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Influence of dietary organic acid, probiotic and antioxidant on the growth performance and nutrient digestibility in growing rabbit

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ABSTRACT

This study was conducted to compare the effects of different feed additives namely, organic acid (citric acid), probiotic (navio plus), and antioxidant (Bio-Sel-E) on the growth performance and nutrient digestibility in growing rabbit to convey a clear message on the use of one specific additive in rabbit diet. Thirty-two crossbred New Zealand White growing rabbit (four to five weeks of age) were randomly assigned to four different groups and reared for a period of fifty six days. Rabbit were fed on green grass (*Hymenachne pseudointerrupta*) and concentrate mixture (2703 Kcal ME/kg, 16.91 % CP). Additives were added at the top of the concentrate mixture, except for the control group. Compared with the control group, rabbit fed different additives showed 9–13% higher growth rate and 7–11% greater growth velocity; and probiotic supplemented group showed the best result ($P<0.05$) in terms of FCR value. Carcass weight (%) increased significantly in probiotic supplemented group, but numerically in organic acid and antioxidant supplemented groups compared with control group. Abdominal fat (%) was significantly decreased in organic acid, probiotic and antioxidant supplemented groups compared with control, however, lowest value (%) was recorded in probiotic supplemented group. Probiotic showed significant effect on pH decreasing trend in ingesta sample up to small intestine. Crude protein digestibility co-efficient (%) value was significantly higher in probiotic supplemented group, but numerically in organic acid and antioxidant supplemented groups compared with control group. Overall results indicated that, among the three different additives: organic acid, probiotic and antioxidant, probiotic may be considered as the best one for the better performance and nutrient digestibility in growing rabbit.

Introduction

Rabbit can play an important role in a small sustainable farming operation. The most common use of rabbit in agricultural industries is for meat, which is high in protein and low in fat, calories, and cholesterol when compared to chicken meat (Petrescu and Petrescu-Mag 2018; Para *et al.*, 2015). Moreover, raising rabbit is a labor-intensive endeavor, and there are many reasons to consider doing so on a small farm: small in size, nutritious, quick economic return and good efficiency in extracting protein from forage with marginal amount of concentrate (Yesmin *et al.*, 2013; Samkol and Lukefahr, 2008). Furthermore, production of organic rabbit is a relatively untapped market. So, there is an opportunity to promote organic rabbit in Bangladesh as a source of healthful and natural meat as well as small farm asset.

Growing criticism on the use of antibiotic growth promoter in animal production fueled the research for non-antibiotic additives, which may have similar impacts in food-producing animals. Increasing worries with food safety led consumers to oppose the usage of antibiotic growth promoters. Among the many alternatives

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probiotics, organic acids and antioxidant rich feed additives are well recognized (El-Hack *et al*., 2022,). According to the scientific reports, administration of probiotics in adequate amounts confer a health benefit on the host animal, such as improve intestinal microbial balance, improve nutrient metabolism, improve growth performance in broilers and, layers (Chowdhury *et al*., 2020; Khatun *et al*., 2010). On the other hand, organic acids creates an acidic environment in the gut that favors the development of beneficial microorganism in broilers, layers, and resulted good performances and digestibility (Khan and Iqbal, 2016; Chowdhury *et al*., 2009). Whereas use of natural antioxidant as feed additive in meat animals can improve performances, influence immune function (Corino and Rossi, 2021), and protect cellular oxidative processes (Surai *et al*., 2019) in broilers. Although, information's are adequate regarding poultry performance and health benefit issues, very limited findings on the effect of such additives in growing rabbit.

Therefore, the present study was conducted to compare the influence of three different additives namely, organic acid (citric acid), probiotic (navio plus), and antioxidant (Bio-Sel-E) on the growth performance, carcass characteristics, ingesta sample pH, nutrient digestibility, and to suggest the best one for growing rabbit.

MATERIALS AND METHODS

A total number of thirty-two newly weaned (four to five weeks of age) healthy crossbred New Zealand White growing rabbit were selected and housed individually in steel cages measuring 1.95 m × 1.80 m × 1.27 m in dimension. J-shaped screened metal feeder and 250 ml bottle drinkers with steel straw were provided in each cage. Rabbit were distributed randomly into four treatment groups in a Completely Randomized Design (CRD) having eight rabbits in each. A concentrate mixture composed of 35% maize, 25% wheat, 10% soybean meal,15% til oil cake, 14% wheat bran, 0.5% common salt, 0.25% premix and 0.25% DL-methionine containing 16.91% CP, 2703 ME (Kcal/kg DM), 0.37% calcium and 0.17% total phosphorus (according to NRC, 1977 recommendation), which was further fortified by feed additives. Additives were added on top of the concentrate mixture to make different treatment groups namely, control (without any additives), citric acid (purchased from local market in Mymensingh, Bangladesh; purity 99.8%, Henan Harvest International Co., Ltd., China) as organic acid; navio plus (Navio plus is the mixture of the following probiotic bacterial and yeast strain: *Bacillus subtilis*, *B. licheniformis, B. megaterium, Lactobacillus acidophilis, L. plantarum, Saccharomyces cerevisiae*, produced by Biovac, Thailand; marketed by ACI Animal Health, Bangladesh) as probiotic; and Bio-Sel-E (produced by Polchem Hygiene Laboratories PVT. LTD, India; marketed by AVON Animal Health, Bangladesh) as antioxidant. Citric acid was added at the rate 2 % with concentrate mixtures (according to Atapattu and Nelligaswatta 2005), whereas others according to recommended doses by respective producers. Concentrate mixture, green grass (local name: dal grass, scientific name: *Hymenachne pseudointerrupta*) and fresh clean water was offered as *ad libitum*.

Growth performance of the experimental rabbit was studied for 56 days, and a conventional digestibility trial was conducted during last 7 days of the experimental period. Feeds left over were recorded and deducted against each animal and 10% of the feces was collected, dried in the sun and stored in the freezer by keeping in polythene bag for further sample preparation and chemical analysis. Rabbits were weighed individually at the beginning of the experiment and weekly interval. Feed conversion ratio (FCR-DM intake / weight gain) was calculated, and growth velocity (GV) was also calculated using the following formula (Handa *et al*., 1995).

GV = $\frac{FW - IW}{IW}$; where, FW = Final body weight; IW=Initial body weight

At the end of the trial, all rabbits were weighted and slaughtered for the measurement of carcass characteristics. For the determination of pH, sample of feed, feces and ingesta of gastrointestinal tract were collected and mixed with water separately and then pH value was measured by pH meter.

Samples of feed, green grass and feces were analyzed for proximate composition following the method of AOAC (1995). Digestibility co-efficient for nutrients was calculated according to McDonald *et al*., 2010. Collected data were analyzed by using "SPSS 11.5" statistical program to compute analysis of variance (ANOVA). Duncan's Multiple Range Test (Duncan, 1955) was done to compare the treatment means at 5% level of significance (Steel and Torrie, 1980).

RESULTS

Growth Performance

The body weight, growth rate, growth velocity, DM intake and FCR are shown in Table 1. Final body weight in rabbit given different additives was varied, although initial BW was almost similar in all groups. Highest final body weight was recorded in probiotic supplemented group (1424 g) and lowest was in control group (1321 g). Organic acid supplemented group showed significantly higher final body weight (1401 g), whereas supplementation of antioxidant results numerically higher final body weight (1387 g) than control group. It is mentionable that, non-significant difference was observed among different additive supplemented groups in terms of final body weight, weight gain, growth rate and growth velocity. However, values for all those parameters were numerically higher in probiotic supplemented group compared with organic acid and antioxidant. Best FCR was found for probiotic supplemented group (4.32) and worse for control group (4.85). Growth benefit effect of dietary probiotics and citric acid and vitamin E in growing rabbit was also observed by few researchers (Kritas *et al*., 2008, Bhatt *et al*., 2017, El-deek *et al*., 2013). Positive effect of probiotics on microfloral growth in the intestine may led to increase feed digestibility, whereas organic acid reduce the gastrointestinal pH and pathogenic microbes thus improve the efficiency of feed utilization by rabbit. On the other hand, antioxidant may enhance the dietary nutrient digestion by reducing the effect of stress on health. Although, effect of above mentioned additives on growing rabbit were studied separately by several researchers, there are no reports on comparison study of these additives on rabbit. Current findings indicated that, rabbit fed probiotic supplemented diet had best (P>0.05) growth performance compared with organic acid and antioxidant. It has been reported that feeding of probiotics may improve growth performances by reducing the harmful microorganisms in gut flora and stimulating the immune system (Kritas *et al*., 2008).

Table 1: Growth performances of rabbit (*n=8*) fed additives supplemented diets

Parameters	Control	Additives supplemented groups			SEM	*P*-value
		Organic acid	Probiotic	Antioxidant		
Initial Weight, g	610±34	615±41	618±28	613±37	8.739	0.993
Final Weight, g	1321^{b}±62	1401^{ab}±77	1424^{a}±89	1387^{ab}±81	12.378	0.049
Total Weight Gain, g	711^{b}±28	786^{ab}±36	806^{a}±61	774^{ab}±44	15.229	0.050
Growth rate, g	12.70^{b}±0.39	14.04^{a}±0.55	14.39^{a}±0.66	13.82^{a}±0.68	0.239	0.035
Growth velocity	1.17^{b}±0.03	1.28^{a}±0.04	1.30^{a}±0.01	1.26^{a}±0.02	0.017	0.001
Total DM intake, g	3448±154	3509±159	3478±187	3488±122	39.12	0.782
Daily DM intake, g	61.57±2.75	62.66±2.84	62.11±3.34	63.74±1.26	0.699	0.782
FCR	4.85^{a}±0.07	4.46^{ab}±0.03	4.31^{c}±0.03	4.62^{ab}±0.25	0.068	0.006

Mean values within the same row with different superscripts are significantly different (P < 0.05); 1Growth rate = weight gain ÷ experimental period; Growth velocity, (final body weight - initial body weight) ÷ initial body weight; DM, dry matter; FCR (feed conversion ratio), dry matter intake ÷ weight gain; All parameters except growth velocity and FCR were calculated per rabbit basis.

Carcass characteristics

Carcass, heart, liver, kidney, spleen and abdominal fat % are shown in Table 2. Data showed that carcass weight in rabbit fed different additives varied among the groups. Highest % of carcass weight was recorded in probiotic supplemented group (55 %) and lowest in control (51 %); other groups showed almost similar (53 %). Fathi *et al*. (2017) reported that, dietary supplementation with probiotics led to increase dressing percentage compared with others. However, heart, liver, kidney, and spleen % values were not significantly differed among the supplemented and control groups. These findings on the carcass characteristics are consistent with several researchers (El-Adway *et al*., 2000; Amaefule *et al*., 2011 and El-deek *et al*., 2013); who reported non-significant effects of dietary probiotics, citric acid, Vitamin E and Selenium on organs weights of rabbit. Interestingly, abdominal fat % was decreased significantly in rabbit fed organic acid and antioxidant supplemented diets compared with control group. Although no such similar report was found on rabbit, according to the findings of some researchers both dietary citric acid and vitamin E inhibits abdominal fat deposition in broilers (Zhang *et al*., 2021; Haq *et al*., 2018). Besides, abdominal fat percent in rabbits fed probiotic added diet was numerically lower (P>0.05) than control group. However, significant effect of dietary probiotics on abdominal fat percent reduction in broilers was reported by several researchers (Yamamoto *et al*., 2007; Saleh *et al*., 2012; Kalavathy *et al*., 2003). The overall findings indicated that all these additives had positive effect on abdominal fat reduction in growing rabbit, may be by inhibiting lipid biosynthesis and promoting fatty acid catabolism thus decreasing the size and/or number of abdominal adipose cells.

The pH of concentrate feed, ingesta sample and feces

The pH values of concentrate feed, ingesta from different parts of gastrointestinal tract and feces are shown in Table 3. The pH value of concentrate feed reflexed the effect of different additives clearly; where organic acid decreased the pH value of concentrate feed, but probiotic and antioxidant did not show such effect. Interestingly, pH of stomach ingesta sample from organic acid and probiotic supplemented groups was significantly lower than that in the control group as well as antioxidant supplemented group. However, pH decreasing tendency of organic acid was not continued in lower parts (small intestine and caecum) of the digestive tract. It is well known that organic acid reduces the pH of diet as well as ingesta from upper part of digestive tract in chicken (Chowdhury *et al*., 2009; Ndelekwute *et al*., 2018), current findings showed the similar effect of organic acid in case of rabbit diet and stomach ingesta. The pH decreasing tendency of organic acid reduced linearly with gradual passing of ingesta from upper to lower part of gastrointestinal tract, may be some other biological secretions are responsible for this neutralization. On the other hand, dietary probiotics leads to decrease pH of stomach ingesta and the effect continued in small intestine, but not in caecum ingesta and feces. Lowering gut pH is considered as one of the potential mechanism of probiotics antagonistic activity against pathogenic bacteria (Abed El-Hack *et al*., 2020). Pathogens normally grow in

Table 2. Carcass characteristics of rabbit ($n=8$) fed additives supplemented diets

Parameters	Control	Additives supplemented groups			SEM	*P*-value
		Organic acid	Probiotic	Antioxidant		
Live weight, g	1359±21	1394±35	1402±13	1374±31	12.368	0.063
Carcass weight, %	51.44^{b}±1.51	53.27^{ab}±1.21	55.92^{a}±1.78	53.53^{ab}±1.32	0.601	0.036
Heart, %	0.38±0.02	0.41±0.02	0.39±0.01	0.41±0.01	0.006	0.116
Liver, %	3.74±0.25	4.05±0.31	4.11±0.28	4.33±0.14	0.089	0.111
Kidney, %	0.81±0.05	0.79±0.03	0.80±0.03	0.80±0.02	0.008	0.914
Spleen, %	0.30±0.01	0.31±0.01	0.28±0.01	0.31±0.01	0.004	0.112
Abdominal fat, %	1.37^{a}±0.01	0.70^{b}±0.11	0.91^{ab}±0.12	0.77^{b}±0.10	0.081	0.012

Mean values within the same row with different superscripts are significantly different (P < 0.05).

Table 3. The pH of concentrate feed, ingesta sample from different parts of gastrointestinal tract and feces of rabbit ($n=8$) fed additives supplemented diets

Parameters	Control	Additives supplemented groups			SEM	*P*-value
		Organic acid	Probiotic	Antioxidant		
Concentrate feed	6.70^{a}±0.10	5.20^{b}±0.10	6.50^{a}±0.10	6.43^{a}±0.21	0.191	0.021
Stomach	4.43^{a}±0.53	3.12^{b}±0.44	3.74^{b}±0.23	4.21^{a}±0.31	0.180	0.015
Small intestine	6.42^{a}±0.41	6.32^{a}±0.52	5.72^{b}±0.31	6.54^{a}±0.23	0.097	0.884
Caecum	5.32±0.21	5.11±0.23	5.03±0.12	5.64±0.24	0.092	0.067
Faces	7.43±0.41	7.21±0.12	7.32±0.13	7.31±0.22	0.066	0.763

Mean values within the same row with different superscripts are significantly different (P < 0.05).

a pH close to 7 or slightly higher; besides, some other useful microorganisms live in an acidic pH (5.8-6.2) and compete with pathogens (Ferd, 1974). Therefore, lower pH leads to decrease pathogenic microbes from gastrointestinal tract and improves nutrient absorption (Boling *et al*., 2001).

Digestibility co-efficient

Digestibility co-efficient values are shown in Table 4. There were no significant differences in the apparent nutrient digestibility co-efficient of dry matter, crude fiber, ether extract and nitrogen free extract of rabbit fed diets supplemented with different additives, except crude protein. Digestibility co-efficient of CP was increased significantly in rabbit fed organic acid supplemented diet.

It was reported that, dietary organic acid increases gastric proteolysis and protein digestibility in broiler chickens (Atapathu and Nelligaswatta, 2005; Gong-YiFeng *et al.*2006). The mechanism may be the increased activity of pepsin and peptides arising from pepsin proteolysis which activate the release of hormones, including gastrin and cholecystokinin, which regulate the digestion and absorption of protein. Numerically higher values for CP digestibility co-efficient in rabbit fed probiotic and antioxidant supplemented diets compared with rabbit in control group indicated that, some unknown facts may suppress the beneficial effects of these additives on rabbit in the current study, as some researchers reported that these additive increased CP digestibility in broiler chickens (Abbasi *et al*., 2020; Mountzouris *et al*., 2010).

Parameters (%)	Control	Additives supplemented groups			SEM	*P*-value
		Organic acid	Probiotic	Antioxidant		
Dry matter	65.21±2.23	69.34±2.71	70.28±1.82	68.11±2.41	1.000	0.064
Crude protein	73.51^{bc}±1.12	79.27^{a}±1.23	78.37^{ab}±1.61	75.84^{ab}±2.54	0.895	0.015
Crude fiber	33.17±2.31	35.53±1.61	34.74±2.40	35.54±2.24	0.565	0.456
Ether extract	55.23±3.41	57.47±2.81	56.12±2.12	57.38±2.50	0.734	0.720
Nitrogen free extract	73.55±0.74	73.12±1.02	72.84±0.87	73.93±1.68	0.307	0.672

Table 4: Digestibility co-efficient of rabbit *(n=8*) fed additives supplemented diets

Mean values within the same row with different superscripts are significantly different (P < 0.05).

Conclusion

It may be concluded that, compared with organic acid and antioxidant, probiotic supplementation showed improved weight gain and feed conversion ratio with apparently no significant changes in carcass traits and abdominal fat content in growing rabbit. Therefore, supplementation of probiotic as feed additives in growing rabbit diet may be very useful considering its direct impact on overall performance of rabbit.

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