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## Βιβλιογραφική αναφορά:

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## Effect of enzyme and yeast-based feed additives on growth, nutrient digestibility, meat quality and intestinal morphology of fattening rabbits

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**ABSTRACT:** Non-antibiotic feed additives are nowadays increasingly used in animal feed industry as more and more reports are surfaced on antibiotic resistance. This study quantified the effects of enzymes and yeast supplementation, individually or in combination, on growth performance, diet digestibility, carcass quality, and ileum morphometry of fattening rabbits. Forty-eight indigenous male weaned rabbits (age,  $37 \pm 2$  days; average body weight (ABW)  $340 \pm 3.2$  g) were assigned into four dietary treatments (16 replicates; 3 rabbits/replicate). The four diets were (i) a basal (control) diet (without additives), containing concentrate (75% of the feed) and wilted mulberry leaves (25% of the total feed); (ii) basal diet plus enzyme blend (driselase-1®; cellulase, amylase and protease;  $2 \text{ g kg}^{-1}$  feed); (iii) basal diet with the addition of *Saccharomyces cerevisiae* (dry yeast TR 100;  $2 \text{ g kg}^{-1}$  feed); (iv) basal diet with the addition of mixture of the enzymes blend and *S. cerevisiae* (1 g each  $\text{kg}^{-1}$  feed). All rabbits had *ad-libitum* access to clean drinking water and experimental diets, with 16 h light for seven-weeks. The results revealed that all additives improved ( $P < 0.05$ ) weight gain (WG) and feed conversion ratio (FCR) but did not alter feed intake than the control group. Although all additives increased ( $P < 0.05$ ) neutral detergent fibre (NDF) digestibility, the dry matter digestibility (DMD) was increased ( $P < 0.05$ ) only by the addition of enzymes blend. Furthermore, all additives increased ( $P < 0.05$ ) carcass yield without any negative effects on meat physical (pH, water release and cooking loss) and chemical (moisture, protein, fat and ash) properties ( $P < 0.05$ ). Moreover, villus height and crypt depth values were improved ( $P < 0.05$ ) with the incorporation of additives. This study demonstrates that addition of enzymes blend and yeast-based additives alone or in combination in rabbit diet improved nutrients digestion and intestinal health, which subsequently improved their growth and carcass quality.

**Keywords:** Enzymes, yeast, mulberry leaves, growth performance, digestibility, meat quality, rabbit

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## INTRODUCTION

Antibiotic growth promoters (AGPs) are still commonly used in animal rations in developing countries, such as Pakistan, with the aim to improve animal performance and reduce the rate of early mortality. However, recent studies show that the use of AGPs can cause serious health issues such as antibiotic resistance (Ali et al., 2017), and subsequently, the European Union banned AGPs in January 2006 (Kabploy et al., 2016). This has triggered enormous research into the exploration of alternate feed additives in an effort to improve gut health, animal performance and to overcome the emerging problem of antibiotic resistance (Wu et al., 2011). In recent past, considerable interest has been established in the use of enzymes and yeast as feed additives (Falcao-e-Cunha et al., 2007). Exogenous enzymes and yeast supplementation in the rabbit diet have been reported to improve growth performance and health status (Chandra et al., 2014). In addition, dietary enzyme supplementation into rabbit diets has been reported to improve nutrient utilization (Falcao-e-Cunha et al., 2007).

Mulberry (*Morus alba*) leaves are getting growing attention in the face of higher price of concentrates ingredients in rabbits diets due to their excellent nutritional value, such as high protein (15-35%), Ca (2.42-4.71%) and P (0.23-0.97) contents (Ayasan and Baylan, 2016), and improved *in vitro* DM digestibility (62.5%; Khan et al., 2019). This has led to a renewed interest in feeding of *Morus alba* leaves to rabbits to improve their growth performance (Khan et al., 2020) and reduce the cost of production and farm profitability.

Rabbit production has increased in Pakistan because it is a good source of affordable meat. In this respect, medium and large scale (semi)-commercial rabbit production systems have gained popularity for providing cheap and high-quality animal protein to the people (Khan et al., 2016). Recent study shows that total meat production and production efficiency of indigenous rabbits can be enhanced by improving feeding methods and management practices (Khan et al., 2017a, 2017b). Just after weaning, rabbits are particularly exposed to enteric diseases particularly diarrhea causing economic losses in terms of mortality; reduce growth and poor feed utilization efficiency due to the transition from feeding on mother's milk to solid food (Bivolarski and Vachkova, 2013). The negative effects of weaning on rabbit health and production

could be reduced by the dietary inclusion of non-antibiotic feed additives, such as probiotics and enzymes (Trocino et al., 2005; Kritis et al., 2008). Literature is available on the beneficial impact of non-antibiotic feed additives on production performance of farm animals. However, literature on the impact of additives (non-antibiotic) on rabbit growth performance is limited. Thus, the present research was executed to find out the effects of inclusion of enzymes blend (cellulase, amylase and protease) and yeast (*Saccharomyces cerevisiae*) on the growth, nutrient digestibility, carcass yield, meat physicochemical properties and ileum morphology of indigenous rabbits.

## MATERIAL AND METHODS

### Management of rabbits and experimental diets

A total of forty-eight healthy male rabbits [age,  $37 \pm 2$  days; average body weight (ABW)  $340 \pm 3.2$  g] were randomly distributed to four dietary groups (16 replicates (3 rabbits per replicate)). Each replicate group was kept in separate cage in the same shed. Each diet (iso-caloric and iso-nitrogenous) was assigned to the 4 replicate groups.

**Table 1.** Nutrient profile of concentrate and mulberry leaves fed to the rabbit

Nutrients <sup>1</sup>	Concentrate <sup>2</sup>	Mulberry leaves <sup>3</sup>
Dry matter	92.1	20.8
Crude protein	18.5	21.8
Neutral detergent fiber	37.7	35.5
Acid detergent fiber	23.4	23.8
Ether extract	4.50	3.31
Total Ash	9.30	15.3
Digestible energy (MJ/Kg)	10.9	11.3

<sup>1</sup>, % of dry matter, unless otherwise stated

<sup>2</sup>Concentrate contained 15% maize grain, 25 % wheat bran, 8% soybean meal, 15% cotton seed cake, 15% maize gluten, 15% palm oil cake, 5% molasses, 1% Di-Ca-Phosphate, 0.1% DL-Methionine, 0.5% sodium chloride, 0.5% vitamin and mineral premix (g/kg: premix provided per kg of diet: 2000 IU Vit. A, 150 IU Vit. D, 8.33 g Vit. E, 0.33 g Vit. K, 0.33 g Vit. B1, 1.0 g Vit. B2, 0.33g Vit. B6, 8.33 g Vit. B5, 1.7 mg Vit. B12, 3.33 g pantothenic acid, 33 mg biotin, 0.83 g folic acid, 200 g choline chloride, 11.7 g Zn, 12.5 g Fe, 16.6 mg Se, 16.6 mg Co, 66.7 g Mg and 5 g Mn)

<sup>3</sup>Mulberry leaves fed to the rabbits are in the green wilted form

The rabbits were fed a basal diet, comprising of concentrate (75%) and wilted mulberry leaves (25%) of the total feed, formulated to fulfil all the nutrients requirements of growing rabbits (Table 1; NRC, 1977). The total amount of basal feed required for the

trial was mixed and divided into four equal portions before pelleting. One portion of the basal diet was taken as the control feed (without feed additives). The remaining three portions were supplemented either with (ii) enzyme blend (driselase-1®; cellulase, amylase and protease; 2 g kg<sup>-1</sup> feed); (iii) *S. cerevisiae* (dry yeast TR 100; 2 g kg<sup>-1</sup> feed); or (iv) with a mixture of the enzymes blend and *S. cerevisiae* (1 g each kg<sup>-1</sup> feed) to get three test diets. Driselase-1 contained cellulase (1,000,000 IU), amylase (700,000 IU) and protease (450,000 IU). Fubon (dry yeast TR 100; live yeast count  $\geq$  10 billion/g) is live yeast product and is provided by Angel Yeast Co. LTD. The four diets were separately pelleted with pellet size of 2 to 2.3 mm. All rabbits received green wilted mulberry leaves (25% portion of the daily feed offered) along with a basal diet. Rabbits had *ad libitum* (measured quantity) access to water and pelleted diets throughout the experiment, with 16 h light during the experimental period. The trial continued for 7 weeks period including a week of acclimatization period to the diets. This study was approved by the Departmental Board of Studies on Ethics, Methodology and Welfare, SBBU Sheringal, KP, Pakistan.

### Growth performance and digestibility assay

On the first day of the trial, body weight (BW) of individual rabbits were noted before morning feeding, and then at the end of each week to compute the daily weight gain (g/day). The feed offered to all experimental rabbits were weighed, and the left-over feed was weighed next morning (8:00 am) to calculate the daily feed intake (FI; g/day). The feed conversion ratio (FCR) was computed for the fattening period. Morbidity and mortality were recorded daily. Morbidity was measured by examining all clinical signs of digestive troubles or sickness of individual rabbit.

A digestibility assay for 6 consecutive days (40 to 46 days of fattening trial) was conducted using 6 randomly selected rabbits from each group. The weight of feed offered, feed refused and fresh hard faeces produced during 24 h were recorded. Before weighing, the faeces were sorted and soft faeces were discarded. Representative faeces samples were collected from each animal, subsampled for dry matter (DM) analysis. The remaining faeces were pooled over 6 days and stored in a freezer (-20 °C) for onward chemical analysis. The experimental diets and rabbit faecal samples were ground ( $\sim$  1 mm particle size). The ground samples were examined for the contents of DM, crude protein (CP; N  $\times$  6.25), ether extract (EE)

and acid detergent fibre (ADF) according to AOAC (2000). Method of Van Soest et al. (1991) was used for the determination of NDF content.

### Carcass traits and meat quality variable

On day 49 of the fattening trial, from each dietary group 6 rabbits were sacrificed for measurement of carcass traits. Prior to slaughter, rabbits were fasted for 10 h, and had free access to clean drinking water. Pre-slaughter weight was recorded. The carcass dissection procedures were followed as suggested by the World Rabbit Science Association recommendation described by Blasco and Ouhayoun (1996). Samples were taken from *longissimus lumborum* muscle (10 g) 24 h post-mortem in triplicate, homogenized with distilled water (50 ml), and analysed for pH value. The water release (%) was calculated using standard method of Grau and Fleischmann, (1957), while the cooking loss were measured using procedure prescribed by Boccard et al. (1981). The chemical profile of *longissimus lumborum* muscle was determined using standard method as per guidelines of AOAC (2000). The vital organs such as liver, heart, kidney and spleen were weighed on digital balance.

### Ileum morphology

Segments ( $\sim$  3.0 cm in length) from the ileum were taken from 3 randomly selected rabbits of each dietary treatment, to determine villus height and crypt depth. For this study, three cross-sections from each ileum specimens were fixed by neutralised formalin (10%; v/v), washed with running water, dehydrated, fixed in paraffin wax, and then divided into 5  $\mu$ m thick segments, and finally marked with hematoxylin-eosin stained (Yan et al., 2017).

### Statistical analysis

The impact of feed additives on growth traits, carcass yield, diet digestibility, ileum morphometry and meat physicochemical properties of rabbits were evaluated by the procedure of PROC MIXED of the Statistical Analysis System (SAS Institute, 2009). For parameters with significant ( $P < 0.05$ ) treatment effect the pairwise differences between mean values were determined by post-hoc test analysis using the Duncan's Multiple Range test.

## RESULTS

Data showing effects of the additives on feed intake (FI), weight gain (WG) and FCR of rabbits is presented in Table 2. Additives in the rabbit diet in-

creased ( $P < 0.05$ ) final BW. Notably, the additives had no effect ( $P > 0.05$ ) on the FI of rabbits. However, the efficiency of feed utilization, as reflected by FCR, was improved ( $P < 0.05$ ) due to addition of the additives in rabbit diets. No mortality was reported in the present study throughout the experimental period.

Data on effects of different feed additives on total tract nutrients digestibility are presented in Table 3. All feed additives increased ( $P < 0.05$ ) NDF digestibility. However, DM digestibility increased ( $P < 0.05$ ) only with the addition of enzymes blend. On the other hand, CP, EE and ADF digestibility did not alter ( $P > 0.05$ ) due to addition of the additives in rabbit diet.

Table 4 summarises data on effects of the additives on carcass yield, dressing percentage and organs weight. Hot and chill carcass yield was higher ( $P < 0.05$ ) in the additives supplemented groups. Similarly, dressing percentage also increased ( $P < 0.05$ ) due to incorporation of the additives in the control diet. Additives did not alter ( $P > 0.05$ ) the organs weight, except for kidney, which was higher ( $P < 0.05$ ) in yeast supplemented group. Incorporation of additives in the diet did not alter ( $P > 0.05$ ) the physical properties (pH, water release and cooking loss) and chemical profile (moisture, protein, fat and ash) of rabbit meat (Table 5).

**Table 2.** Effects of enzyme and yeast-based diet on growth performance of fattening rabbit

Body Weight, BW, g	Diets <sup>1</sup>				SEM	Significance
	Control	Enzyme	Yeast	Enzyme-yeast		
Initial, day 42	425 ± 3.54	428 ± 2.21	427 ± 1.56	424 ± 2.60	0.183	ns
Weight, day 56	611.8 ± 3.67	628.7 ± 3.47	633.6 ± 2.15	621.9 ± 3.27	0.453	ns
Weight, day 77	1030 <sup>b</sup> ± 4.96	1063 <sup>a</sup> ± 4.34	1059 <sup>a</sup> ± 4.26	1047 <sup>ab</sup> ± 4.17	1.684	**
Final, FW, day 90	1206 <sup>b</sup> ± 6.56	1247 <sup>a</sup> ± 3.22	1244 <sup>a</sup> ± 6.11	1243 <sup>a</sup> ± 3.51	4.143	***
Average daily gain, ADG, g						
During days 43-56	13.34 ± 0.23	14.34 ± 0.25	14.76 ± 0.17	14.14 ± 0.19	1.667	ns
During days 57-77	19.94 <sup>b</sup> ± 0.43	20.68 <sup>a</sup> ± 0.42	20.26 <sup>a</sup> ± 0.38	20.23 <sup>a</sup> ± 0.32	1.189	**
During days 78-90	13.5 <sup>b</sup> ± 0.16	14.15 <sup>a</sup> ± 0.23	14.22 <sup>a</sup> ± 0.18	15.09 <sup>a</sup> ± 0.25	2.196	**
During days 43-90	16.27 <sup>b</sup> ± 0.05	17.06 <sup>a</sup> ± 0.06	17.02 <sup>a</sup> ± 0.17	17.06 <sup>a</sup> ± 0.14	1.345	**
Feed intake, FI, g/d						
Concentrate	54.3 ± 0.115	56.4 ± 0.125	57.1 ± 0.267	55.8 ± 0.345	1.189	ns
Mulberry leaves	37.55 ± 1.45	32.79 ± 1.56	33.13 ± 1.67	33.68 ± 1.78	3.445	ns
Total intake, g/d	91.85 ± 2.13	89.19 ± 2.45	90.23 ± 3.15	89.48 ± 2.78	2.834	ns
Total intake during d 43-90	4409 ± 5.48	4281 ± 5.81	4331 ± 6.12	4295 ± 4.89	0.021	ns
F/G ratio	4.99 <sup>a</sup> ± 0.030	4.62 <sup>b</sup> ± 0.025	4.69 <sup>b</sup> ± 0.141	4.66 <sup>b</sup> ± 0.061	0.065	**

In the same row, values carrying different superscript letter <sup>a,b</sup> means significant difference at  $P < 0.05$ ; SEM, standard error of mean, F/G, feed to gain ratio; ns, non-significant; \*\* for  $P < 0.01$ ; \*\*\* for  $P < 0.001$ ; <sup>1</sup>Control, basal diet (blend of concentrate (75%) and wilted mulberry leaves (25%) without the inclusion of enzyme and yeast; Enzyme, kg<sup>-1</sup> basal diet contained 2 g enzyme blend (cellulase, amylase and protease); Yeast, kg<sup>-1</sup> basal diet contained 2 g *Saccharomyces cerevisiae*; Enzyme-yeast, kg<sup>-1</sup> basal diet contained blend of enzyme (cellulase, amylase and protease) and *S. cerevisiae* (1 g each)

**Table 3.** Effects of enzyme and yeast-based diet on total tract apparent nutrient digestibility of fattening rabbit

Nutrients	Diets <sup>1</sup>				SEM	Significance
	Control	Enzyme	Yeast	Enzyme-yeast		
DM	69.8 <sup>a</sup> ± 0.45	71.9 <sup>b</sup> ± 1.37	71.7 <sup>ab</sup> ± 0.21	70.9 <sup>ab</sup> ± 0.44	0.623	**
CP	55.5 ± 0.85	54.4 ± 0.35	55.0 ± 0.13	55.4 ± 0.14	0.174	ns
EE	82.8 ± 0.12	80.9 ± 0.05	80.6 ± 0.05	81.8 ± 0.43	0.138	ns
NDF	43.5 <sup>a</sup> ± 0.09	48.5 <sup>b</sup> ± 0.55	49.6 <sup>b</sup> ± 0.07	48.7 <sup>b</sup> ± 0.47	0.729	***
ADF	33.5 ± 0.11	34.6 ± 0.83	35.5 ± 0.15	35.2 ± 0.13	0.117	ns

Values are presented as mean with standard deviation

In the same row, values carrying different superscript letter <sup>a,b</sup> means significant difference at  $P < 0.05$

DM, Dry matter, CP, crude protein, EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre

SEM, standard error of mean; ns, non-significant; \*\* for  $P < 0.01$ ; \*\*\* for  $P < 0.001$

<sup>1</sup>Control, basal diet (blend of concentrate (75%) and wilted mulberry leaves (25%) without the inclusion of enzyme and yeast;

Enzyme, kg<sup>-1</sup> basal diet contained 2 g enzyme blend (cellulase, amylase and protease); Yeast, kg<sup>-1</sup> basal diet contained 2 g *Saccharomyces cerevisiae*;

Enzyme-yeast, kg<sup>-1</sup> basal diet contained blend of enzyme (cellulase, amylase and protease) and *S. cerevisiae* (1 g each)

**Table 4.** Effects of enzyme and yeast-based diet on carcass weight (CW), dressing percentage and organs weight of fattening rabbit

	Diets <sup>1</sup>				SEM	Significance
	Control	Enzyme	Yeast	Enzyme-yeast		
Hot CW, g	692 <sup>b</sup> ± 6.56	734 <sup>a</sup> ± 6.85	735 <sup>a</sup> ± 4.58	739 <sup>a</sup> ± 9.29	5.732	**
Chill CW, g	643 <sup>b</sup> ± 6.07	685 <sup>a</sup> ± 6.42	686 <sup>a</sup> ± 4.54	689 <sup>a</sup> ± 9.20	5.529	**
Reference CW, g	543 <sup>b</sup> ± 6.56	585 <sup>a</sup> ± 6.85	586 <sup>a</sup> ± 4.72	589 <sup>a</sup> ± 9.29	5.751	**
Dressing percentage	57.4 <sup>b</sup> ± 0.23	58.9 <sup>a</sup> ± 0.36	59.1 <sup>a</sup> ± 0.23	59.4 <sup>a</sup> ± 0.69	0.342	**
Liver, g	57.9 ± 0.21	57.1 ± 0.15	56.8 ± 1.15	57.1 ± 0.40	0.509	ns
Kidney, g	8.82 <sup>ab</sup> ± 0.07	8.69 <sup>b</sup> ± 0.05	8.84 <sup>a</sup> ± 0.02	8.51 <sup>c</sup> ± 0.05	0.043	**
Heart, g	4.03 ± 0.01	4.01 ± 0.02	4.12 ± 0.12	3.99 ± 0.02	0.05	ns
Lungs, g	8.78 ± 0.45	8.86 ± 0.67	8.98 ± 0.47	8.82 ± 0.33	0.298	ns
Spleen, g	0.68 ± 0.01	0.67 ± 0.02	0.69 ± 0.02	0.67 ± 0.01	0.012	ns

In the same row, values carrying different superscript letter <sup>a, b, c</sup> means significant difference at  $P < 0.05$ . ns, non-significant; \*\*,  $P < 0.01$ , SEM, standard error of mean

<sup>1</sup>Control, basal diet (blend of concentrate (75%) and wilted mulberry leaves (25%) without the inclusion of enzyme and yeast; Enzyme, kg<sup>-1</sup> basal diet contained 2 g enzyme blend (cellulase, amylase and protease); Yeast, kg<sup>-1</sup> basal diet contained 2 g *Saccharomyces cerevisiae*; Enzyme-yeast, kg<sup>-1</sup> basal diet contained blend of enzyme (cellulase, amylase and protease) and *S. cerevisiae* (1 g each)

**Table 5.** Effects of enzyme and yeast-based diet on physical properties and chemical profile of *longissimus lumborum* muscles of rabbit

Physical properties	Diets <sup>1</sup>				SEM	Significance
	Control	Enzyme	Yeast	Enzyme-yeast		
pH24	5.76 ± 0.03	5.75 ± 0.04	5.76 ± 0.03	5.70 ± 0.10	0.096	ns
Release water (%)	14.5 ± 0.14	14.9 ± 0.19	15.2 ± 0.12	15.0 ± 0.08	0.45	ns
Cooking loss (%)	34.8 ± 0.17	34.5 ± 0.20	34.5 ± 0.23	34.8 ± 0.74	0.44	ns
Chemical profile (%)						
Moisture	72.1 ± 0.04	71.9 ± 0.07	72.0 ± 0.03	71.9 ± 0.02	0.774	ns
Crude protein	21.4 ± 0.48	21.5 ± 0.03	21.2 ± 0.18	21.3 ± 0.21	0.37	ns
Fat	1.82 ± 0.03	1.87 ± 0.02	1.89 ± 0.03	1.89 ± 0.04	0.023	ns
Ash	1.29 ± 0.12	1.26 ± 0.11	1.27 ± 0.10	1.28 ± 0.12	0.987	ns

pH24, ultimate pH determined at 24 h post-mortem; SEM, standard error of mean; ns, non-significant

<sup>1</sup>Control, basal diet (blend of concentrate (75%) and wilted mulberry leaves (25%) without the inclusion of enzyme and yeast; Enzyme, kg<sup>-1</sup> basal diet contained 2 g enzyme blend (cellulase, amylase and protease); Yeast, kg<sup>-1</sup> basal diet contained 2 g *Saccharomyces cerevisiae*; Enzyme-yeast, kg<sup>-1</sup> basal diet contained blend of enzyme (cellulase, amylase and protease) and *S. cerevisiae* (1 g each)

**Table 6.** Effects of enzyme and yeast-based diet on the ileum morphometry of fattening rabbit

Diets <sup>1</sup>	Villus height, $\mu\text{m}$	Crypt depth, $\mu\text{m}$	Villus height crypt depth ratio
Control	840.8 <sup>a</sup> ± 3.95	137.1 <sup>a</sup> ± 1.22	6.13 <sup>a</sup> ± 0.06
Enzyme	1192 <sup>b</sup> ± 2.18	117.3 <sup>b</sup> ± 1.63	10.2 <sup>c</sup> ± 0.15
Yeast	1177 <sup>b</sup> ± 10.3	119.2 <sup>b</sup> ± 1.57	9.88 <sup>bc</sup> ± 0.22
Enzyme-yeast	1178.4 <sup>b</sup> ± 2.26	120.6 <sup>b</sup> ± 0.93	9.77 <sup>b</sup> ± 0.06
SEM	4.676	1.115	0.114
Significance	***	***	***

In the same column, values carrying different superscript letter <sup>a, b, c</sup> means significant difference at  $P < 0.05$ , SEM, standard error of mean, \*\*\*,  $P < 0.001$

<sup>1</sup>Control, basal diet (blend of concentrate (75%) and wilted mulberry leaves (25%) without the inclusion of enzyme and yeast; Enzyme, kg<sup>-1</sup> basal diet contained 2 g enzyme blend (cellulase, amylase and protease); Yeast, kg<sup>-1</sup> basal diet contained 2 g *Saccharomyces cerevisiae*; Enzyme-yeast, kg<sup>-1</sup> basal diet contained blend of enzyme (cellulase, amylase and protease) and *S. cerevisiae* (1 g each)

Data on effects of the additives on ileum morphology are shown in Table 6. Incorporation of additives increased ( $P < 0.05$ ) villus height and reduced ( $P < 0.05$ ) crypt depth. Nevertheless, villus height to crypt depth ratio only increased in enzymes blend supplemented group ( $P < 0.05$ ).

## DISCUSSION

The AGP use in the animal feed has been widely practiced in developing countries including Pakistan for decades. Research has established that the addition of AGP in animal diets, maintain healthy gut ecosystem and improves the animal performance (Cas-

tanon, 2007). However, due to growing incidence of antibiotic resistance (Falcão-e-Cunha et al., 2007; Ali et al., 2016), the antibiotic use has been discouraged in animal feeds. Alternatively, non-antibiotic feed additives, mostly enzymes and probiotics are increasingly used in animal feeds to maintain gut health, improve nutrient utilization and promote animal performance (Mateos et al., 2010; Abudabos et al., 2015; Ayasan and Inci, 2019). This study provides a comprehensive insight on the effects of enzymes (cellulase, amylase and protease) blend and yeast-based additives on growth performance, carcass quality traits, nutrients utilization, ileum morphology and meat physicochemical quality of native rabbits in Northern Pakistan.

Our results showed that inclusion of enzymes blend, yeast or their mixture in the rabbit diet increased WG and improved FCR as compared to the control group (without feed additives), highlighting their potential as an alternative to AGP in rabbit diets. The observed improvement in growth and FCR could be attributed to the positive effects of enzymes and yeast probiotics on the microflora of gut and cecum, such as due to the higher production of beneficial volatile fatty acids in the cecum (Guedes et al., 2009; Pinheiro et al., 2009). The increased production of these volatile fatty acids, particularly butyrate, suppresses the pathogenic bacteria in the gut (Mourao et al., 2006), and improve feed digestibility and gut health. Our findings are in accordance with Chandra et al., (2014), who found significant effects of supplementation of probiotics (*Saccharomyces boulardii* and *Pediococcus acidilacticii* 50% each) and Kemzyme HF (containing fibrolytic, proteolytic and lipolytic enzymes) alone or in combination on BW gain of the rabbits. Considering the carcass yield, in the present study probiotics and/or enzyme blend supplemented groups supported higher carcass weight and dressing percentage than the control group. Shanmuganathan et al. (2004) reported that exogenous enzymes (cellulases and proteases) and yeast culture (Yea-Sacc1026 at 200 ppm) remarkably increased (24.7%) the carcass yield of rabbit, which could be attributed to better nutrient utilization. In the present study, only weight of the kidney is higher in supplemented diet, while Shanmuganathan et al. (2004) reported that enzyme and yeast supplements increased the weights of liver, pancreas and caecum of rabbits. However, the reasons for heavier organs observed with additives are not clear.

In the present study, the physical properties (pH, cooking loss and water release), and chemical profile of meat were not altered by enzymes and yeast inclusion in the diets. Rotolo et al. (2014) found that live yeast (*S. cerevisiae* var. *boulardii*) supplementation in rabbit diet did not affect meat physicochemical traits. Similarly, Simonova et al. (2009) reported that bacteriocinogenic and probiotic strain supplemented diet had no significant effects on physicochemical properties of rabbit meat. Studies have established that rabbit meat is of high nutritional value due to high protein (21.2%; Khan et al., 2016) and linolenic acid (C18:3n-3) contents (Khan et al., 2018). Nonetheless, the chemical composition reported in the present study fell within normal range of rabbit meat.

It is an established fact that unhealthy digestive tract has been linked with gastro-intestinal diseases, inefficient digestive functions and lower feed efficiency (Tang et al., 2009). Intestinal villus height and crypt depth are vital indices for the intestinal health status (Jia et al., 2010). Morphometric variations such as shorter villi in the small intestine are linked with lower absorption of the nutrients, feed utilization efficiency and animal performance (Xu et al., 2003). In our database, the supplementation of enzymes and yeast in the rabbit diet resulted in longer villi, lower crypts depth and higher villus height to crypt depth ratio in the ileum, which may have contributed to the improved feed efficiency and growth performance as compared to the control group.

The favourable effects of the yeast-based diet on maintenance of gut health contributed towards improvement in nutrient digestibility (Mateos et al., 2010). In the present study, enzymes and yeast-based supplemented groups supported higher DM and NDF digestibility, suggesting more efficient utilization of these nutrients in the intestinal tract, which contributed to an improved FCR as compared to the control group (without feed additives). Our findings are consistent with Bhatt et al. (2016), who reported that digestibility of NDF improved with the dietary dry yeast addition in rabbit diet. The significant positive effects of probiotics on nutrient digestibility, growth performance and feed efficiency in combination with maintenance of healthier intestinal morphology are expected to have practical implication for rabbit's productivity and meat quality.

## CONCLUSIONS

The results of this study report the effects of en-

zymes (cellulase, amylase and protease) blend and yeast (*S. cerevisiae*)-based probiotic supplementation on growth performance, nutrients digestibility, carcass quality and ileum morphology of rabbits. Supplementation of *S. cerevisiae* and enzymes blend alone or in combination supported better ileum morphometry, and increased DM and NDF digestibility. As a result, feed conversion efficiency and weight gain of

rabbits significantly improved with supplementation of the additives. These results suggest that inclusion of probiotics can improve intestinal health, feed utilization efficiency and rabbit productivity, which can potentially improve the profitability of rabbit farms.

## CONFLICT OF INTEREST

None declared.

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