



Effects of Supplemented Growth Promoters on Performance and Intestinal Morphology in Broilers Reared under Different Stocking Densities

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ABSTRACT

The aim of the present study was to investigate the influence of different growth promoters on broiler performance and intestinal morphology reared under various stocking densities. A total of 900 day old (Ross-308) straight run broilers were randomly divided into fifteen treatment groups according to a completely randomized design. A 5 × 3 factorial arrangement of treatment was employed. Treatments were consisted of four growth promoters (antibiotics, prebiotics, probiotics, and symbiotics) and a control group with three stocking densities (0.046, 0.056, 0.065m²). Each treatment was replicated 6 times with 10 birds in each. Regarding growth performance, feed intake ($p=0.0008$), body weight ($p=0.0085$) and feed conversion ratio ($p=0.0001$) were improved with the supplementation of symbiotic in the diet whereas lower body weight was noted in the control group. In terms of intestinal morphology, villus height ($p=0.0011$) and villus to crypt ratio ($p=0.0001$) were significantly higher in the symbiotics treatment groups compared to the control group. Moreover, birds reared under 0.065m² stocking density showed improvement in body weight, feed conversion ratio and livability as compared to the other treatment groups. It can be concluded that the supplementation of symbiotics in the diet of commercial broiler reared under 0.065m² stocking density had a positive influence on overall growth performance and intestinal morphology.

INTRODUCTION

Poultry products (meat and eggs) is one of the major food producing enterprise all over the world. The trend in poultry farming is changing from birds per unit area to meat production per unit area and farmer usually place more birds than standard to increase profit (Skrbic *et al.*, 2008). However, this overcrowding exposes the birds to stress and microbial attack. The use of various antibiotics to combat this disease threat create resistance in humans as well as in the animals. The resistance which is created by bacterial action in the body proliferates via food chain into the intestinal micro flora of humans and also may transfer through plasmid to new generation. The main purpose in banning antibiotics usage in feed in numerous countries is to minimize the resistance of antibiotics and bacterial colonies into part of farm animals (Smith *et al.*, 2007). However, the complete removal of antibiotics in feed results in poor growth and more disease attacks, therefore the scientists are working on suitable alternatives for antibiotics. Among different replacements, prebiotics, probiotics, and symbiotic are found to have direct effect on bird's performance and gut health under optimum environmental conditions (Muir *et al.*, 2000).

The efficiency of probiotics may enhance the fusion of different strain of probiotics into the colonies of endogenous bacteria, which



stimulates the growth and activities of both exogenous and endogenous bacteria (Suskovic *et al.*, 2001). The mixture of prebiotics and probiotics as named symbiotics may apply to the synergistic effect on growth and colonies multiplication of beneficial micro-organism which ultimately exert positive effect on the health of the intestine and absorption of the nutrient in the host (De Vrese & Schrezenmeir, 2008). There is a considerable biological advantage with respect to growth performance and intestinal morphology in poultry production (Awad *et al.*, 2009). Numerous studies reported effect of different prebiotics, probiotics and their combinations on the performance of commercial broilers however their interaction with different stocking densities are still neglected and require further investigations. Therefore, the present study was conducted to evaluate the effect of alternative sources of antibiotic growth promoters and their effect on growth performance and intestinal morphology of commercial broilers reared under various stocking densities.

MATERIALS AND METHODS

In this study, 900 commercial straight run broiler chicks (Ross-308) were acquired from the commercial

Table 1 – Calculated Ingredient and nutrient composition of basal diet.

Ingredient	Inclusion Level %
Corn	57.67
Wheat bran	1.25
Soybean meal	29.72
Canola meal	6.12
soy oil	1.48
l-Lys HCl	0.19
dl-Met	0.28
l-Thr	0.08
Salt	0.38
Limestone	1.21
Phytase(10000FTU)	0.01
Dicalcium phosphate	1.46
Trace Vitamin/Min premix	0.15
Total	100
Nutrient	
EE%	4.24
CP%	22.1
AME, kcal/kg	2,950
Calcium, %	0.95
Available P, %	0.38
Sodium, %	0.18
Digestible Lys, %	1.18
Digestible Met, %	0.58
Digestible Met + Cys, %	0.84
Digestible Thr, %	0.74

hatchery and allocated into 15 treatment groups. A 5 × 3 factorial arrangement of treatments were applied according to a completely randomized design. The treatments consisted of 4 growth promoters [antibiotics (lincomycine 0.15%), probiotics (*bacillus subtilis* 0.5%), prebiotics (mannan oligosaccharides 0.6%) and symbiotics (combination of prebiotic and probiotics 1 %)] and a control group and 3 stocking densities (0.046m², 0.056m², 0.065m²). The treatments were replicated 6 times with 10 birds in each. The dimensions for each replicate were 0.46 m², 0.56 m², 0.65m², respectively. Day old chicks were vaccinated against Newcastle Disease (ND) and Infectious Bronchitis (IB) at the hatchery. The chicks were reared on deep litter system where rice husk was used as bedding material with a thickness of 3 inches. In each replicate, one round feeder and three nipple drinkers were installed for *ad libitum* feeding and watering. During brooding, the temperature and humidity were optimized at 33±1.1°C and 62±3% whereas, in rearing period 22-24°C temperature, 65-70% humidity, were maintained. A 24 hours lighting (30 lux) schedule was followed while vaccines were administered according to the schedule (Table 2).

Table 2 – Vaccination Schedule for experimental birds.

Day	Vaccine	Route
1	ND+IB	Eye Drop
5	ND+IB	Eye Drop
12	IBD	Spray
19	ND+IB	Drinking water

ND = Newcastle Disease; IB = Infectious Bronchitis; IBD = Infectious Bursal Disease.

Parameters studied

Growth Performance Traits

The body weight of each bird was recorded on a weekly basis and weight gain was calculated by using the following formula:

Body Weight Gain (g) = Final Body Weight (g) – Initial Body Weight (g)

Feed intake (g) was recorded on a daily basis by using the follow formula:

Feed Intake (g) = Feed offered (g) – Feed residue (g)

Weekly feed conversion ratio was calculated as follows:

$$\text{Feed Conversion Ratio} = \frac{\text{Feed Intake (g)}}{\text{Weight Gain (g)}}$$

The mortality (%) was recorded on a daily basis if any and calculated as the number of birds died as relative to the total number of birds multiplied by 100.



Intestinal Health

After slaughtering at 35 days of age, 5 ileum samples of each treatment group were collected to determine the Villus height (um), Crypt depth (um) and Villus to crypt ratio by the method adopted by Houshmand *et al.* (2011).

Statistical Analysis

The collected data were analyzed through factorial ANOVA technique. General Linear Model was used in SAS software (version, 9.1). Significant treatment means were separated through Duncan's Multiple Range (DMR)-test (Duncan, 1955). The following mathematical model was applied:

$$Y_{ijk} = \mu + T_i + S_j + (TS)_{ij} + \hat{\alpha}\epsilon_{ijk}$$

Where,

Y_{ijk} = Observation of depended variable recorded on i^{th} and j^{th} treatment

μ = Population mean

T_i = Effect of i^{th} treatment ($i = 1, 2, 3, 4, 5$)

S_j = Effect of j^{th} stocking density ($j = 1, 2, 3$)

$(TS)_{ij}$ = Interaction effect between treatment and stocking density

$\hat{\alpha}\epsilon_{ijk}$ = Residual effect associated with i^{th} and j^{th} treatment $NID \sim 0, \sigma^2$

RESULTS AND DISCUSSION

Feed intake

All treatments separately as well as in interaction showed variations in feed intake (Table-3). The birds fed the control diet showed the highest feed intake whereas the birds fed symbiotic in the diet showed the lowest ($p=0.0008$) feed intake than that of other groups. The probable reason of these results might be that the supplementation of growth promoters like symbiotics in broiler diets enhance the biological

functions of the beneficial microbes in the gut of the host birds and increase the nutrient absorption thereby decreasing the feed intake (Sara *et al.*, 2016). Our findings are in agreement with those of Dozier *et al.* (2006), who reported a linear decrease in feed intake with increasing population density.

As far as the stocking density is concerned, non-significant differences were observed among all treatment groups. The interaction of growth promoter and stocking density indicated that the birds reared at 0.056m² stocking density in the control group showed the highest ($p \leq 0.05$) feed intake, while the birds supplemented with symbiotic as growth promoter and reared at 0.065m² stocking density showed the lowest ($p \leq 0.05$) feed intake compared to the other groups. Similar results were found by Tong *et al.*, (2012) who observed significantly higher feed intake and growth of broilers reared at different stocking densities. Similarly, Mehmood *et al.*, (2012a) found significantly higher feed intake in the birds reared at 0.065m²/bird than that of 0.056m² and 0.046 m²/bird stocking densities.

Body weight

Different treatments, including growth promoters and stocking density separately and in interaction, showed pronounced effect on the body weight (Table-3). Among the growth promoters, symbiotics showed the highest ($p=0.0085$) body weight, while the control group showed the lowest body weight compared to that of other treatment groups. Increased body weight in symbiotic group may likely be attributed to the beneficial effects of growth promoters, stimulating activity of one or a limited number of bacteria in the colon, and thus improves host health and body weight (Ashayerizadeh *et al.*, 2009). The symbiotics supplementation also promoted favorable condition in the intestine for the colonization of beneficial microflora, facilitated better growth performance of broiler chicks. As far as the

Table 3 – Effect of growth promoters on live performance (mean \pm standard errors) of broiler chickens raised at various stocking densities.

Traits	Growth promoters					p-value	Stocking densities (m ²)			p-value
	Control	T1	T2	T3	T4		0.046	0.056	0.065	
FI (Kg)	3.71.00 ^a ± 49.43	3.52.07 ^b ± 51.46	3.59.93 ^{ab} ± 34.63	3.47.27 ^b ± 43.53	3.46.60 ^b ± 44.62	0.0008	3554.8 ± 37.93	3559.20 ± 39.66	3525.12 ± 33.22	0.7588
BW (Kg)	2.09.88 ^b ± 21.69	2.13.90 ^b ± 18.52	2.11.08 ^b ± 24.33	2.15.07 ^{ab} ± 34.34	2.21.55 ^a ± 23.72	0.0085	2087.66 ^b ± 15.57	2140.23 ^{ab} ± 25.19	2187.60 ^a ± 15.62	0.0014
FCR	1.78 ^a ± 0.03	1.65 ^{bc} ± 0.02	1.71 ^{ab} ± 0.02	1.63 ^{bc} ± 0.04	1.57 ^c ± 0.03	0.0001	1.71 ^a ± 0.02	1.68 ^{ab} ± 0.02	1.62 ^b ± 0.01	0.0152
Mort%	6.00 ± 1.32	6.67 ± 1.29	6.00 ± 1.32	7.33 ± 1.26	6.67 ± 1.30	0.9325	10.40 ^a ± 1.17	4.80 ^b ± 0.71	4.40 ^b ± 0.81	0.0001

Superscripts on different means within row differ significantly at $p \leq 0.05$; FI = Feed Intake; BW = Body Weight; FCR = Feed Conversion Ratio; Mort = Mortality; T1: Antibiotics; T2: Prebiotics; T3: Probiotics; T4: Symbiotics.



stocking density is concerned, birds reared at 0.065m² stocking density showed the highest ($p \leq 0.05$) body weight followed by those reared at 0.056m² and 0.046m². Increased body weight on 0.065m² stocking density in the current study may be explained by the fact that more rearing space facilitated the birds to exhibit his maximum potential for faster growth rate (Sahota *et al.*, 2012).

Feed Conversion Ratio

Overall means of feed conversion ratio (FCR) showed significant differences among the various treatment groups (Table-3). The symbiotic fed birds had improved ($p=0.0001$) FCR compared to that of other treatment groups. The improved FCR in symbiotic fed group might be attributed to the improved intestinal

environment as it is reported that feeding symbiotic in the diet reduces the intestinal pH and increases digestive enzyme activity in gastrointestinal tract (Samli *et al.*, 2007). Similar to these findings, Oliva Das *et al.* (2016) indicated a significant improvement in FCR in birds fed different growth promoters such assymbiotic than those fed with the control diet.

As far as the stocking density is concerned, non-significant differences were observed among all groups. The interaction of growth promoters and stocking densities exhibited non-significant difference in feed conversion ratio. Similarly, Thitaram (2005) investigated the effect of isomalto-oligosaccharide (IMO), as a growth promoters, in a concentration of 1%, 2% and 4%, but found no differences in feed conversion ratio compared to the control group.

Table 4 – Interaction effect of growth promoters and stocking densities on live performance (mean \pm standard errors) of broiler.

Treatments	Stocking densities	Parameters			
		Feed Intake(kg)	Body weight(kg)	FCR	Mortality %
Control	0.046m ²	3.66.00 ^{ab} \pm 90.73	2.06.40 ^c \pm 45.27	1.79 ^{ab} \pm 0.04	12.00 ^a \pm 2.49
	0.056m ²	3.83.20 ^a \pm 60.58	2.10.00 ^{bc} \pm 38.69	1.83 ^a \pm 0.04	4.00 ^{bc} \pm 1.63
	0.065m ²	3.62.80 ^{ab} \pm 95.50	2.12.25 ^{bc} \pm 27.65	1.71 ^{abcd} \pm 0.05	6.00 ^{abc} \pm 2.66 ^c
Antibiotics	0.046m ²	3.56.20 ^b \pm 99.84	2.10.85 ^{bc} \pm 32.75	1.69 ^{abcd} \pm 0.05	10.00 ^{ab} \pm 2.98
	0.056m ²	3.47.00 ^b \pm 86.13	2.11.25 ^{bc} \pm 33.03	1.65 ^{bcd} \pm 0.05	6.00 ^{abc} \pm 1.63
	0.065m ²	3.53.00 ^b \pm 88.32	2.18.60 ^{abc} \pm 26.77	1.62 ^{cde} \pm 0.04	4.00 ^{bc} \pm 1.63
Prebiotics	0.046m ²	3.66.00 ^{ab} \pm 63.27	2.07.75 ^c \pm 32.18	1.77 ^{abc} \pm 0.02	12.00 ^a \pm 2.49
	0.056m ²	3.57.00 ^b \pm 72.72	2.11.90 ^{bc} \pm 60.46	1.70 ^{bcd} \pm 0.05	4.00 ^{bc} \pm 1.63
	0.065m ²	3.53.80 ^b \pm 37.10	2.14.60 ^{abc} \pm 26.45	1.65 ^{bcd} \pm 0.02	6.00 ^{abc} \pm 1.63
Probiotics	0.046m ²	3.40.80 ^b \pm 73.65	2.08.55 ^c \pm 36.18	1.65 ^{bcd} \pm 0.06	10.00 ^{ab} \pm 2.98
	0.056m ²	3.47.80 ^b \pm 85.99	2.16.75 ^{abc} \pm 91.10	1.64 ^{bcd} \pm 0.10	4.00 ^{bc} \pm 1.63
	0.065m ²	3.53.20 ^b \pm 67.70	2.21.90 ^{ab} \pm 25.34	1.59 ^{de} \pm 0.03	4.00 ^{bc} \pm 1.63
Symbiotics	0.046m ²	3.49.00 ^b \pm 76.90	2.14.75 ^{abc} \pm 25.38	1.64 ^{bcd} \pm 0.04	8.00 ^{abc} \pm 2.49
	0.056m ²	3.47.00 ^b \pm 90.92	2.22.25 ^{ab} \pm 39.79	1.56 ^{de} \pm 0.03	6.00 ^{abc} \pm 1.63
	0.065m ²	3.41.80 ^b \pm 68.60	2.27.65 ^a \pm 47.01	1.51 ^a \pm 0.04	2.00 ^c \pm 1.33
<i>p</i> -value		0.0037	0.0021	0.0055	0.0090

Superscripts on different means within row differ significantly at $p \leq 0.05$

Mortality

Mean mortality percentage were significant among stocking density and their interaction with growth promoters (Table-3). The lowest mortality was recorded in birds reared under 0.065m² stocking density whereas maximum percentage was observed in 0.046m². Most likely the explanation of this trend is that the mortality rate is inversely proportional to the stocking density, as the stocking density increases the mortality rate also increases (Dozier *et al.*, 2006). In addition, birds remain under permanent stress in smaller spaces which deteriorate the immune status. However, some studies indicated that different stocking densities did not lead to the apparent differences in the incidence of mortality (Tong *et al.*, 2012).

Intestinal health

Villus height

Means of different growth promoters, symbiotic found significantly higher ($p=0.0011$) values of villus height as compared to other treatment group, but a non-significant difference was observed in stocking densities (Table-5). The interaction among different treatment groups exhibited higher villus height in symbiotics supplemented groups reared at 0.065m² stocking densities, while the lowest was found in antibiotics supplemented group reared at 0.065m² stocking density. The longer ileal villi observed in broiler chickens that were fed on diets supplemented with probiotic and symbiotic may be due to the



Table 5 – Effect of growth promoters on histological parameters (mean ± standard errors) of broiler chickens raised at various stocking densities.

Traits	Growth promoters					p-value	Stocking densities (m ²)			p-value
	Control	T1	T2	T3	T4		0.046m ²	0.056m ²	0.065m ²	
VH	779.85 ^b ±106.90	745.16 ^b ±78.45	818.24 ^b ±22.68	1027.35 ^a ±40.91	1100.03 ^a ±95.99	0.0011	947.75 ±47.07	875.10 ±57.98	859.51 ±86.32	0.4540
CD	192.77 ±26.93	174.87 ±14.89	169.39 ±9.46	166.06 ±16.26	137.53 ±12.35	0.1629	176.51 ±13.03	160.34 ±15.70	167.51 ±11.50	0.6270
V:C	4.13 ^c ±0.37	4.85 ^{bc} ±0.77	5.02 ^{abc} ±0.34	7.18 ^{ab} ±0.95	8.36 ^a ±0.60	0.0001	5.96 ±0.54	6.39 ±0.73	5.38 ±0.53	0.0580

Superscripts on different means within row differ significantly at $p \leq 0.05$; VH: Villus height; CD: Crypt Depth; V:C: Villus to Crypt Ratio; T1: Antibiotics; T2: Prebiotics; T3: Probiotics; T4: Symbiotics

enhanced short chain organic acids formation induced by probiotics. Thus the increase in the acidity in the gut, which reduces the growth of many pathogenic or non-pathogenic intestinal bacteria, reduce intestinal colonization and reduce infectious processes, ultimately decrease inflammatory processes at the intestinal mucosa, which increase villus height (Loddi *et al.*, 2004). The results were in line with the findings of Mirza (2009) who found a significant increase in ileum villus height at 42 days as a result of symbiotic supplementation to the broiler diet.

Crypt depth

The overall means of growth promoters and stocking densities showed non-significant differences (Table-5). However; the interaction between growth promoters and stocking density revealed significant differences which might be due to intestinal epithelial cells originated in the crypt which migrate along the surface of the villus to the villus tip and extrude the intestine within 48 to 96 hours (Potten, 1998). The

shortening of the villus height and thickness of the crypt may lead to the poor absorption of nutrients and enhance the gastrointestinal secretion which ultimately found poor growth performance (Xu *et al.*, 2003)

Villus to crypt ratio

Comparing means among growth promoters and stocking densities showed significant results in villus to crypt ratio (Table-5). Symbiotics supplemented group showed significantly higher ($p=0.0001$) villus to crypt ratio compared to the other groups while, stocking density remained non-significant. In interaction the probiotics supplemented group had the higher values of villus to crypt ratio reared under 0.056m² stocking density, while the antibiotics supplemented group showed the lowest values reared at 0.065m² floor space. The supplementation of symbiotics in the diet enhance the villus to crypt ratio which ultimately had the positive effect on the absorption of nutrients and increase the body weight of the birds (Catalá-Gregori *et al.*, 2008). Similar findings are seen in a study by

Table 6 – Interaction effect of growth promoters and stocking densities on histological parameters (mean ± standard errors) of broiler.

Treatments	Stocking densities	Parameters		
		Villus height (µm)	Crypt depth (µm)	Villus: Crypt (µm)
Control	0.046m ²	827.30 ^{bcd} ±37.99	241.70 ^a ±43.95	3.70 ^{fg} ±0.52
	0.056m ²	755.98 ^{cd} ±243.08	202.56 ^{abc} ±57.84	3.57 ^{fg} ±0.18
	0.065m ²	756.25 ^{cd} ±253.11	134.04 ^{bcd} ±26.93	5.12 ^{def} ±0.86
Antibiotics	0.046m ²	1035.89 ^{abc} ±63.51	152.78 ^{bcd} ±0.58	6.79 ^{cd} ±0.44
	0.056m ²	748.68 ^{cd} ±56.33	162.98 ^{abcd} ±35.74	5.63 ^{def} ±1.58
	0.065m ²	450.91 ^d ±58.86	208.84 ^{abc} ±23.83	2.14 ^g ±0.03
Prebiotics	0.046m ²	719.81 ^{cd} ±3.08	185.81 ^{abcd} ±16.73	3.97 ^{fg} ±0.37
	0.056m ²	874.89 ^{bc} ±6.99	189.86 ^{abcd} ±0.89	4.61 ^{def} ±0.06
	0.065m ²	860.01 ^{bc} ±26.87	132.50 ^{bcd} ±4.63	6.49 ^{cd} ±0.02
Probiotics	0.046m ²	980.81 ^{abc} ±55.48	179.94 ^{abcd} ±22.51	5.84 ^{def} ±1.04
	0.056m ²	1166.11 ^{abc} ±71.97	103.32 ^d ±6.72	11.29 ^a ±0.04
	0.065m ²	935.14 ^{abc} ±13.37	214.91 ^{ab} ±13.63	4.41 ^{efg} ±0.34
Symbiotics	0.046m ²	1174.95 ^{ab} ±138.93	122.36 ^{cd} ±6.37	9.50 ^{ab} ±0.64
	0.056m ²	829.87 ^{bcd} ±5.02	142.99 ^{bcd} ±32.64	6.85 ^{cd} ±1.52
	0.065m ²	1295.26 ^a ±207.70	147.24 ^{bcd} ±36.89	8.73 ^{ab} ±0.15
p-value		0.0121	0.0234	0.0001

Superscripts on different means within row differ significantly at $p \leq 0.05$.



Peric *et al.*, (2010) who states that the supplementation of prebiotics (fructose oligosaccharides) in the diet observed significant difference in gut morphology of the broilers and reduce the villus to crypt ratio in the intestine.

CONCLUSIONS

An inference could be drawn that the supplementation of symbiotics in the diet of commercial broilers can improve the growth performance and intestinal morphology which enhance the absorption in gastro intestinal tract. Furthermore, the birds reared at 0.065m²/bird stocking density had a positive influence on the growth performance and villus height.

REFERENCES

- Ashayerizadeh A, Dabiri N, Ashayerizadeh O, Mirzadeh KH. Effect of dietary antibiotics, probiotics and prebiotics as growth promoters on growth performance, carcass characteristics and hematological indices of broiler chicken. *Pakistan Journal of Biological Sciences* 2009;12(1):52-57.
- Awad WA, Ghareeb K, Abdel-Raheem S, Bohm J. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histo-morphology of broiler chickens. *Poultry Science* 2009;88:49-55.
- Catalá-Gregori P, Mallet S, Travel A, Orengo J, Lessire M. Efficiency of a prebiotic and a plant extract alone or in combination on broiler performance and intestinal physiology. *Canadian Journal of Animal Science* 2008;88:623-629.
- Dozier WA, Thaxton JP, Purswell JL, Olanrewaju HA, Branton SL, Roush WB. Stocking density effects on male broilers grown to 1.8 kilograms of body weight. *Poultry Science* 2006;85:344-351.
- Duncan DB. Multiple range and multiple F tests. *Biometrics* 1955;11:1-42.
- Houshmand M, Azhar K, Zulkifli I, Bejo MH, Kamyab A. Effects of non-antibiotic feed additives on performance, nutrient retention, gut pH, and intestinal morphology of broilers fed different levels of energy. *Journal of Applied Poultry Research* 2011;20:121-128.
- Loddi MM, Moraes VMB, Nakaghi LSO, Tucci FM, Hannas MI, Ariki JA. Mannan oligosaccharide and organic acids on performance and intestinal morphometric characteristics of broiler chickens. *Proceedings of the 20th Annual Symposium on Computational Geometry*; 2004; Brooklyn, NY. USA. p.45.
- Mehmood S, Akram M, Sahota AW, Javed K, Hussain J, Jatoi AS. Effect of different stocking densities on growth performance, abdominal fat yield and immune response of broilers. *Abstracts of the National Science Conference on "Agriculture and food security issues in global environmental perspectives"*; 2012a; Rawalakot, PAK. Azad Kashmir: The University of Poonch; 2012. 282 p.
- Mirza RA. Using prepared symbiotic in early feeding of broiler chicken and its effect on some productive, physiological and histological performance and carcass characteristics [thesis]. Erbil (IRQ): University of Salahddin Erbil, Agriculture College; 2009.
- Muir WI, Bryden WL, Husband AJ. Immunity, vaccination and the avian intestinal tract. *Developmental and Comparative Immunology* 2000;24:325-342.
- Oliva D, Patil SS, Savsani HH, Padodara RJ, Garg DD, Marandi S, et al. Effect of dietary prebiotics, probiotics and synbiotics as feed additives on blood profile and broiler performance. *International Journal of Science, Environment and Technology* 2016;5(5):3546-3552
- Perić L, Milošević N, Žikić D, Bjedov S, Cvetković D, Markov S, et al. Effects of probiotic and phytogetic products on performance, gut morphology and caecal microflora of broiler chickens. *Archives of Animal Breeding* 2010;53:350-359.
- Potten CS. Stem cells in the gastrointestinal epithelium, numbers, characteristics and death. *Philosophical Transaction of the Royal Society Biological Sciences* 1998;353:821-830.
- Sahota AW, Mehmood S, Akram M, Jatoi AS, Javed K. Growth performance, breast yield and economics in sexed broiler chickens influenced by different phase feeding programs. *Abstracts of National Science Conference on "Agriculture and food security issues in global environmental perspectives"*; 2012; Rawalakot, PAK. Azad Kashmir: The University of Poonch; 2012. p.280.
- Samli HE, Senkoğlu N, Koc F, Kanter M, Ağa A. Effects of Enterococcus faecium and dried whey on broiler performance, gut histomorphology and microbiota. *Archives of Animal Nutrition* 2007;61:42-49.
- Sara k, Elmoghazy G, Madiha SI. The Effects of Pro, Pre and Symbiotic on Growth Performance in Broilers. *Alexandria Journal of Veterinary Sciences* 2016;51(2):222-227.
- SAS Institute. SAS user's guide: statistics. Version 9.1. Cary; 2003.
- Skbric Z, Pavlovski Z, Lukic M, Blagojevic M. Carcass quality of broilers reared in lower stocking density and in conditions of discontinuous light program. *Book of Proceedings. 1st Mediterranean Summit of WPSA "Advances and Challenges in Poultry Science"*; 2008; Porto Carras GR; 2008. p. 1028-1032.
- Smith JL, Drum DJ, Dai Y, Kim JM, Sanchez S, Maurer JJ, et al. Impact of antimicrobial usage on antimicrobial resistance in commercial *Escherichia coli* strains colonizing broiler chickens. *Applied Environmental Microbiology* 2007;73:1404-1414.
- Suskovic J, Kos B, Goreta J, Matosic S. Role of lactic acid bacteria and bifidobacteria in synbiotic effect. *Food Technology and Biotechnology* 2001;39:227-235.
- Thitaram SN, Chung CH, Day DF, Hinton A, Bailey JS, Siragusa GR. Isomaltooligosaccharide increases cecal bifidobacterium population in young broiler chickens. *Poultry Science* 2005;84:998-1003.
- Tong HB, Lu J, Zou JM, Wang Q, Shi SR. Effect of stocking densities on growth performance, carcass yield and immune status of local chicken breeds. *Poultry Science* 2012;91:667-673.
- Xu ZR, Hu CH, Xia MS, Zhan XA, Wang MQ. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poultry Science* 2003;82:1030-1036.