The effect of isoamylase application in cassava root meal on broiler growth performance, feed retention time, and metabolite profile

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Abstract. The objectives of this study were to evaluate isoamylase application on the cassava root meal (CRM) and identify its effect on broiler growth performance, feed retention time, and metabolite profile. The experiment used 270 sexed broilers strain Lohman with an initial body weight of 45.88 ± 0.41 g were allotted into 3 treatments and 6 replications with 15 birds in each pen for 35 days. Dietary treatments were T0: Basal Ration + 0% CRM, T1: Basal Ration + 50% CRM, T2: Basal Ration + 50% CRM + 0.05% isoamylase. The results showed that the inclusion 50% of CRM both with and or without isoamylase was increased ($P<0.01$) feed intake and feed conversion ratio (FCR) and gave the same effect ($P>0.05$) on body weight, body weight gain, carcass weight, and carcass percentage during the overall period compared to fed T0. The treatment had no significant difference in coefisien total starch digestibility, metabolizable energy, and blood glucose levels. In conclusion, the inclusion of 50% cassava root meal with and or without isoamylase to subtitute corn in ration does not affect the broiler growth performance however FCR was higher than the control.

1 Introduction

Feed costs in poultry production constitute more than 70% of production costs [1], where energy feed sources occupy the largest portion of 70% - 75% in rations [2]. Corn is predominantly used as animal feed raw material, especially poultry feed in Indonesia. However, the availability of maize, especially feed maize, becomes scarce especially in every rainy season and causes an increasing in the price of maize in the market. The increasing in feed raw material costs has accelerated the demand to find alternative feed ingredients that can replace this material with lower production costs. In order to meet the needs of carbohydrates, cassava is the third food crop in Indonesia after rice and maize. Cassava (*Manihot* spp) is the highest carbohydrate source among staple crops and can potentially replace maize as an energy source in poultry feed. Starch is a major component of carbohydrates which is a source of energy. The main composition of cassava starch generally

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consists of amylose (AMS) and amylopectin (AMP). Cassava starch contains 17% amylose and 83% amylopectin, compared with maize starch which has 28% amylose and 72% amylopectin [3,4]. [5] Reported that high levels of resistant starch in cassava probably consisted of 82.85% amylopectin with 5.79% branch associations and 17.25% amylose with 0.48% branch associations. This causes cassava starch difficult to digest and utilize by poultry. One way to increase the amylose content in cassava root meal (CRM) is adding isoamylase. Isoamylase, also known as a debranched enzyme, hydrolyzes the branch chain α-1.6 glucosidic in glucose, amylopectin, and beta-limit dextrin. An increasing hydrolyzed of branch chains in amylopectin is expected to increase the amylose content. Thus it can increase nutrient availability and broiler growth performance.

Amylase was able to increase starch digestibility as well as reduce the presence of glucose as a potential substrate for non-beneficial bacteria at the end of the digestive tract [6]. [7] Found that feed containing 50% peeled cassava meal suplemented by cocktail enzymes (Maxigrain) produced the same production as a ration containing 100% maize. [8] Reported that the addition of carbohydrase and phytase significantly increased the body weight and metabolizable energy in poultry fed cassava pellets and mash. Supplementation of α-1.6 isoamilase in broiler feed increased nutrient digestibility, metabolizable energy, and broiler productivity [9]. This study was designed to evaluate isoamylase application on the CRM and identify its effect on broiler growth performance, feed retention time, and blood glucose level.

2 Material and Methods

2.1 Material

The main material in this study used cassava root meal from UD. Setia Flour Product (Bogor, Indonesia) and Isoamylase (EC 3.2.1.68) was from Bacillus licheniformis, purchased from Creative Enzymes (USA), Creative Biomart, Inc. This enzyme has optimal activity at pH around 5.6 - 7.0 and at temperatures between 50 - 55 ° C. Enzyme activity ≥ 10,000 Ug-1. The diet was formulated according to the recommendations of Leeson and Summers (2005) for a type of high-density diet for open cages (Table 1). The ration was given in the form of crumble for the starter period (0-21 days) and pellets for the finisher period (22 - 35 days). Feed and drink were given ad libitum. One-day-old sexed Lohmann (270 chicks), obtained from PT Japfa Comfeed Indonesia Hatchery, vaccinated with New castle Disease, Infectious Bursal Disease, and Avian Influenza, and raised for 35 days. The cages (1 m x 1 m x 0.8 m) were randomly alloted of 3 treatments (6 replications with 15 chicks each). Dietary treatments in this study were T0: Basal ration + 0% CRM (control); T1: Basal ration + 50% CRM; T2: Basal ration + 50% CRM + 0.05% Isomylase (500 Ug-1).

2.2 Methods

2.2.1 Growth Performance

Feed intake (FI), body weight (BW), body weight gain (BWG), feed conversion ratio (FCR) were measured weekly. Weight and percentage of digestive tracts and carcass, and intestinal length were observed at 35 days of age.

2.2.2 Feed Retention Time

Observation of retention time at 30th and 90th minutes was carried out at 22 days of age and 60th minutes at 23 days of age. Feed retention time was measured by placing broiler chickens on individual cages and calculating the amount of feed left in the crop from the amount of feed intake for 2 hours before the chicken was fasted for 24 hours, then slaughtered at 30th,60th, and 90th minutes.

$$Feed\ retention\ time\ (\%) = \frac{feed\ in\ the\ crop\ (g)}{feed\ intake\ (g)}\ x\ 100\% \quad (1)$$

Table 1. Feed rations and nutrient compositions of broilers

Ingredients	Starter (0-21 days)		Finisher (22-35 days)	
	T0	T1/T2	T0	T1/T2
Maize	55.00	27.50	55.00	27.50
CRM	0.00	27.50	0.00	27.50
Soy Bean Meal	28.00	28.00	27.35	27.35
Meat Bone Meal	2.00	2.000	2.820	2.820
Corn Gluten Meal	9.590	9.660	6.420	7.420
Crude palm oil	1.650	1.700	4.320	4.630
CaCO3	0.240	0.070	0.250	0.050
DCP	2.460	2.490	2.900	1.850
NaCl	0.060	0.090	0.040	0.040
L-Methionine	0.220	0.190	0.200	0.180
L-Lysine	0.270	0.300	0.200	0.170
Premix[a]	0.500	0.500	0.500	0.500
Isoamylase	-	-/0.05	-	-/0.05
Total (%)	100	100	100	100
Nutrient Composition				
Dry Matter (%)	87.99	88.07	85.72	88.23
Crude Protein (%)	23.04	23.00	21.50	21.50
Crude Fiber (%)	2.41	3.12	2.29	3.08
Extract Ether (%)	4.29	3.59	6.70	6.55
Ash (%)	5.61	5.98	5.31	5.52
Metabolizable Energy ($kcalkg^{-1}$)	3005.88	3000	3150	3150
Ca (%)	0.96	0.96	0.87	0.87
P available (%)	0.48	0.48	0.44	0.44
P total (%)	0.78	0.75	0.72	0.70
Lysine (%)	1.44	1.44	1.29	1.29
Methionine (%)	0.56	0.56	0.51	0.51
Methionine + Cystine (%)	0.97	0.90	0.83	0.82
Salt (%)	0.23	0.22	0.17	0.17

[a]Per kg diet: Copper 16 mg, Iodine 1,25 mg, Iron 40 mg, Manganese 120 mg, Selenium 0,3 mg, Zinc 100 mg. Vitamin A 12000 IU, Vitamin D3 5000 IU, Vitamin E 75 IU, Vitamin K3 3 mg, Riboflavin (B2) 8 mg, Nicotin Acid 60 mg, Panthotenic Acid 15 mg, Pyridoxin (B6) 4 mg, Biotin 0,15 mg, Folic acid 2 mg, Vitamin B12 0,016 mg, Choline 1600 ppm.

2.2.3 Starch digestibility coefficient in small intestine

At the age of 33 days, broiler chickens were placed in individual cages and calculated the amount of feed intake for 2 hours after the chickens were fasted for 24 hours, then slaughtered. Digesta in the small intestine was accommodated in plastic and dried in an oven 60 ºC until the weight was constant. The dried samples were analyzed for its starch content. The results of the analysis of starch content in the digesta were going to be compared with the total content of starch consumed by chicken.

$$starch\ digestibility\ coefficien = 1 - \frac{starch\ content\ in\ digesta\ (g)}{starch\ content\ in\ feed\ (g)} \quad (2)$$

2.2.4 Feed metabolizable energy

Metabolizable energy measurements based on [10] were carried out by placing two groups of broilers on individual cages. The first group was fasted for 48 hours, then excreta was collected for 24 hours. The second group was fasted for 24 hours, then fed for 2 hours and calculated the amount of feed intake. Excreta was collected fot 24 hours after completion of feeding. Excreta was dried and analyzed for gross energy content (Calorimetry Bomb).

$$AME\ (kcalkg^{-1}) = \frac{(GEf-FI)-(GEEp-E)}{FI} x\ 1000 \quad (3)$$

$$TME\ (kcalkg^{-1}) = \frac{(GEf-FI)-[(GEEp-E)-(GEEe\ x\ Ee)]}{FI} x\ 1000 \quad (4)$$

*GEf: Groos energy feed (kkalkg^{-1}), GEEp: gross energy excreta (kkalkg^{-1}), GEEe: gross energy endogenous excreta (kkalkg^{-1}), FI: feed intake (g), E: excreta (g), Ee: endogenous excreta (g).

2.2.5 Blood Glucose Levels

Blood sampling was taken at 34 day of age via branchial vein. Blood sampling was taken as ± 2 ml using 3 ml disposable syringe. The blood was put into a vacuum tube that has been filled with Ethylenediaminetetraacetic acid (EDTA) and homogenized. Serum blood was taken for glucose analysis (Enzymatic Colorimetric AKL Method 20101803460).

2.2.6 Statistical Analysis

The dietary experimental design used Completely Randomized Design (CRD) 3 treatments 6 replications. Data were analyzed using analysis of variance (ANOVA) and significantly different data continued with Duncan's multiple range test [11] using SPSS software (SPSS® version 16.0).

3 Result and Discussion

3.1 Broiler Growth Performance and Feed Retention Time

The results on the broiler growth performance showed that the inclusion of CRM up to 50% both with or without isoamylase addition were highly significant (P <0.01) increasing FI, BW, and BWG at starter period. There were no differences on FI, BW, and BWG but FCR highly significant (P <0.01) increased at finisher period. Feed intake and FCR highly significant (P <0.01) increased during the overall period compared to control. The control

treatment had feed retention which tended to be higher in the range of 30-90 minutes. In contrast, lower feed retention occurred in treatments T1 and T2.

Table 2. Effect of dietary treatments on the growth performance of broilers

Parameters	Treatments			P Value
	T0	T1	T2	
BW DOC (g bird^{-1})	46.29	45.87	45.76	
Starter (0-21 days)				
FI (g bird^{-1})**	957.54 ± 46.22b	1215.08 ± 35.36a	1219.20 ± 58.83a	0.000
BW(g bird^{-1})**	731.89 ± 50.69b	884.84 ± 41.13a	871.11 ± 19.35a	0.000
BWG (g bird^{-1})**	685.60 ± 51.29b	838.97 ± 41.11a	825.35 ± 19.56a	0.000
FCR (FIBWG^{-1})	1.39 ± 0.06	1.45 ± 0.06	1.47 ± 0.06	0.135
Finisher (22-35 days)				
FI (g bird^{-1})	1769.79 ± 87.59	1897.80 ± 93.89	1845.42 ± 114.84	0.115
BW(g bird^{-1})	1756.38 ± 136.85	1819.82 ± 80.61	1805.66 ± 125.30	0.623
BWG (g bird^{-1})	1024.49 ± 98.38	934.97 ± 47.47	934.55 ± 118.56	0.190
FCR (FIBWG^{-1})**	1.73 ± 0.06b	2.03 ± 0.06a	1.99 ± 0.10a	0.002
Cumulative (0-35 days)				
FI (g bird^{-1})**	2725.11±119.71b	3112.89 ± 121.45a	3064.63 ± 120.70a	0.000
BW(g bird^{-1})	1756.38 ± 136.85	1819.82 ± 80.61	1805.66 ± 125.30	0.623
BWG (g bird^{-1})	1710.09 ± 137.01	1773.95 ± 79.99	1759.91 ± 125.58	0.619
FCR (FIBWG^{-1})**	1.59 ± 0.05b	1.75 ± 0.05a	1.74 ± 0.05a	0.004

** The numbers on the same line and followed by different letters indicate a highly significant difference at the test level of 1%.
FI: Feed Intake; BW: Body Weight; BWG: Body Weight Gain; FCR: Feed Conversion Ratio

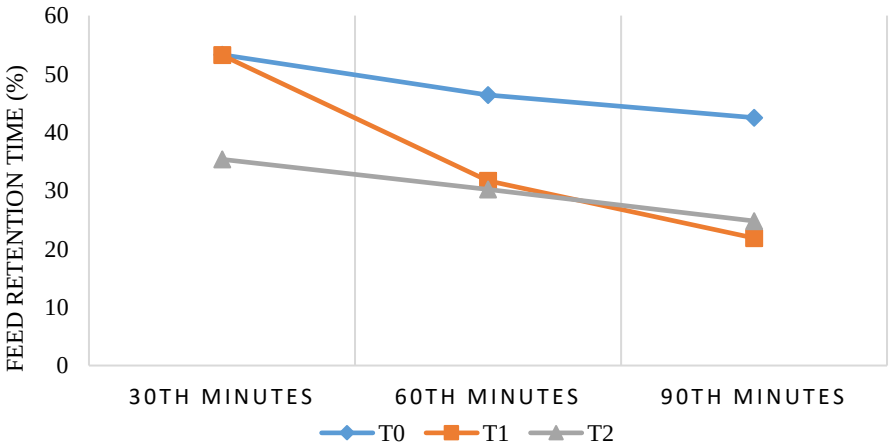

Fig. 1. Feed retention time (The amaount of left feed in crop) at 30th, 60th, and 90th minutes

The highly significant of FCR was due to BW and BWG in the treatment showed the same results with the control. [9] revealed that 300 and 600 Ukg α-1.6 isoamylase were able to increase BW, decrease FI, and achieve the same effect from controls but FCR was much higher than control. The higher of FI in the treatments compared to control was due to faster feed rate in the digestive tract. The higher inclusion of CRM will increase the content of amylopectin in the ration. Amylopectin which has branch chains tends to be less soluble in water. Components of fiber which were insoluble in water cause a faster feed rate [12,13]. Amylopectin in cassava had a relatively long chain length and caused cassava starch to be difficult to digest by poultry [5]. In addition, amylose has a molecular weight of about 100 kDa, amylopectin has a higher molecular weight of 104 - 106 kDa [14]. Increasing of feed rate in this study were supported by a lower feed retention in the treatments (Figure 1).

Higher feed retention rates allow feed to be in contact with digestive tract enzymes more optimally in order to increase feed digestibility. Crop filling was highly dependent on feed volume in the proventriculus and gizzard, whose capacity in chickens was estimated to reach 5-10 g of feed [15]. When these organs were fully filled, feed storage occurred [16]. After a while, when the gizzard was emptied, muscle contraction caused the passage of the digesta moved to a further part of the intestinal tract [17]. Therefore, the function of the anterior digestive tract was closely related, and affected the peristalsis further part of the digestive system. Furthermore, ad libitum feeding caused reducing physical use of crop by chickens [18] because the chance of feed in crop was only about 30 minutes according to digesta rate [19].

Higher feed retention in the control was caused by a slower feed rate compared to the other treatments which can be seen from the highly significant ($P < 0.01$) increasing on gizzard weight (Table 2). Higher gizzard weight indicated a higher process of mechanical digestion. A less developed gizzard would function as a transit organ from the mechanical digestive organs, with the implication of accelerating retention time [20]. Conversely, lower feed retention occured at T1 and T2. This was due to the difference in the AMS / AMP ratio which affected on the starch structure. Starch was arranged in a very complex and large structure, where amorphous and crystalline layers alternately form stiff and semi-crystalline granules, with sizes varying from 1 to 50 μm [21]. The semi-crystalline layer consisted of alternating layers of α-glucan crystals which extended from successive branches of amylopectin and amorf amylopectin branches [22]. The increasing amylopectin content would increase the formation of semi-crystalline layers so that it had the potential to increase the feed rate. [23] stated that the degree of crystallinity was inversely proportional to the stach digestion rate, and [24] proved this by concluding that slow starch digestibility was associated with a regular structure of crystalline and amorphous layers alternating in starch granules. [24] revealed that the digestion of starch granules started from the surface pores and interior channels, which allow amylase to enter the interior and digest granules gradually from the inside.

3.2 Carcass and Visceral Organs

The inclusion of CRM up to 50% subtituted maize was highly significant ($P < 0.01$) reducing both the weight and percentage of gizzard. Addition of isoamylase decreased ($P < 0.05$) the

percentage of liver. The treatments did not affect (P> 0.05) on weight and percentage of small intestines, abdominal fat, and carcass as well as lenght of small intestines.

The treatments was highly significant reducing both weight and percentage of gizzard. This was due to the decreasing corn which had a larger particle size than CRM in rations. Thus it reduced mechanical digestive activity in the gizzard and made gizzard less develop. The use of coarse particles or grains had been aimed to stimulate gizzard growth, improve mechanical function, increase digesta peristalsis, and increase gizzard volume, in order to increase retention time [25, 26]. Gizzard would shrink feed particle size to 100-200 μm before entering the small intestine [27, 28]. The higher particle size of the feed in the gizzard, the longer it would take to reach the size of the feed that can enter the small intestine.

The liver was the main organ for synthesis and metabolism, and served for glucose absorption of around 25% to 35% [29]. After consuming carbohydrates, 33% of glucose was absorbed by the liver [30]. Glucose derived from starch degradation was transported to the blood to maintain sufficient concentration to supply energy to other tissues [31, 32]. The results of the observations also showed that the treatment had no effect on the weight and percentage of the proventriculus. This is not in accordance with [33, 34]. The effect of the treatment in this study did not affect the weight, percentage, and length of the small intestine (duodenum, jejenum, ileum). This result was in line with the research [35, 36].

Table 3. Effect of dietary treatments on weight and percentage of carcass, visceral organ, and intestinal length of broilers aged 35 days

Parameters	Treatments			P Value
	T0	T1	T2	
Proventriculus (g)	10.66 ± 2.25	10.50 ± 2.94	7.83 ± 1.32	0.084
Proventriculus percentage (%)	0.61 ± 0.15	0.58 ± 0.18	0.43 ± 0.06	0.097
Gizzard (g)**	23.33 ± 3.50a	18.00 ± 1.67b	14.50 ± 0.06c	0.000
Gizzard percentage (%)**	1.33 ± 0.15a	0.99 ± 0.13b	0.80 ± 0.10c	0.000
Liver (g)*	41.33 ± 4.22ab	44.00 ± 6.63a	36.33 ± 2.87b	0.044
Liver percentage (%)*	2.37 ± 0.15a	2.41 ± 6.63a	2.00 ± 0.11b	0.015
Duodenum (g)	16.66 ± 4.22	18.33 ± 4.58	15.83 ± 1.60	0.511
Duodenum percentage (%)	0.95 ± 0.20	1.00 ± 0.22	0.87 ± 0.10	0.521
Jejenum (g)	26.66 ± 8.01	30.16 ± 6.11	27.77 ± 4.22	0.622
Jejenum percentage (%)	1.52 ± 0.42	1.65± 0.31	1.53 ± 0.23	0.754
Ileum (g)	20.83 ± 4.53	24.16 ± 6.24	20.30 ± 3.26	0.354
Ileum percentage (%)	1.19 ± 0.22	1.32 ± 0.32	1.12 ± 0.19	0.413
Duodenum (cm)	38.00 ± 1.41	39.00 ± 4.42	36.50 ± 4.76	0.539
Jejenum (cm)	81.91 ± 12.69	91.50 ± 9.97	81.91 ± 12.15	0.289
Ileum (cm)	80.16 ± 12.96	89.25 ± 10.74	81.08 ± 13.41	0.401
Abdominal fat (g)	26.50 ± 7.81	28.00 ± 5.29	33.83 ± 5.38	0.136
Abdominal fat percentage (%)	1.51 ± 0.40	1.54 ± 0.31	1.87 ± 0.30	0.175
Carcass weight (g)	1249.3 ± 111.22	1288.16 ± 91.19	1278.00 ± 62.40	0.747
Carcass percentage (%)	71.75 ± 2.26	70.70 ± 2.80	70.67 ± 1.30	0.636

** The numbers on the same line and followed by different letters indicate a highly significant difference at the test level of 1%.

* The numbers on the same line and followed by different letters indicate a significant difference at the test level of 5%.

The results did not affect the weight and percentage both of abdominal fat and carcass. Higher abdominal fat in the treatment group was a factor that contributes to the increasing in carcass weight due to higher body weight. The result of this study was in accordance with [37] found that the use of cassava in the form of pellets and chips were able to replace corn in broiler feed by up to 50%. This was supported by the fact that cassava products did not have an adverse effect on carcass weight, abdominal fat, and carcass composition. The opposite results are shown in the [33] although the result did not record a significant effect of feed treatment on carcass percentage, data showed a tendency to decrease with increasing use of cassava leaf concentrates and cassava tubers.

3.3 Broiler Chicken Metabolite Profile

The inclusion of 50% CRM in rations both with and without isoamylase did not give a significant difference to the starch digestibility coefficient in the small intestine. The results also showed no significant differences in AME, TME, and blood glucose levels in all treatments.

Table 4. Effect of dietary treatments on starch digestibility coeficient in small intestine, metabolizable energy, and blood glucose level on broiler

Parameters	Treatments			P Value
	T0	T1	T2	
Starch digestibility coefficient	0.98 ± 0.010	0.98 ± 0.001	0.97 ± 0.004	0.162
AME (kcal kg^{-1} feed)	3142.89 ± 220.84	3185.42 ± 168.67	3167.95 ± 172.83	0.950
TME (kcal kg^{-1} feed)	3317.73 ± 272.95	3292.95 ± 158.63	3313.50 ± 167.63	0.984
Blood glucose ($mgdL^{-1}$)	169.65 ± 9.78	155.21 ± 16.43	150.54 ± 22.30	0.161

AME: Apparent Metabolizable Energy; TME: True Metabolizable Energy.

The inclusion of 50% CRM in rations both with or without isoamilase did not give a significant difference to the coefficient starch digestibility in small intestine. The results of the study on starch digestibility coefficient in small intestine were 0.97 - 0.98. [38] found that the total starch digestibility coefficient had increased by 0.96 at the age of 3 days of broiler chicken. The same authors also found starch digestibility increased ($P < 0.01$) linearly as grew older in fast-growing broilers but not in slow-growing layer chickens. [39] found that 50% of poultry showed ileal starch digestion above 0.94, when fed with pellet diet containing 38.5% of whole wheat. Better results were obtained from [40] revealed ileal starch digestion was 0.98 in a diet containing 44% of whole wheat with other pellet ingredients. The whole process of starch digestion and absorbtion were carried out during short retention times in the duodenum and jejunum, estimated by [41] for about 1 hour.

Starch digestibility was able to affect AME in feed [42, 43]. The results showed no significant differences in AME and TME in all treatments. This was due to there was no significant difference in starch digestibility coefficient. [37] reported that metabolizable energy (ME) consumption decreased ($P < 0.001$) in the treatment fed cassava pellet and chips without enzyme supplementation, but increased ($P < 0.001$) in all enzyme-supplemented feeds. Supplementation of 200 and 400 Ukg-1 α-1.6 Isoamylase in feed did not have a significant effect on ME compared to control [44]. The results of this study, TME on T1 and T2 tended to be lower compared to control, indicating that to hydrolyze or digest CRM requires higher energy.

The results of blood glucose levels ranged from 150.54 to 169.65 mgdL-1. [45] stated that glucose levels in broilers were higher than mammals, ranging from 180 to 250 mgdL-1.

Chicken in a state of hypoglycemia if the blood glucose level was 137 mgdL-1 and hyperglycemia in blood glucose levels 363 mgdL-1 [46]. The results of this study did not show significant differences in all treatments but the blood glucose levels were still in normal standards. The diet containing CRM with or without isoamylase addition tended to have lower blood glucose levels compared to the control ration (155.21 mgdL-1 and 150.54 mgdL-1). The same result was shown by [44] reported that there was no significant difference in broiler serum glucose concentration between the dietary treatments supplemented with 1500 and 3000 Ukg-1 α-Amylase (A. oryzae), 480 and 960 Ukg-1 α-1.4 Amylase (B. subtilis) and 200 and 400 Ukg-1-1.6 Isoamylase (B. subtilis). Previous research had shown that glucose absorption was not a factor that limits starch utilization [47].

4. Conclusion

It can be concluded that the application of 0.05% isoamylase in CRM was able to reduce the amylose / amylopectin ratio which was close to the amylose / amylopectin ratio in corn and increase the reducing sugar content. The inclusion of 50% CRM with or without isoamylase to subtitute corn in the ration did not affect growth performance, intestinal starch digestibility coefficient, AME, TME, and blood glucose levels of broiler chickens but had a higher FCR compared to control.

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