzymes in the diets had no significant effects on production performance throughout the study period. In agreement with this finding, Barekatain *et al.*[9] reported that supplementation with 0.025% xylanase and 0.02% protease in corn-soybean meal diets had no synergistic effect on the productive performance of broiler chickens during 1-21 days of age. The ineffective impact of enzyme supplementation may have been due to: (1) The limitation of substrate on which the enzymes could act[8], (2) The xylanase was digested by the exogenous protease[17] and (3) The xylanase may not have been fully utilized when the protease was added[9]. It has been reported that ducks could digest NSP more efficiently than chickens[18]. In general, ducks consume more drinking water than chickens (4.2:1 vs 2.3:1)[19]. A higher water intake would result in a higher digesta water content, which could translate into lower digesta viscosity that reduces the negative impact of the high viscosity of feed ingredients[20]. Therefore, there could be reduced efficiency from enzyme supplementation in ducks compared to chickens.

Carcass yields: The effects of multi-enzymes supplementation in the diets on the carcass yields of ducks at 45 days of age are presented in Table 6. There were no significant interactions between the nutrient content (D) and enzyme supplementation (E), a reduction in the nutrient content (D) significantly increased the percentage of wing weight, where as supplemental multi-enzymes (E) significantly reduced subcutaneous fat, abdominal fat and body fat accumulation.

It was not surprising that there were no significant differences in the carcass components (except wing weight), since the ducks fed the LD diet could compensate their feed intake to meet their nutrient requirements. This result indicated that as long as the ducks could increase the amount of feed intake to meet nutrient requirements, carcass components such as edible meat (breast and leg meat) would not be negatively affected by applying the LD diet. Supplementation of multi-enzymes in the duck diets did not improve breast and leg meat production for either the CD or LD diets. Similarly, Zakaria *et al.*[21] reported that supplementation with multi-enzymes could not improve carcass characteristics such as the whole carcass weight, dressing carcass, breast, thighs and wings of broiler chicken. Interestingly, multi-enzyme supplementation clearly reduced the fat accumulation (abdominal and subcutaneous fat) of ducks, although the mechanism of this phenomenon is unclear and further investigation is required.

Small intestinal morphology: The effects of supplemental multi-enzymes in the diet on the small intestinal morphology

Table 6: Effect of supplemental multi-enzymes in diet on carcass yields of meat-type ducks at 45 days (body weight%)

		Diet (D)			p-value			
Item	Enzyme (E)	CD	LD	Average	D	E	D×E	SEM
Carcass	NS	80.44	80.23	80.33				
	S	80.43	80.47	80.45	0.72	0.61	0.60	0.11
	Average	80.43	80.35	80.39				
Breast meat	NS	14.24	14.34	14.29				
	S	13.79	14.22	14.01	0.40	0.36	0.60	0.15
	Average	14.02	14.28	14.15				
Leg meat	NS	11.62	11.82	11.72				
	S	12.00	11.96	11.98	0.70	0.19	0.54	0.09
	Average	11.81	11.89	11.85				
Wing	NS	7.60	7.68	7.64				
	S	7.61	7.78	7.70	<0.05	0.29	0.40	0.03
	Average	7.61^{b}	7.73^{a}	7.67				
Subcutaneous fat	NS	17.95	17.46	17.70^{A}				
	S	17.12	17.04	17.08^{B}	0.26	<0.05	0.41	0.14
	Average	17.53	17.25	17.39				
Abdominal fat	NS	1.26	1.23	1.25^{A}				
	S	1.17	1.10	1.14^{B}	0.31	<0.05	0.63	0.02
	Average	1.21	1.67	1.19				
Body fat accumulation	NS	19.21	18.69	18.95^{A}				
	S	18.28	18.14	18.21^{B}	0.25	<0.05	0.50	0.15
	Average	18.75	18.42	18.58				
Fat index[1]	NS	0.75	0.78	0.76				
	S	0.76	0.79	0.78	0.13	0.47	0.93	0.01
	Average	0.75	0.78	0.77				

NS: Non-supplemented, S: Supplemented, [1]Fat index: Breast meat weight:subcutaneous fat weight ratio, ^{a,b}Means within the same row without the same superscript letter are significant different(p<0.05), ^{A,B}Means within the same column without the same superscript letter are significant different (p<0.05)

Table 7: Effect of supplemental multi-enzymes in diet on small intestinal morphology of meat-type ducks at 45 days

		Diet (D)			p-value			
Item	Enzyme (E)	CD	LD	Average	D	E	D×E	SEM
Villus height (µm)								
Duodenum	NS	1,067.01	1,164.11	1,115.56				
	S	1,086.71	982.41	1,024.13	0.98	0.18	0.15	40.44
	Average	1,074.89	1,073.26	1,074.00				
Jejunum	NS	1,102.43	1,025.74	1,064.08				
	S	1,051.14	1,078.35	1,063.23	0.62	0.99	0.43	37.77
	Average	1,079.11	1,046.78	1,063.72				
Ileum	NS	1,093.34	1,045.44	1,069.39				
	S	1,021.76	1,033.51	1,028.81	0.77	0.64	0.76	47.77
	Average	1,064.71	1,039.47	1,050.94				
Crypt depth (µm)								
Duodenum	NS	350.98	308.06	329.52				
	S	324.44	340.88	334.30	0.50	0.84	0.22	13.58
	Average	340.36	324.47	331.69				
Jejunum	NS	315.99	329.97	322.98				
	S	287.54	336.83	309.45	0.08	0.42	0.33	8.53
	Average	303.06	332.72	317.18				
Ileum	NS	317.81	299.52	308.66				
	S	270.22	310.01	294.09	0.75	0.44	0.13	9.34
	Average	298.78	304.76	302.04				
Villus height/crypt depth ratio								
Duodenum	NS	3.11^{b}	3.80^{a}	3.45				
	S	3.37^{ab}	2.97^{b}	3.13	0.51	0.23	<0.05	0.16
	Average	3.21	3.38	3.31				
Jejunum	NS	3.51	3.15	3.33				
	S	3.73	3.19	3.49	0.09	0.52	0.95	0.13
	Average	3.61	3.17	3.40				
Ileum	NS	3.47	3.53	3.50				
	S	3.87	3.35	3.56	0.56	0.84	0.37	0.17
	Average	3.63	3.44	3.53				

NS: Non-supplemented, S: Supplemented, [a,b]Means within the same row without the same superscript letter are significant different ($p<0.05$)

(villus height, crypt depth and villus height/crypt depth ratio) of ducks at 45 days of age are presented in Table 7. There was a significant interaction between D and E for the villus height/crypt depth ratio of the duodenal segment. A reduction in the nutrient content (D) and supplementation of multi-enzymes (E) did not significantly affect any parameters.

The quality of the feed and nutrition can influence physiological functions in the gastrointestinal tract[22]. The morphology of the small intestine can be used as an indicator of gut health and gut function[23]. Feeding the LD diet to ducks seemed to increase the crypt depth ($p = 0.08$) and villous height: crypt depth ratio ($p = 0.09$) of the jejunum (the main absorptive site). It is generally known that poor gut morphology is positively related to the depth of the crypt or the ratio of villous height: crypt depth[24]. In agreement with the poor FCR of ducks fed the LD diet, this indicated that feeding a low nutrient density diet adversely affected the gut morphology, so that the ducks attempted to increase their feed intake to make up for the deficit in their nutrient requirements.

In broiler chickens, supplemental multi-enzymes in wheat-soybean meal diets reduced the intestinal weight[25], reduced the relative length of the ileum[8] and increased the villi height and villus height: crypt depth ratio[26]. However, in present study, there was no significant effect of diet supplemented with multi-enzymes on the small intestinal morphology of the ducks. This may have been caused by using the broken rice as an energy source, since it contains less fiber and higher nutrient digestibility than corn[27,28]. Therefore, the effects of multi-enzyme supplementation on the morphology may be less pronounced in ducks fed broken rice. The effect of interaction between nutrient density and enzyme supplementation on the villous height: crypth ratio in the segments of the duodenum suggested that there is a complicated mechanism at work regarding the quality of feed and exogenous enzyme supplementation on gut morphology and feed utilization.

CONCLUSION

In this study, the reduction in the levels of protein and energy in the diet retarded overall productive performance (growth rate and FCR) of the ducks during their early life, while the ducks were able to increase the amount of feed intake to meet their nutrient requirement during the later grower period. Supplementation with multi-enzymes did not significantly improve the productive performance and gut morphology but clearly reduced fat accumulation.

ACKNOWLEDGMENTS

This study is supported in part by the Graduate Program Scholarship from The Graduate School, Kasetsart University. The authors would like to express their gratitude to DSM Nutritional Products (Thailand) Company Limited, Grimaud Frères Selection (France) and Duck King (Thailand) Company Limited for their support. The contribution is also gratefully recorded of the Animal Farm Research and several staff members at the Department of Animal Science, Bangkhen Campus, Kasetsart University, Thailand for suggestions, guidance and support throughout this study.

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