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Effect of Fiber Degrading Enzyme on the Utilization of Dietary Fiber in Japanese Quails (*Coturnix japonica*)

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Abstract

The experiment was carried out to investigate the effect of a fiber degrading enzyme on dietary fiber utilisation in Japanese quails. Three hundred day-old quail chicks were randomly assigned to one of four treatment groups (A, B, C and D), each with three replicates and each with 25 quail birds. Four iso-nitrogenous and iso-caloric diets (CP: 21%; ME: 2850 kcal/kg) were developed. Diet "A" contained no exogenous enzyme, whereas diets (B, C and D) contained 200, 300 and 400 g/ton of feed, respectively, of Rovabio[®] Advances T-Flex 25 granulated exogenous enzyme. Chicks were fed ad libitum, and data on growth performance was collected. After the trial was completed, three birds from each replicate were slaughtered at random to determine carcass parameters and meat quality. Body weight increased (P < 0.05) in birds given Rovabio[®] enzyme, but feed intake and FCR were not significantly different (P > 0.05) across all treatments. The enzyme supplementation had no effect on carcass characteristics or water holding capacity. Meat pH was lower (P < 0.05) in birds fed Rovabio[®] enzyme at 400 g/ton of feed. When compared to the control diet, enzyme supplementation significantly improved fiber digestibility (P < 0.05). Protein and fat digestibility, on the other hand, remained unaffected across all treatments (P > 0.05). The results show that adding Rovabio[®] enzyme at a rate of 200 g/ton of feed improves body weight, fiber digestibility and economics efficiency. © 2022 Friends Science Publishers

Keywords: Enzyme; Fiber; Digestibility; Non-starch polysaccharides; Meat quality

Introduction

In developing countries, the poultry industry is experiencing some critical issues with animal feed prices, which are unpredictable in the current scenario, as raw ingredients, primarily corn and soybean, have been increasing in price day by day (Alagawany and Attia 2015). As a result, there is a need to consider some low-cost resources, such as agricultural by-products and other crops, which are significantly less expensive than conventional feed stuff. As a result, developing countries are paying more attention to non-traditional feed resources such as vegetable and fruit waste and agricultural by-products. These ingredients are high in Non-Starch Polysaccharides (NSPs), which poultry cannot break down or digest, so some exogenous enzymes must be supplemented (Amerah *et al.* 2005).

The current food scarcity necessitates raising quail birds to fill the protein gap in the human diet (Chimote *et al.* 2009). Quail meat is an excellent source of animal protein. Quail birds have a shorter life span and are resistant to disease. Feed costs could be reduced, and poultry performance improved by including fiber splitting enzymes such as glucanase and xylanase in feed (Ashok and Prabakaran 2012). Most of the feed ingredients contained undigested NSPs such as xylan, glucan, cellulose, pectin, lignin, and arabinoxylans, which reduce feed utilization, availability of metabolizable energy, and the performance of the birds. In poultry diets, technologically advanced enzymes and their by-products from natural fermentation were supplemented. It has not been found to harm the health of end-users. In poultry diets, enzymes are used to hydrolyze the fiber content, primarily NSPs of cereal grains (Cowieson and Adeola 2005). Due to the populations of gut microbes, exogenous dietary enzymes can affect the lumen content of broilers. Because of the presence of NSPs such as xylan, glucan, and arabinoxylan, feeds with a high nutritional profile respond to a lesser extent. However, the addition of xylanase and glucanase to poultry diets plays a critical role in alleviating the anti-nutritional factor NSPs. The addition of xylanase to poultry diets containing wheat rather than corn has been found to be beneficial (Machado et al. 2020).

Exogenous enzymes' mode of action varies depending on the arabinoxylan content of cereal grains (Cowieson *et al.* 2010). The high content of NSPs in various cereal grains

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and cereal by-products reduces their use in bird diets due to anti-nutritional factors that increase intestine digesta viscosity. Fiber degrading enzymes have also been widely used in barley and wheat-based diets to reduce viscosity in the small intestine through NSP cleavage. Furthermore, these enzymes cleave cell walls, increasing the absorption and digestibility of sugar molecules derived from hemicellulose (Albertsen et al. 2011). Milling by-products contain higher levels of NSPs, limiting birds' efficient utilization of feed. Among these NSPs are cellulose, -glucans, arabinoxylans, and pectin. NSPs were found to have anti-nutritive activity even at low concentrations (50 g/kg) in broiler diets. The anti-nutritive activity of NSPs was reduced by soaking the cereals in water or supplementing them with NSP degrading enzymes (Nguyen and Morgan 2021). In a recent study published by Sentürk et al. (2021) corn was replaced with barley at two different energy levels: 2800 and 2900 Kcal/kg. They discovered that barley had a negative impact on feed intake and egg production.

When the fiber degrading enzyme xylanase was added to broiler diets, it improved performance by 2–4% when compared to the control diet without the enzyme. Diets containing more grains, such as wheat, oat, rye, and barley, outperformed corn and sorghum-based broiler diets (Cowieson *et al.* 2010). The exogenous enzyme in poultry feed primarily hydrolyzes the feed's NSP content. Corn, wheat, soybean meal, and canola meal, in that order, contain 8.3, 10.2, 25.7 and 18 percent NSPs (Ward 2014). There have been few studies to evaluate the use of different types of enzymes and combinations of enzymes in quail diets. The current study was carried out to investigate the effect of various supplemental ratios of exogenous enzymes on the utilization of dietary fiber in Japanese quails.

Materials and Methods

Trial location

This study was conducted at the University of Agriculture, Faisalabad, to assess the effect of fiber degrading enzyme supplementation in quail chicks (*Coturnix coturnix japonica*).

Preparation and cleaning of poultry house

Before the experiment began, the poultry house was thoroughly whitewashed and fumigated with $KMnO_4$ and formalin to ensure complete disinfection. For disinfection, the experiment's drinkers and feeders were washed with water and dried in the sun. Cleaning and disinfection of the house with equipment were completed one week before the arrival of the chick.

Experimental birds and diet

Three hundred day-old Japanese quail chicks were divided into one control and three treatment groups, with each group having three replicates and each replicate containing 25 quails (Coturnix coturnix japonica). Four experimental diets were prepared with equal nitrogenous and caloric value (CP: 21%; ME: 2850 kcal/kg) (Table 1 and 2). Diet "A" contained no exogenous enzyme, whereas diets (B. C. and D) contained 200, 300, and 400 g per tonne of Rovabio® Advances T Flex 25 granulated exogenous enzyme, respectively. Endo-1,4-xylanase 6250 Visco unit/g and endo-1,3(4)-glucanase 4300 Visco unit/g are both present in Rovabio® Advances T Flex 25 granulated exogenous enzyme. It was a fungal enzyme, talaromyces versatilis which was used to culture it. Chicks were reared for 42 days during the experiment, with the same environmental conditions and management practices applied to all treatments. During the experimental trial, light was provided by electrical bulbs in the poultry house, and birds were given ad libitum feed and water.

Data recording and bodyweight

The body weight of each quail chick was measured upon arrival and then every week for all experimental groups using an electrical weighing balance.

Feed intake

Feed intake was calculated for each replicate by subtracting the refusal feed quantity from the total amount of feed provided throughout the week. The following relationship was used to calculate chick feed intake.

$$Feed intake/bird (g) = \frac{Offered Feed (g) - Residual Feed(g)}{No. of chicks per replicate (n)}$$

Feed conversion ratio (FCR)

The efficiency of feed was estimated by measuring the feed conversion ratio. The following formula was applied to calculate FCR:

$$FCR = \frac{Feed intake (g)}{Weight gain (g)}$$

Carcass characteristics

On the 42nd day of the experiment, three birds were chosen at random from each replicate. Birds were slaughtered and their feathers were removed after their live body weight was recorded. Along with the head and shanks, visceral organs were removed. The percentage weights of the carcass, chest, thigh, liver, gizzard, and heart were calculated using the formulas below:

Dressing % age =
$$\frac{\text{Dressed weight of bird (g)}}{\text{Live weight of the bird (g)}} \times 100$$

Chest weight % age = $\frac{\text{Chest weight (g)}}{\text{Carcass weight(g)}} \times 100$

Legs weight % age =
$$\frac{\text{Legs weight (g)}}{\text{Carcass weight(g)}} \times 100$$

Organ weight % age = $\frac{\text{Organ weight (g)}}{\text{Bird live weight(g)}} \times 100$

Meat quality

Breast meat samples were collected after slaughter and delivered to the National Institute of Food and Technology (NIFSAT), University of Agriculture, Faisalabad, to determine the water holding capacity and pH of the meat.

Water holding capacity

A sample of 15 g of chopped meat was taken and placed in a centrifuge tube. After that, 22.5 mL (0.6 N NaCl) was added to it. The sample was homogenized and centrifuged at 5000 rpm for 10 min. Water was drained from the tubes, which were then weighted. The following formula was used to calculate water holding capacity (WHC):

WHC % =
$$\frac{W1 - W2 - 15}{15} \times 100$$

* 15 is constant factor.

Meat pH procedure

A sample of 1 g chopped meat was taken and 10 mL water was added to it. The sample was homogenized, and the pH was determined using a pH meter.

Nutrient digestibility

The marker method was used to assess nutrient digestibility (indirect). As a result, AIA (Acid insoluble ash) Celite® was added at 1% to the diet of birds and fed to them. The fecal samples were collected during the trial's final week. Polythene sheets were placed beneath each pen, and feces were collected twice daily. For each treatment, the collected samples were properly homogenized and placed in plastic bags. Feed and fecal samples proximate analysis were recorded by (AOAC 2000). The following formula was used to determine nutrient digestibility:

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"Digestibility coefficient % = 100 - (100 \times \frac{\% \text{ marker in feed}}{\% \text{ marker in feces}} \times \frac{\% \text{ nutrient in feaces}}{\% \text{ nutrient in feed}})
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Economics

Cost of production per live weight was recorded on the basis of feed cost and live bird weight (Wadood *et al.* 2022).

Statistical analysis

Statistical Analysis System (SAS v. 9.1 for Windows) was used to investigate the collected data using a Completely Randomized Design. Tukey's test was used to compare means (Steel *et al.* 1997).

Results

Growth performance

Table 3 compares mean values for growth parameters (feed intake, body weight, and FCR). Birds fed enzyme diets of 200, 300 and 400 g/ton gained more weight (P < 0.05) than the control group diet. The addition of enzymes had no effect on feed intake or FCR (P > 0.05).

Carcass characteristics

Table 4 shows the dressing percentage, breast and thigh yield, and giblet organ weight. Statistical analysis revealed that the addition of enzyme had no effect on carcass response (P > 0.05).

Meat quality

Table 5 shows a comparison of mean values for meat quality (pH and water holding capacity). The pH of meat was lower (P < 0.05) in birds fed Rovabio® enzyme at 400 g/ton of feed, but enzyme addition had no effect on WHC.

Nutrient digestibility

Table 6 compares mean values for nutrient digestibility (crude fiber, crude fat, and crude protein). Fiber digestibility was significantly improved (P < 0.05) with enzyme supplementation at 200 g/ton compared to the control diet. Protein and fat digestibility, on the other hand, remained unaffected across all treatments (P > 0.05).

Economics efficiency

Cost of production per 100 g live weight was lower in birds received Rovabio® enzyme at 200 g/ton of feed (Table 7).

Discussion

The current study found that diets supplemented with an exogenous fiber degrading enzyme significantly improved body weight (P < 0.05). This could be because increased fiber digestibility leads to increased nutrient availability and weight gain. Cowieson and Adeola (2005) found similar results, reporting that exogenous enzyme supplementation in the diet reduced digesta flow rate in the intestinal tract, improved nutrient utilization, and increased bird body weight. Increased soluble NSP concentrations are well known to cause increased digesta viscosity and decreased nutrient digestion and absorption. Glucanase breaks down polymeric chains into smaller pieces, which reduces gut viscosity and thus improves the nutritive value of NSP-rich

Table 1: Ingredients composition of experimental diet

Ingredients	Experin	Experimental diets						
0	A	В	С	D				
Maize	47.21	47.0	47.0	47.0				
Wheat grain	5.0	5.0	5.0	5.0				
Corn Gluten 60%	2.5	2.5	2.5	2.5				
Rice polishing	5.0	5.0	5.0	5.0				
Soybean meal	17.32	17.32	17.23	17.13				
Canola meal	7.0	7.0	7.0	7.0				
Sunflower meal	7.88	7.88	7.88	7.88				
Fish meal	3.0	3.0	3.0	3.0				
Vegetable oil	1.48	1.48	1.48	1.48				
Limestone (Chips)	0.91	0.91	0.91	0.91				
Di-calcium phosphate	1.30	1.30	1.30	1.30				
Vit. min. premix	0.5	0.5	0.5	0.5				
L-Lysine Sulphate	0.69	0.69	0.69	0.69				
DL-Methionine	0.14	0.14	0.14	0.14				
L-Threonine	0.08	0.08	0.08	0.08				
Rovabio [®] Advance	0	0.20	0.30	0.40				
Total	100	100	100	100				

Rovabio® Advance is fiber degrading enzyme developed by Adisseo, A Bluestar Company, Europe

Vitalink[®] is a vitamins premix; each Kg of it supplied the following: 20,000 KIU Vitamin A; 5400 KIU Vitamin D3; 48 g Vitamin E; 4 g Vitamin K₃; 4 g Vitamin B₁; 4.3 g Vitamin B₂; 59.5 g Niacin; 0.20 g Biotin; 20 g Pantothenic acid; 7.6 g Vitamin B₆; 1.7 g Folic Acid; 0.012 g Vitamin B₁₂

Nutrimin[®] is a minerals premix; each Kg of it supplied the following: 120 g ZnSO₄; 120 g CuSO₄; 140 g MnSO₄; 10 g FeSO₄; 1.8 g Iodine

Table 2: Nutrients composition of experimental diets

Nutrients	А	В	С	D
M.E (kcal/kg)	2850.00	2850.00	2850.00	2850.00
Crude protein	21.00	21.00	21.00	21.00
Ether extract	4.93	4.93	4.93	4.93
Crude fiber	5.00	5.00	5.00	5.00
Calcium	0.98	0.98	0.98	0.98
Avail. P	0.44	0.44	0.44	0.44
Dig. Lysine	1.24	1.24	1.24	1.24
Dig. M+C	0.82	0.82	0.82	0.82
Dig. Threonine	0.79	0.79	0.79	0.79

grains (Smits and Annison 1996). Cowieson and Ravindran (2008) discovered that feeding a corn-soy based diet containing multiple enzymes (protease, xylanase, and amylase) improved weight gain in broiler birds. Avila *et al.* (2012) reported that when broilers were fed a diet supplemented with fiber degrading and phytase enzymes, their body weight increased.

Tiwari *et al.* (2010) reported contradictory results, demonstrating that an enzyme cocktail (amylase, protease and xylanase) had no effect on broiler weight gain. Exogenous NSPase enzyme addition did not improve weight gain in broilers, according to West *et al.* (2007). Body weight may not be increased because there is an insufficient substrate in the negative control diets or the diet contains nutrient levels above the birds' requirements, causing enzyme results to be inclusive. This research was conducted on a corn-soya diet, whereas most studies have been conducted on wheat and barley-based diets.

When quail bird diets were supplemented with varying levels of enzymes or without enzyme, feed intake was not affected (P > 0.05). The findings contradict the findings of

Table 3: Growth performance of Japanese quails fed varying levels of exogenous fiber degrading enzyme (1–42 days)

	Dietar	y treatn	nents*	SEM	P-Value	
	А	В	С	D		
Feed intake (g)	607	668	691	678	33.6	0.359
Body Weight (g)	141 ^b	170 ^a	171 ^a	168 ^a	5.35	0.011
FCR	4.32	3.96	4.03	4.04	0.257	0.770

A = Control group 0 g enzymeB = Treatment group 200 g enzyme

C = Treatment group 300 g enzyme

D = Treatment group 400 g enzyme

SEM = Standard error of the mean

a-b Values in the same row not followed by a common superscript differ significantly

 Table 4: Carcass characteristics of Japanese quails fed varying levels of exogenous fiber degrading enzyme

	Dietary	treatme	SEM	P-Value		
	А	В	С	D		
Live Weight (g)	161	179	171	182	6.86	0.147
Dressing %	54.41	52.97	55.59	52.67	1.27	0.350
Upper Half %	64.48	65.42	65.06	66.51	0.637	0.166
Lower Half %	35.46	36.10	34.58	34.66	0.689	0.369
Heart %	0.90	0.87	0.86	0.81	0.031	0.303
Liver %	2.50	2.26	2.48	2.77	0.190	0.322
Gizzard %	2.67	2.67	2.53	3.12	0.167	0.093

A = Control group 0 g enzyme

B = Treatment group 200 g enzyme

C = Treatment group 300 g enzyme

D = Treatment group 400 g enzyme

SEM = Standard error of the mean

 $Upper \ half = Breast, \ wings, \ neck \ and \ spine$

Lower half = Drum stick, thigh and back

Grecco *et al.* (2019) who demonstrated that feed intake increased when birds were unable to obtain nutrients from feed, as was observed when 140 kacl/kg of reduced calories from the control group was offered to birds. Although xylanase has secondary properties such as -amylase, glucanase, protease, and cellulose activity, these may have no effect on feed digestibility. Selle *et al.* (2016) discovered that adding Xylanase and phytase to a wheat-containing broiler diet improved feed intake.

The feed conversion ratio was unaffected by any of the dietary treatments. This could be due to a non-significant effect on feed intake and data variation. The findings are consistent with those of West et al. (2007) who found that feeding Rovabio to birds had no effect on FCR. This could be because the dietary nutrients they provided were higher than the birds' recommendations. More research should be done by reducing the amount of energy and amino acids in the experimental diet. Mussini et al. (2011) discovered that varying levels of Octa zyme (β -mannanase) supplementation had no effect on FCR. This could be because the basal diet already contained enough nutrients to support broiler bird growth, or it could be due to late enzyme supplementation at 19 to 33 days. According to Yu et al. (2007) enzyme supplementation in broiler diet had no effect on feed conversion ratio. Cowieson and Adeola (2005) discovered contradictory results, demonstrating that feed efficiency was significantly improved when the broiler was fed cocktail enzymes with low energy diet. As a result of the enzymeenhanced reduction in viscosity of digests, which results in more nutrients available for birds via enzyme cocktail supplementation, an improvement in FCR was observed.

Dressing percentage and relative carcass weight, as well as thigh, heart, and liver percentage weight were unaffected (P > 0.05) in birds fed a control diet or a diet supplemented with varying levels of exogenous fibrolytic enzymes. Current research findings are consistent with those of Alagwany et al. (2018) found that combining enzyme with sunflower meal had no effect on carcass characteristics other than slowing rate change in broiler birds. Cho et al. (2012) concluded that supplementing low energy broiler diets with an exogenous fiber degrading enzyme (Endopower®) did not improve gizzard and liver weight. West et al. (2007) concluded that even when supplemented with a basal diet, enzyme supplementation had no effect on carcass parameters. The current study results were partially related to those who supplemented multi-enzyme to quail diets and found no effect on carcass characteristics, except liver weight was increased, which could be due to a high sunflower portion in the diet. The carcass parameters changed significantly when they supplemented enzyme with sunflower levels, which was not the case when only multi-enzymes were supplemented in the control diet (Tüzün et al. 2020).

Bilal et al. (2017) established that interactions between dietary SFM level and enzyme accumulation in broiler feeds changed the relative liver, gizzard, and thigh weights. Watee-Kongbuntad and Lumyong (2006) discovered that incorporating exogenous enzymes into broiler diets significantly improved broiler carcass weight.

The addition of enzyme to the diets of quail birds had no effect on the WHC of the meat. Hussein et al. (2020) discovered that multi-enzyme preparation in a low energy diet had no effect on meat WHC and other parameters except pH as meat test was performed 24 h later on breast meat. Meat pH increased in the control diet versus the less energy enzyme supplemented diet. The pH change could be explained by changes in response to slaughter stress, temperature, time, glycogen content at slaughter, and bird weight. Mnisi and MLambo (2018) investigated whether meat quality parameters could be preserved by supplementing with exogenous protease. In vivo trials have shown that diet phenolic content can affect meat quality. Exogenous enzyme supplementation had no effect on the meat quality of quail birds in the current scenario. These findings were consistent with those of Zakaria et al. (2010) who used a combination of different fiber degrading enzymes in broiler diets and discovered no difference in the meat water holding capacity of these birds. Ismail et al. (2006) reported contradictory findings, observing an increase in meat WHC when broiler diets were supplemented with fiber degrading enzymes.

The supplementation of enzyme improved crude fiber digestibility but had no effect on the level of enzymes in the diet. Exogenous enzymes increase the activity potential of endogenous enzymes in meat-type quail birds. Another

Table 5: Meat quality of Japanese quails fed varying levels of exogenous fiber degrading enzyme

		Dietary	SEM	P-value					
	А	В	С	D					
ьH	5.99ª	5.99ª	5.82 ^{ab}	5.55 ^b	0.07	0.008			
WHC	17.81	16.40	18.87	13.98	2.56	0.587			
A = Control group 0 g enzyme									
2 - Treatm	- Tractment group 200 g on turne								

C = Treatment group 300 g enzyme

D = Treatment group 400 g enzyme SEM = Standard error of the mean

a-b Values in the same row not followed by a common superscript differ significantly

Table 6: Nutrient digestibility of Japanese quails fed varying levels of exogenous fiber degrading enzyme

	Dietary	treatment	SEM	P-value						
	А	В	С	D	_					
Crude fiber	77.00 ^b	89.78 ^a	84.29 ^{ab}	84.88 ^{ab}	2.44	0.037				
Crude fat	83.84	81.00	82.66	86.90	3.33	0.658				
Crude protein	59.36	70.96	58.95	60.26	3.99	0.182				
A = Control group 0 g enzyme										
D T	T									

B = Treatment group 200 g enzyme

C = Treatment group 300 g enzyme

D = Treatment group 400 g enzyme

SEM = Standard error of the mean

a-b Values in the same row not followed by a common superscript differ significantly

Table 7: Economics efficiency of Japanese quails fed varying levels of exogenous fiber degrading enzyme

Treatments	Dietar	y treatn	SEM	P-val		
	А	В	С	D		
Day old bird cost	8	8	8	8	-	-
Total feed cost / bird	33.69	38.63	40.74	40.74	2.88	0.359
Miscellaneous	15	15	15	15	-	-
Production cost / bird	56.69	61.63	63.74	63.74	2.88	0.37
Av. body weight (g)	141 ^b	170 ^a	171 ^a	168 ^a	5.35	0.011
Production cost / 100 gm live weight	40.21 ^a	36.25°	37.28 ^b	37.94 ^b	1.45	0.03
A - Control group () a answere						

A = Control group 0 g enzyme

B = Treatment group 200 g enzyme C = Treatment group 300 g enzyme

D = Treatment group 400 g enzyme

SEM = Standard error of the mean

^c Values in the same row not followed by a common superscript differ significantly

reason could be the ability of xylanase in the small intestine to break down the backbone of arabinoxylan into smaller parts (xylose and arabinose). These smaller fragments improve nutrient digestibility by lowering digesta viscosity. Grecco et al. (2019) discovered an increase in neutral fiber digestibility with xylanase supplementation in a diet. Cozannet et al. (2017) concluded that the multi-carbohydrase enzyme complex (xylanase and arabinofuranosidase) improved crude fiber digestibility compared to the control group diet. Yadav and Sah (2005) found that xylanase supplementation in a nutrient-deficient diet improved crude fiber digestibility. These findings contradict the findings of Ponte et al. (2004) who discovered that fiber degrading enzymes had no effect on the digestibility of crude fiber in a broiler diet. All dietary treatments had no effect on crude fat digestibility. In contrast to these findings, Stefanello et al. (2016) discovered that broiler chicks fed corn-soybean mealbased diets containing varying levels of xylanase improved their crude fat digestibility by 2-3%. Romero et al. (2014) concluded that a corn and soybean meal-based diet supplemented with two different enzyme groups (xylanase and amylase) (xylanase, amylase, and protease) increased crude fat digestibility significantly.

In the current study, there was no difference (P > 0.05) in crude protein digestibility across all dietary treatments. Cowieson and Ravindran (2008) discovered that after incorporating xylanase, amylase, and protease into the broiler diet, ileal protein and nitrogen digestibility remained unaffected. Olukosi *et al.* (2007) discovered that a soybean and corn-based diet supplemented with a combination of enzymes (xylanase, amylase, and protease) improved protein digestibility. The variation in results caused by the addition of enzymes to feed and dietary energy may be related to the amount of substrate exposed to the enzyme or the availability of energy from the dietary ingredients themselves (Adeola and Cowieson 2011).

Conclusion

The results show that adding Rovabio[®] enzyme at a rate of 200 g/ton of feed improves body weight, fiber digestibility and economics efficiency.

Author Contributions

RM and MS did experimental work and manuscript writing; MS and HN designed the experiment; FA and HS performed data analysis; MS and MMAH prepared the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest

Data Availability

Data is available

Ethics Approval

All the experimental protocols were reviewed and approved by the Departmental Scrutiny Committee of the University of Agriculture, Faisalabad

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