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# EFFECT OFLYSOZYME, TRIBUTYRIN, BACILLUS AMYLOLIQUEFACIENS SC06 AND ENRAMYCIN ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY AND CARCASS CHARACTERISTICS OF BROILER DURING FINISHER PHASE

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The objective of current research was to evaluate lysozyme, bacillus amyloliquefaciens SC06 and tributyrin as an alternative of enramycin in broiler chicken. Four isocaloric and isonitrogenous experimental diet contained either enramycin, lysozyme, tributyrin or bacillus amyloliquefaciens SC06 were prepared. Three hundred mix sexed broiler chicks were distributed into4experimental treatments with five replicates in each experimental treatment. Results showed that feed intake and feed efficiency during finisher phase was not influenced by any treatment ($P$ >0.05). Similarly, body weight gain was not influenced by experimental treatments ($P$ >0.05). However, dry matter digestibility was higher in tributyrin and enramycin as compared to lysozyme and B.A SC06 ($P$<0.05). Lysozyme, B.A SC06 and tributyrin showed better ether extract digestibility as compared to enramycin ($P$<0.05). Tributyrin reflected highest crude fiber digestibility as compared to enramycin, lysozyme and B.A SC06 treatments ($P$<0.05). Slaughtering parameter represented that dressing %, breast meat weight %, relative heart weight % and relative gizzard weight % did not differ in all experimental treatments ($P$>0.05). However, highest thigh meat yields % was seen in enramycin treatment and tributyrin treatment ($P$<0.05). Based on performance parameters, it is concluded that lysozyme, bacillus amyloliquefaciens SC06 and tributyrin can successfully replace enramycin in broiler finisher rations.
**Keywords:** Lysozyme; Tributyrin; Bacillus Amyloliquefaciens SC06; Broiler; Growth; digestibility; carcass

## INTRODUCTION

Antibiotics have been extensively used in the feed of animals (Huyghebaert *et al.,* 2011) to enhance boy weight gain and feed efficiency (Huyghebaert *et al.,* 2011). Recently, many countries have banned the antibiotics in the feed as growth promoters due to microbial resistance to antibiotics (Walia *et al.,* 2019). However, in developing countries, poultry feed industry is still using antibiotics in the feed of poultry birds as growth promoters to improve birds performance and to protect birds from various diseases (Huyghebaert *et al.,* 2011). Recently, scientist of developed countries and developing countries are trying to find alternative sources to prepare antibiotic free diets (Hassan *et al.,* 2010; Hussein and Selim, 2018). In the current scenario people are working on many antibiotic replacers like probiotics, prebiotics, immuno-modulating enzymes, organic acids and herbs to substitute antibiotics in broiler diet to improve growth performance and immunity against diseases (Liu *et al.,* 2010; Gong *et al.,* 2017; Hussein and Selim, 2018; John, 2019).
It has been reported that exogenous enzymes like xylanases and beta-glucanases in the feed act as an alternative antibacterial growth promoter (Huyghebaert *et al.,* 2011) by reducing the propagation of pathogenic bacteria (Jackson *et al.,* 2003). Lysozyme is also exogenous enzyme used in poultry feed and known to breaks the glycosidic bonds of peptidoglycan found in protective layer of bacterial cell wall (Zhang *et al.,* 2010). Lysozyme inclusion in diet of broiler chicken prevents necrotic enteritis, stop α-toxin production, influences bacterial number in gut, provide immunity and improve broiler performance, hence, it has the potential to be used as an alternative of antibiotic (Liu *et al.,* 2010; Zhang *et al.,* 2010).
The use of probiotics in the diet of poultry is due to its positive impact on gut health and better utilization of nutrients (Huyghebaert *et al.,* 2011). Bacillus amyloliquefaciens SC06 is probiotic and is being widely used in poultry ration to improve the growth performance of birds. Bacillus amyloliquefaciens SC06 induce autophagy which provide prevention against bacterial infection and improve gut health which ultimately increase body weight gain, feed efficiency and immunity in growing chicken (Rajput *et al.,* 2014; Wu *et al.,* 2017).
Butyric acid is an organic acid and known to improve growth performance and immunity of growing birds (Nari *et al.,* 2020). It is prime energy source of enterocytes and considered

important for the development of lymphoid tissues associated with gut (Friedman and Bar-Shira, 2005). Moreover, Leeson *et al.* (2005) observed that butyric acid supplementation in the ration of broilers enhance body weight gain and feed efficiency in growing broilers.

Butyric acid, lysozyme and some probiotic strains of bacillus have crucial role in improving gut health and performance of broilers. Therefore, a study on comparative efficacy of enramycin, butyric acid, lysozyme and bacillus amyloliquefaciens SC06 was conducted. The objective of study was to find a suitable replacer of enramycin without affecting the performance of growing broiler. Our hypothesis was that butyric acid, lysozyme and bacillus amyloliquefaciens SC06 will successfully replace enramycin.

## MATERIALS AND METHODS

***Experimental diet***: Diets were formulated to be isocaloric and isonitrogenous for finisher phases (d 22 to 35) (Table 1). Experimental diets were fed to experimental groups for two weeks started from day 22 and finished at day 35 of age. All the raw material and ingredients for feed formulation of the experimental rations was provided by Asia Feed (Pvt. Ltd.). The data of chemical composition of ingredient used in formulation of the experimental rations were taken from Brazilian Table for Poultry and Swine. All diets were formulated on digestible amino acids basis keeping lysine as reference amino acid as described in a recent study (Abdullah et al. 2019). Four experimental rations were prepared, and experimental diets were in form of pellet. Each experimental diet contained either enramycin, lysozyme, B.A SC06 or tributyrin according to manufacturer recommended level. Enramycin, lysozyme, B.A SC06 and tributyrin were procured from Polaris International (Pvt. Ltd. Lahore, Pakistan).

***Experimental birds housing and management***: Three hundred day-old cobb strain broiler chicks (mix sex) with average weight of 48 g were provided by Asia Hatcheries (Pvt. Ltd.) Lahore. Chicks were weighed and distributed into four treatments. Distribution of chicks in each treatment was done in such a manner that each experimental treatment had 5 replicates, and each replicate contained 15 chicks. Rice straw was used as litter material and litter was speared in each pen having dimension 4ˈ× 3ˈ× 2.5ˈ feet. Each experimental unit was reared in separate pen. *Ad-libitum* feed intake was ensured throughout the experimental trial as described in recent study (Hussain *et al.*, 2018; Hussain *et al.*, 2020). Birds had free access fresh drinking water round the clock. Shed temperature and ventilation was maintained with the help of an electric water cooler, and ceiling fans. Standard management conditions were ensured in the shed throughout the experimental trial. Birds were free from thrust and hunger as described in literature (Aziz ur Rahman *et al.*, 2017; Aziz ur Rahman *et al.*, 2019). Birds were ensured to perform normal behavior throughout study, keeping in view the behavior parameters as described in literature (Muhammad *et al.*, 2016).

**Table 1. Composition of basal diet.**

| Ingredients | Quantity (g/kg) |
|---|---|
| Corn | 614.41 |
| Canola meal | 70.00 |
| Soybean meal | 251.00 |
| Chips | 19.00 |
| MCP | 10.00 |
| Salt | 1.50 |
| Sodium bicarbonate | 2.10 |
| Lysine sulphate | 5.00 |
| DL-Methionine | 1.70 |
| L-Threonine | 1.80 |
| L-valine | 0.50 |
| L-Isoleucine | 1.30 |
| Sunflower oil | 17.00 |
| Vitamin and Mineral Premix1 | 5.00 |
| Phytase 10000 FTU/Kg | 0.10 |
| Betaine | 1.30 |
| Total | 1000.00 |
| Nutrients (g/kg) | |
| Dry Matter | 888.00 |
| Metabol. Energy KCal/Kg | 2900.00 |
| Crude Protein | 193.50 |
| Crude Fat | 41.90 |
| Crude Fiber | 41.20 |
| Calcium | 9.00 |
| Phosphorous | 3.30 |
| Lysine | 11.00 |
| Methionine | 4.50 |
| Met+Cyst. | 7.40 |
| Threonine | 7.80 |
| Tryptophan | 2.00 |
| Arginine | 10.90 |
| Isoleucine | 7.90 |
| Valine | 7.90 |

[1]Vitamin and Mineral Premix: Each kilogram contained: Vit. A, 7 000 I.U; Vit. D3, 2 500 I.U; Vit. E, 30 mg; of Vit. K3 1 mg; Vit. B1, 1.5 mg; Vit. B2, 4 mg; Vit. B6, 2 mg; Vit. B12, 0.02 mg; niacin, 30 mg; folic acid, 0.55 mg; pantothenic acid, 10 mg; biotin, 0.16 mg; choline chloride, 400 mg; Copper, 20 mg; Iron, 70 mg; Manganese, 100 mg; Zinc, 70 mg; Iodine, 0.4 mg and Selinium, 0.5 mg

***Data Collection:*** The weekly data of total intake of feed was obtained by the difference method. To obtain average weekly feed intake per broiler, the intake of feed per week per pen was divided by total number of broilers in each replicate. The weekly feed intake was corrected with mortality to avoid any chance of error (Sharif *et al.*, 2018). The experimental birds were weighed one by one at the first day of experiment, and after that weekly body weight was done till the completion of experiment. The weekly gain in body weight was determined as the difference between the initial body weight of birds at the start of week and final body weight at the end of week (Rehman *et al.,* 2019). The number of total mortalities was recorded daily in each experimental treatment throughout the experimental period. The feed efficiency ratio (FCR) was measured by dividing the quantity of experimental feed consumed in a given duration by the weight gain at the same duration.

On termination day of trial three mature broilers from each replicate, with nearly similar live body weight, were chosen. Experimental mature broilers were weighed individually, subjected to a day feed withdrawal time. After withdrawal period, all the birds were weighed again. Weighed birds were slaughtered for determination of carcass parameters and internal organs weight. The carcass without internal organs (giblets) was weighed. Weighed giblets were expressed as a %age of its live body weight and measured as the carcass yield. Moreover, the weight of the thigh, breast, gizzard, liver (without gall bladder), spleen, and heart was recorded and its relation to the live body weight of the birds, in percentages, was calculated.

Nutrient diegestibility was determined on $35^{th}$day of age by using marker method. The marker used for nutrient digestibility was acid insoluble ash (Celite®). On $31^{st}$ day age of chicks, acid insoluble ash digestibility marker (Celite®) was mixed in experimental rations. Celite® mixed experimental rations were fed to birds in all experimental treatments. Feeding of Celite® mixed experimental rations continued till the end of trial. On $35^{th}$ day of age, polythene sheets were put under each pen, to collect droppings twice a day. Particle like feather, feed, or dust was removed from the feces carefully. The collected fecal samples were transferred to a plastic bag. Plastic bag contained fecal samples were transferred into refrigerator to freeze samples at −20◦C.

***Chemical analysis:*** Experimental feeds and fecal samples within the replicate were pooled to single sample. Samples were placed in polythene bags carefully as described in previous studies (Iamam-ul-Haq et al. 2019; Shahzad et al. 2019). Pooled samples were dried by using the hot air oven at 65◦C (Su*et al.,* 2013; Li *et al.,* 2014; Zhang *et al.,*2015; Wang *et al.,* 2016). The dried feeds and fecal samples were grinded and passed through a 0.5 mm sieve. These grinded samples were stored at -20◦C. The experimental rations were further analyzed for crude protein (CP), dry matter (DM), and ether extract (EE) by using standard methods (AOAC, 2005) with some modification as described in literature (Niu *et al.,* 2017; He *et al.,* 2018; Li *et al.,* 2019). For determination of CP, the N content was determined by the Kjeldahl procedure and CP was further calculated as N contents × 6.25 as described in recent studies (Xia *et al.,* 2018a; Xia *et al.,* 2018b; Xia *et al.,* 2018c; Chen *et al.,* 2019). For determination of fat contents in the feed and fecal sample, soxhlet apparatus was used. The fecal samples were analyzed for N by using Kjeldahl apparatus and acid insoluble ash was determined in the ashed samples of diet and feces.

***Statistical analysis:*** Collected data were analyzed by using Statistical Analysis System (SAS, 10.1 for windows) and applying analysis of variance technique under Completely Randomized Design. Mean values were compared using Tukey's test (Steel *et al*. 1997).

## RESULTS

***Performance parameters***: Growth performance data are presented in Table 2. Results showed that feed intakeduring finisher phase were not influenced by addition of either enramycin, lysozyme, B.A SC06, or tributyrin in the diet (P >0.05). Similarly, body weight gain was not affected by addition of either enramycin, lysozyme, B.A SC06, or tributyrin in the finisher diet of growing broilers (P >0.05). Moreover, FCR was also similar in all rations contained either enramycin, lysozyme, B.A SC06, or tributyrin(P >0.05).

***Digestibility assay***: Results of nutrient digestibility are showed in Table 3. Results revealed that DM, EE, crude fiber (CF)and nitrogen free extract digestibility were different among the experimental groups fed finisher diet contained either enramycin, lysozyme, B.A SC06, or tributyrin (*P*<0.05). Results showed that DM digestibility was higher in tributyrin and Enramycin as compared to lysozyme and B.A SC06 (*P*<0.05). However, lowest digestibility of DM was seen in broilers fed diet that contained either enramycin, lysozyme, B.A SC06, or tributyrin(*P*<0.05). Broiler fed the finisher diet contained either lysozyme, B.A SC06 and tributyrin showed better EE digestibility as compared to enramycin (*P*<0.05). Birds fed finisher diet contained tributyrin reflected highest CF digestibility as compared to lysozyme and B.A SC06 (*P*<0.05). Similarly, tributyrin and enramycin fed groups of broilers reflected highest nitrogen

**Table 2. Effect of replacing enramycin with lysozyme, tributyrin and bacillus amyloliquefaciens SC06 on growth performance of broiler.**

| **Parameter** | **Dietary Treatments** | | | | **SEM1** | ***P*-value** |
|---|---|---|---|---|---|---|
| | **Enramycin** | **Lysozyme** | **B.A SC062** | **Tributyrin** | | |
| Feed intake(g/bird) | 2124.80 | 2121.50 | 2142.50 | 2123.30 | 31.55 | 0.59 |
| Body weight (g/bird) | 1323.90 | 1328.60 | 1301.20 | 1286.50 | 32.99 | 0.78 |
| Feed conversion ratio | 1.60 | 1.59 | 1.65 | 1.65 | 0.03 | 0.48 |

[1]SEM; standard mean mean, [2]Bacillus amyloliquefaciens SC06, Means with different superscripts in a row differ significantly (*P*<0.05)

**Table 3. Effect of replacing enramycin with lysozyme, tributyrin and bacillus amyloliquefaciens SC06 on nutrient digestibility of broilers**

| Digestibility % | Dietary Treatments | | | | [1]SEM1 | *P*-value |
|---|---|---|---|---|---|---|
| | Enramycin | Lysozyme | [2]B.A SC062 | Tributyrin | | |
| Nutrients | | | | | | |
| Dry matter | 76.85$^{a}$ | 73.78$^{b}$ | 75.35$^{ab}$ | 76.57$^{a}$ | 0.47 | 0.0012 |
| Crude protein | 76.58 | 76.44 | 76.31 | 74.31 | 0.75 | 0.1495 |
| Ether extract | 86.96$^{ab}$ | 84.57$^{a}$ | 89.11$^{a}$ | 88.69$^{a}$ | 0.89 | 0.0094 |
| Crude fiber | 42.13$^{b}$ | 44.40$^{ab}$ | 42.10$^{b}$ | 45.83$^{a}$ | 0.69 | 0.0020 |
| Ash | 39.90 | 39.94 | 37.73 | 43.47 | 1.46 | 0.0867 |
| Nitrogen free extract | 84.69$^{a}$ | 80.49$^{b}$ | 82.60$^{ab}$ | 84.32$^{a}$ | 0.62 | 0.0008 |

[1]SEM; standard mean mean, [2]Bacillus amyloliquefaciens SC06. Means with different superscripts in a row differ significantly ($P$<0.05)

**Table 4. Effect of replacing enramycin with lysozyme, tributyrin and bacillus amyloliquefaciens SC06 on carcass characteristics of broilers.**

| Parameters | Dietary Treatments | | | | SEM1 | *P*-value |
|---|---|---|---|---|---|---|
| | Enramycin | Lysozyme | B.A SC062 | Tributyrin | | |
| Dressing % | 62.93 | 62.62 | 62.86 | 61.75 | 0.86 | 0.75 |
| Breast meat weight % | 34.74 | 36.49 | 35.60 | 34.19 | 0.96 | 0.38 |
| Thigh meat weight% | 23.71$^{a}$ | 22.04$^{ab}$ | 22.81$^{ab}$ | 23.16$^{a}$ | 0.33 | 0.01 |
| Relative Heart weight% | 0.36 | 0.36 | 0.35 | 0.32 | 0.01 | 0.27 |
| Relative Gizzard weight% | 0.98 | 1.02 | 0.99 | 1.09 | 0.05 | 0.13 |
| Relative Liver weight% | 1.95$^{b}$ | 2.10$^{ab}$ | 2.30$^{ab}$ | 2.46$^{a}$ | 0.12 | 0.04 |

[1]SEM; standard mean mean, [2]Bacillus amyloliquefaciens SC06. Means with different superscripts in a row differ significantly ($P$<0.05)

free extract digestibility as compared to lysozyme and B.A SC06 ($P$<0.05). However, the lowest nitrogen free extract digestibility was observed in broilers fed diet contained lysozyme as compared to other experimental treatments ($P$<0.05).

***Carcass characteristics***: Slaughtering parameter data are presented in Table 4. Dressing % did not differ in groups fed finisher diet contained either enramycin, lysozyme, B.A SC06, or tributyrinat 35$^{th}$ day age ($P$>0.05). Similarly, at 35$^{th}$ day of age, breast meat yield was not changed in groups fed finisher diet contained either enramycin, lysozyme, B.A SC06, or tributyrin($P$>0.05). Highest thigh meat yields % was seen in enramycin treatment and tributyrin treatment ($P$<0.05) but it was comparable with lysozyme and B.A SC06 treatment. Results showed that relative percent weight of heart was similar in broilers fed finisher diet contained either enramycin, lysozyme, B.A SC06, or tributyrin ($P$>0.05). Similarly, relative percent weight of gizzard was not changed in experimental broilers fed finisher ration contained either enramycin, lysozyme, B.A SC06, or tributyrin ($P$>0.05). Highest relative percent weight of liver was seen in tributyrin experimental ration and lowest relative percent weight of liver was seen in enramycin experimental treatment ($P$<0.05).

## DISCUSSION

Findings of current research explored that feed intake was similar in all birds fed diet contained either lysozyme, bacillus amyloliquefaciens SC06, tributyrin or enramycin. Finings of similar intake with lysozyme, organic acid, probiotic and antibioticmix diets in broilers are in accordance with the results of previous researcher who stated that lysozyme, organic acids and probiotics had similar effect on intake of broiler (Humphrey *et al.*, 2002; Huyghebaert *et al.*, 2011; Zhang *et al.*, 2013). Interestingly, dietary treatments also did not change the body weight of experimental birds. Numerous studies reported beneficial effects of antibiotics supplementation on body weight gain of broiler chicks (Cavazzoni *et al.*, 1998; Dumonceaux *et al.*, 2006). For instance, Dumonceaux *et al*. (2006) observed improved body weight gain in broilers fed a diet contained virginiamycin as compared to control. Similarly, improved body weight gain in broiler has also been observed in the study of Cavazzoni *et al*. (1998) who found that virginiamycin supplementation in the diet of broiler increased the weight gain. Donabedian *et al*. (2003) reported that feeding virginiamycin supplemented diet to the broilers increased 13% mean body weight of growing broilers. Based on literature, the use of antibiotics in the diet is fully justified by broiler feed manufacturer to improve the growth performance. However, the findings of the current study illustrate that supplementation of antibiotics, exogenous enzyme, organic acid and probiotics had similar impact on performance including body weight of growing broilers. The findings of current research were similar with the results of previous researcher who stated that probiotics, organic had similar effect on body weight gain as compared to prebiotics (Fatufe and Matanmi, 2011; Rodjan *et al.*, 2018). In the current study, tributyrin was used a source of butyric acid (organic acid), released from tributyrin by the intestinal lipase (Li *et al.,* 2015), which may influence on body wight of broiler on the same way as enramycin. Dalmasso *et al*. (2008) stated that butyric acid act as main source of energy for intestinal epithelial cells and play a critical role in stimulating epithelial cell proliferation and differentiation. Furthermore, it has also been reported that butyric acid also has anti-inflammatory effects (Huyghebaert *et al.,* 2011) and paly an important role to strengthen the gut mucosal barrier by increasing production of antimicrobial peptides in mucous and by stimulating the expression of tight junction proteins (Mariadason *et al.,* 1997; Schauber *et al.,* 2003; Peng *et al.,* 2007). Therefore, it could be assumed that butyric acids from tributyrin, can play a role as an antibiotic growth promoter in broiler feed industry. In the current study weight gain of birds fed probiotics was similar with enramycin fed birds. It has been reported that the performance of broilers increased by feeding with direct feeding of beneficial microbes due to a positive modulation of the gut microbiota (Keeney and Finlay, 2011; Qiu *et al.,* 2018). Recent studies reported that gut microbiota have positive impact on growth (Qiu *et al.,* 2019a; Qiu *et al.,* 2019b; Qiu *et al.,* 2020). The current study of feeding bacillus amyloliquefaciens SC06 probiotics as replacement of enramycin is in agreement with study who reported similar findings on performance of broilers (Lei *et al.,* 2015). The similar weight gain in birds fed enramycin and lysozyme could be explained by enhanced gut antioxidant, immune genes and significant increase in intestinal villi by lysozyme, which concomitantly increase the intestinal surface area for nutrients absorption. The presence of healthy and functional gut is imperative to maintain high feed efficiency and excellent production index (Abdel-Latif *et al.,* 2017). Findings of current research work are similar with the finding of Humphrey *et al*. (2002), and they reported that lysozyme did not affect body weight as compared with antibiotics. Therefore, similar body weight gain in broiler with lysozyme, organic acid, probiotic and antibiotic represents that they have similar mechanism of action that increase nutrient uptake, decrease population of harmful bacteria in gut and hence improve the growth of the birds.

In the current study higher nutrient digestibility was observed in birds fed tributyrin as compared with all other treatments except ash digestibility in lysozyme. These results are similar with the research work of Ghazalah *et al*. (2011) who stated that dietary supplementation of organic acids improved nutrient digestibility of broilers. Moreover, Garcia *et al.* (2007) also reported that supplementation of formic acid in broiler finisher diet improved apparent ileal digestibility of DM and CP. Increase in nutrient digestibility is due to decrease in pH of chyme, crop, and increase in activity of α-galactosidase, and pepsin (Afsharmanesh and Pourreza, 2005; Ao *et al.,* 2009). However, lysozyme effect on improved digestibility of nutrient is unclear. Further basic trails are needed to investigate the effect of lysozyme on digestibility.

Similar carcass characteristics in all treatments in current study representing equal significance of antibiotics replacers used in current study. These findings are similar with the findings of pervious researcher (Denli *et al.,* 2003; Yalçinkaya *et al.,* 2008; Youssef *et al.,* 2017) and they reported that supplementation of organic acids, probiotics and prebiotics has no effect on dressing percentage, breast and thigh meat yield of broilers. Similarly, other researchers also reported that organic acid and probiotic diet of broilers did not influence the dressing yield and carcass characteristics (Kopecký *et al.,* 2012; Attia *et al.,* 2013). However, lysozyme and enramycin showed higher thigh meat yield. Present study reported carcass parameters for the first time by using lysozyme in diet, which could be justified by similar mechanism of action of lysozyme and antibiotic (enramycin) (Liu *et al.,* 2010).

***Conclusions*****:** Enramycin, lysozyme, bacillus amyloliquefaciens SC06 and tributyrin have similar results on performance parameters like feed intake, body weight gain and feed conversion ratio. Based on findings of performance parameter, it is recommended that enramycin can be successfully replaced with lysozyme, bacillus amyloliquefaciens SC06 and tributyrin in the diets of broilers during finisher phase.

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## REFERENCES

Abdel-Latif, M.A., H. Ali, A.R. Elbestawy, R. Ghanem, S.A. Mousa and H.S.A. El-Hamid. 2017. Exogenous dietary lysozyme improves the growth performance and gut microbiota in broiler chickens targeting the antioxidant and non-specific immunity mRNA expression. PLoS ONE 12:e0185153.

Abdullah, H.M., L.R. Bielke and Y.A. Helmy. 2019. Effect of arginine supplementation on growth performance and immunity of broilers: A Review. J. Glob. Inno. Agri. Soci. Sci. 7:141- 144.

Aziz ur Rahman, M., X. Chuanqi, S. Huawei and C. Binghai. 2017. Effects of hay grass level and its physical form (full length vs. chopped) on standing time, drinking time, and social behavior of calves. J. Vet. Behav. 21:7-12.

Aziz ur Rahman, M., C. Xia, L. Ji, B. Cao and H. Su. 2019. Nutrient intake, feeding patterns, and abnormal behavior of growing bulls fed different concentrate levels and a single fiber source (corn stover silage). J. Vet. Behav. 33:46-53.

Afsharmanesh, M. and J. Pourreza. 2005. Effects of calcium, citric acid, ascorbic acid, vitamin D 3 on the efficacy of microbial phytase in broiler starters fed wheat-based diets I. Performance, bone mineralization and ileal digestibility. Inter. J. Poult. Sci. 55: 22-43

AOAC. 2005. Official methods of analysis 17th ed. Washington, DC: Association of Official Analytical Chemists.

Ao, T., A. Cantor, A. Pescatore, M. Ford, J. Pierce and K. Dawson. 2009. Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. Poult. Sci. 88:111-117.

Attia, Y.A., A.E.A. El-Hamid, H.F. Ellakany, F. Bovera, M.A. Al-Harthi and S.A. Ghazaly. 2013. Growing and laying performance of Japanese quail fed diet supplemented with different concentrations of acetic acid. Ital. J. Anim. Sci. 12: e37.

Cavazzoni, V., A. Adami and C. Castrovilli. 1998. Performance of broiler chickens supplemented with Bacillus coagulans as probiotic. Br. Poult. Sci. 39:526-529.

Chen, D., C.Yuanwei,Z. Huabing, X. Kequan, T. Jing, T. Qiyuan and M.A Rahman. 2019. Effects of replacing whole-plant corn silage with whole-plant rice silage and rice straw on growth performance, apparent digestibility and plasma parameters in growing angus cross bred beef cattle. Int. J. Agric. Biol:1116-1122.

Dalmasso, G., H.T.T. Nguyen, Y. Yan, L. Charrier-Hisamuddin, S.V. Sitaraman and D. Merlin. 2008. Butyrate transcriptionally enhances peptide transporter PepT1 expression and activity. PLoS ONE 3.

Denli, M., F. Okan and K. Celik. 2003. Effect of dietary probiotic, organic acid and antibiotic supplementation to diets on broiler performance and carcass yield. Pak. J. Nutri. 2:89-91.

Donabedian, S., L.A. Thal, P. Bozigar, T. Zervos, E. Hershberger and M. Zervos. 2003. Antimicrobial resistance in swine and chickens fed virginiamycin for growth promotion. J. Microbiol. Methods 55:739-743.

Dumonceaux, T.J., J.E. Hill, S.M. Hemmingsen and A.G. Van Kessel. 2006. Characterization of intestinal microbiota and response to dietary virginiamycin supplementation in the broiler chicken. Appl. Environ. Microbiol. 72:2815-2823.

Fatufe, A. and I. Matanmi. 2011. Effect of probiotics, organic acids or their mix on the growth performance of starting cockerels. Arch. Zootec. 60

Friedman, A. and E. Bar-Shira. 2005. Effects of nutrition on development of immune competence in chicken's gut-associated lymphoid system. In: Proceedings of the 15th European Symposium on poultry nutrition, Balatonfüred, Hungary, 25-29 September.Pp 247-255.

Garcia, V., P. Catala-Gregori, F. Hernandez, M. Megias and J. Madrid. 2007. Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. J. Appl. Poult. Res. 16:555-562.

Ghazalah, A., A. Atta, K. Elkloub, M.E. Moustafa and R.F. Shata. 2011. Effect of dietary supplementation of organic acids on performance, nutrients digestibility and health of broiler chicks. J. Appl. Poult. Res. 16:555-562.

Gong, M., D. Anderson, B. Rathgeber and J. MacIsaac. 2017. The effect of dietary lysozyme with EDTA on growth performance and intestinal microbiota of broiler chickens in each period of the growth cycle. J. Appl. Poult. Res. 26:1-8.

Hassan, H., M. Mohamed, A.W. Youssef and E.R. Hassan. 2010. Effect of using organic acids to substitute antibiotic growth promoters on performance and intestinal microflora of broilers. Asian-Australas. J. Anim. Sci. 23:1348-1353.

He, Y., W. Niu, Q. Qiu, C. Xia, T. Shao, H. Wang, Q. Li, Z. Yu, Z. Gao and M.A.U. Rahman. 2018. Effect of calcium salt of long-chain fatty acids and alfalfa supplementation on performance of Holstein bulls. Oncotarget 9:3029.

Humphrey, B.D., N. Huang and K.C. Klasing. 2002. Rice expressing lactoferrin and lysozyme has antibiotic-like properties when fed to chicks. J. Nut. 132:1214-1218.

Hussain, M., A. Mahmud, J. Hussain, S. Qaisrani, S. Mehmood and A. Rehman. 2018. Subsequent effect of dietary lysine regimens fed in the starter phase on the growth performance, carcass traits and meat chemical composition of aseel chicken in the grower phase. Braz. J. Poultry Sci. 20:455-462.

Hussain, M., A. Mahmud, J. Hussain, S.N. Qaisrani, S. Mehmood, S. Ahmad and A.U. Rehman. 2020. Effect of dietary amino acid regimens on growth performance and bodyconformation and immune responses in Aseel chicken. Indian J. Anim. Res. 54:53-58.

Hussein, E. and S. Selim. 2018. Efficacy of yeast and multi-strain probiotic alone or in combination on growth performance, carcass traits, blood biochemical constituents, and meat quality of broiler chickens. Livest. Sci. 216:153-159.

Huyghebaert, G., R. Ducatelle and F. Van Immerseel. 2011. An update on alternatives to antimicrobial growth promoters for broilers. Vet. J. 187:182-188.

Iamam-ul-Haq, M.S. Naeem, R.M. Amir, M. Ilyas, F. Shabir, I. Ahmad, B. Ali, Z. Farid and H.A. Raza. 2019. Growth and yield response of spring maize (zea mays l.) Under different potassium doses and irrigation regimesJ. Glob. Inno. Agri. Soci. Sci. 7:135-139.

John, A.S. 2019. Broiler production without antibiotics: United States field perspectives. Anim. Feed Sci. Technol. 250:93-98.

Keeney, K.M. and B.B. Finlay. 2011. Enteric pathogen exploitation of the microbiota-generated nutrient environment of the gut. Curr. Opin. Microbiol. 14:92-98.

Kopecký, J., C. Hrnčár and J. Weis. 2012. Effect of organic acids supplement on performance of broiler chickens. Scientific Papers Animal Science and Biotechnologies 45:51-54.

Leeson, S., H. Namkung, M. Antongiovanni and E. Lee. 2005. Effect of butyric acid on the performance and carcass yield of broiler chickens. Poult. Sci. 84:1418-1422.

Lei, X., X. Piao, Y. Ru, H. Zhang, A. Péron and H. Zhang. 2015. Effect of Bacillus amyloliquefaciens-based direct-fed microbial on performance, nutrient utilization, intestinal morphology and cecal microflora in broiler chickens. Asian-Australas. J. Anim. Sci. 28:239.

Li, L., Y. He, M. Aziz-ur-Rahman and B. Cao. 2014. Effects of different dietary energy and rumen-degradable protein levels on rumen fermentation, nutrients apparent digestibility and blood biochemical constituents of chinese crossbred yellow bulls. Pak. Vet. J. 34:367-371.

Li, J., Y. Hou, D. Yi, J. Zhang, L. Wang, H. Qiu, B. Ding and J. Gong. 2015. Effects of tributyrin on intestinal energy status, antioxidative capacity and immune response to lipopolysaccharide challenge in broilers. Asian-Australas. J. Anim. Sci. 28:1784.

Li, L., M.A.U. Rahman, R. Li, J. Hu, Q. Wang and A. Zhang. 2019. Resistant starch type 4 affects colon morphology, fermentation and microflora in rats. Int. J. Agric. Biol. 22: 665-668.

Liu, D., Y. Guo, Z. Wang and J. Yuan. 2010. Exogenous lysozyme influences Clostridium perfringens colonization and intestinal barrier function in broiler chickens. Avian Pathol. 39: 17-24.

Mariadason, J.M., D.H. Barkla and P.R. Gibson. 1997. Effect of short-chain fatty acids on paracellular permeability in Caco-2 intestinal epithelium model. Amer. J. Physio. Gastro. Liver Physio. 272: G705-G712.

Muhammad, A.U., C.Q. Xia and B.H. Cao. 2016. Dietary forage concentration and particle size affect sorting, feeding behaviour, intake and growth of Chinese holstein male calves. J. Anim. Physiol. Anim. Nutr. 100:217-223.

Nari, N. and H.A.Ghasemi. 2020. Growth performance, nutrient digestibility, bone mineralization, and hormone profile in broilers fed with phosphorus-deficient diets supplemented with butyric acid and Saccharomyces boulardii. Poult. Sci. 99: 926-935.

Niu, W., Y. He, C. Xia, M.A.U. Rahman, Q. Qiu, T. Shao, Y. Liang, L. Ji, H. Wang and B. Cao. 2017. Effects of replacing Leymus chinensis with whole-crop wheat hay on Holstein bull apparent digestibility, plasma parameters, rumen fermentation, and microbiota. Sci. Rep. 7:1-12

Peng, L., Z. He, W. Chen, I.R. Holzman and J. Lin. 2007. Effects of butyrate on intestinal barrier function in a Caco-2 cell monolayer model of intestinal barrier. Pedi. Res. 61:37-41.

Rehman, A., H. Hesheng, M. Rahman, X. Jingyi, L. Shuang, G. Yannan and S.H. Qamar. 2019. Evaluation of efficacy of compound chinese medicinal herbs against mycoplasma synoviae using different lab tests in mouse and chicken. Int. J. Agric. Biol. 22:647-654.

Rajput, I.R., A. Hussain, Y.L. Li, X. Zhang, X. Xu, M.Y. Long, D.Y. You and W.F. Li. 2014. Saccharomyces boulardii and Bacillus subtilis B10 modulate TLRs mediated signaling to induce immunity by chicken BMDCs. J. Cell. Biochem. 115:189-198.

Qiu, Q., C. Gao, M. Aziz ur Rahman, B. Cao and H. Su. 2020. Digestive Ability, Physiological Characteristics, and Rumen Bacterial Community of Holstein Finishing Steers in Response to Three Nutrient Density Diets as Fattening Phases Advanced. Microorganisms 8: 335

Qiu, Q., C. Gao, Z. Gao, Y. He, B. Cao and H. Su. 2019a. Temporal Dynamics in Rumen Bacterial Community Composition of Finishing Steers during an Adaptation Period of Three Months. Microorganisms 7:410.

Qiu, Q., T. Shao, Y. He, A.U.R. Muhammad, B. Cao and H. Su. 2018. Applying real-time quantitative PCR to diagnosis of freemartin in Holstein cattle by quantifying SRY gene: a comparison experiment. PeerJ. 6:e4616.

Qiu, Q., Y. Zhu, X. Qiu, C. Gao, J. Wang, H. Wang, Y. He, B. Cao and H. Su. 2019b. Dynamic variations in fecal bacterial community and fermentation profile of Holstein steers in response to three stepwise density diets. Animals 9:560.

Rodjan, P., K. Soisuwan, K. Thongprajukaew, Y. Theapparat, S. Khongthong, J. Jeenkeawpieam and T. Salaeharae. 2018. Effect of organic acids or probiotics alone or in combination on growth performance, nutrient digestibility, enzyme activities, intestinal morphology and gut microflora in broiler chickens. J. Anim. Physiol. Anim. Nutr. 102:e931-e940.

Schauber, J., C. Svanholm, S. Termen, K. Iffland, T. Menzel, W. Scheppach, R. Melcher, B. Agerberth, H. Lührs and G. Gudmundsson. 2003. Expression of the cathelicidin LL-37 is modulated by short chain fatty acids in colonocytes: relevance of signalling pathways. Gut 52:735-741.

Shahzad, M., W.H. Ghani, M. Ayyub, Q. Ali, H.M. Ahmad, and M. Faisal. 2019. Performance of some wheat cultivars against aphid and its damage on yield and photosynthesis. J. Glob. Inno. Agri. Soci. Sci. 7:105-109.

Sharif, M., M. Shoaib, M.A.U. Rahman, F. Ahmad and S.U. Rehman. 2018. Effect of distillery yeast sludge on growth performance, nutrient digestibility and slaughter parameters in Japanese quails. Sci. Rep. 8:8418.

Su, H., Y. Wang, Q. Zhang, F. Wang, Z. Cao, M.A.U. Rahman, B. Cao and S. Li. 2013. Responses of energy balance, physiology, and production for transition dairy cows fed with a low-energy prepartum diet during hot season. Trop. Anim. Health Prod. 45:1495-1503.

Walia, K., M. Sharma, S. Vijay and B.R. Shome. 2019. Understanding policy dilemmas around antibiotic use in food animals & offering potential solutions. Indian. J. Med. Res. 149:107.

Wang, C., A. Muhammad, Z. Liu, B. Huang and B. Cao. 2016. Effects of ensiling time on banana pseudo-stem silage chemical composition, fermentation and in sacco rumen degradation. J. Anim. Plant Sci. 26:339-346.

Wu, Y., Y. Wang, H. Zou, B. Wang, Q. Sun, A. Fu, Y. Wang, Y. Wang, X. Xu and W. Li. 2017. Probiotic Bacillus amyloliquefaciens SC06 induces autophagy to protect against pathogens in macrophages. Front. Microbiol. 8:469.

Xia, C., M. Aziz Ur Rahman, H. Yang, T. Shao, Q. Qiu, H. Su and B. Cao. 2018a. Effect of increased dietary crude protein levels on production performance, nitrogen utilisation, blood metabolites and ruminal fermentation of Holstein bulls. Asian-Australas. J. Anim. Sci. 31:1643.

Xia, C., Y. Liang, S. Bai, Y. He, A.U.R. Muhammad, H. Su and B. Cao. 2018b. Effects of harvest time and added molasses on nutritional content, ensiling characteristics and in vitro degradation of whole crop wheat. Asian-Australas. J. Anim. Sci. 31:354-362.

Xia, C.Q., A.U.R. Muhammad, W. Niu, T. Shao, Q. Qiu, S. Huawei and B. Cao. 2018c. Effects of dietary forage to concentrate ratio and wildrye length on nutrient intake, digestibility, plasma metabolites, ruminal fermentation and fecal microflora of male Chinese Holstein calves. J. Integr. Agric. 17:415-427.

Yalçinkaya, İ., T. Güngör, M. Başalan and E. Erdem. 2008. Mannan oligosaccharides (MOS) from Saccharomyces cerevisiae in broilers: Effects on performance and blood biochemistry. Turk. J. Vet. Anim. Sci. 32:43-48.

Youssef, I., A.S. Mostafa and M.A. Abdel-Wahab. 2017. Effects of dietary inclusion of probiotics and organic acids on performance, intestinal microbiology, serum biochemistry and carcass traits of broiler chickens. J. Worl. Poul. Res. 7:57-71.

Zhang, G., G.F. Mathis, C.L. Hofacre, P. Yaghmaee, R.A. Holley and T.D. Durance. 2010. Effect of a radiant energy–treated lysozyme antimicrobial blend on the control of clostridial necrotic enteritis in broiler chickens. Avian Dis. 54:1298-1300.

Zhang, Z., J. Cho and I. Kim. 2013. Effects of Bacillus subtilis UBT-MO2 on growth performance, relative immune organ weight, gas concentration in excreta, and intestinal microbial shedding in broiler chickens. Livest. Sci. 155:343-347.

Zhang, X., M.A.U. Rahman, Z. Xue, X. Wang, Y. He and B. Cao. 2015. Effect of Post-pubertal Castration of Wannan Cattle on Daily Weight Gain, Body Condition Scoring and Level of Blood Hormone. Int. J. Agric. Biol. 17:334-338.