The Effect of Early Feeding and Feed Additives on Lymphoid Organs, Intestinal Microbiology and Meat Peroxidation of Broiler

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Abstract: Early feeding of newly hatched chicks may improve immunity system and intestinal development. This trial was carried out to evaluate the effect of immediate access to nutritional supplement post hatch and feed additives on lymphoid organs, meat stability and gut microbiology of broiler chicks under commercial condition. A total of 1200 male chicks were allotted according to two group in hatchery: Fed groups received early feeding (Vitagel®) during transport until 24 hours post hatch and deprived groups were restricted within transport box for 24 hours post hatch without Vitagel®. In farm, the birds divided into negative control (basal diet) and positive control (Availmycin as antibiotic; *Bacillus subtillis* as probiotic, and Bioherbal® as medicinal plants. When broiler chicks were fed immediately post hatch decreased detrimental bacteria (*Clostrodium* Spp and E.Coli) ($P<0.05$).The friendly bacteria (*Lactobacilli* and *Bifidobacteria*) were not changed ($P<0.05$). Feed additives showed significantly reduced ($p<0.001$) detrimental bacteria and increased friendly bacteria ($p<0.05$). The interactive effect between early feeding supplement and feed additives affected intestinal microbiology ($P<0.01$) through increasing friendly bacteria and reducing detrimental bacteria. At market age, Immediate access to feed increased meat peroxidation (MAD) of broiler chicks in fresh samples (at one day of chilling -18 ^{0}c) and stored sample (at 30day of chilling -18 ^{0}c ($P<0.05$), while feed additives had the similar effect. Interactive effects was observed for meat stability ($P<0.05$). In conclusion, early feeding by Vitagel® may be suitable for broiler chicks that exposure to nutritional and hatchery stresses.

[Yahya Sabah Abdulameer, Mehrdad Modirsanei, Mohammad Mehdi Kiaei, Behzad Mansoori, Mohsen. **The Effect of Early Feeding and Feed Additives on Lymphoid Organs, Intestinal Microbiology and Meat Peroxidation of Broiler.** *Life Sci J* 2016;13(7):30-40]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 4. doi:10.7537/marslsj130716.04.

Key word: Initial period, nutritional supplement, Broiler meat Stability

1.Introduction

The newly hatched chicks may be more acceptances to pathogens and oxidative factors because their immune system is still immature in early period of life. Delay in collection chicks from hatchery trays can culminate immunity and oxidative stress of the newly hatched chicks. Furthermore, it is believed that high yield breed, although advantage to industry, have put more problems on the growing bird, resulting in histological and biochemical conversion of the muscle tissue that are presumed to impair some biochemical traits (Petracci & Cavani, 2012). Therefore the experiments which help the broiler to resist the pathogenic bacteria are desirable and beneficial to increase meat productivity and quality of meat. The studies that explain a role of delayed access to feed on body resistant of pathogens and reduced of pathogenic bacteria are limited. Previous experiments had an opinions about delayed access to feed on losing body weight, decrease development of immune organs and irregular morphology in small intestine (Uni & Ferket, 2004; Yadav *et al*. 2010) and how early access to feed post hatch had a rapid development of cell activity that were increased skeletal growth and development of lymphoid organ and immune response (Dibner & Knight, 1999; Bhanja *et al.* 2010).

However because of unfixed nutritional requirements for neonatal chick spread of hatching time and ability of neonatal chicks to adapt quickly on exogenous feed without hen were pushed to experience of early feeding within transport boxes for attenuating stressors after hatching of broiler chickens. There are tended about using of microbial for producing some enzymes that are regarded as robust anti stress and release bioactive peptides from diverse protein to prevent of oxidative stress (Correa *et al*. 2011; Pan *et al*. 2011).

Feed additives have been shown to stimulate mucosal immunity manipulation of gut function and enhanced microbial ecosystem (Gudev, *et al*. 2004;

Mathivanan *et al*. 2007; Rahimi *et al*. 2011) of young or stressed broiler chicks.

Also at present, there is a high tendency to use early feeding post hatch or feed additives like probiotic and herbal plants which contain several compounds that have antioxidant activity and considered as a simple method for improvement of oxidative stability. In addition, there are very little studies available on the effects of delayed feeding or immediate access to feed post hatch on meat stability. The efficacy of nutritional supplement (Vitagel®) or feed additives in early period of life on intestinal microbiology and meat perioxdation was determined in this trial.

2.Materials and Methods

The study was carried out at the University of Tehran, Animal housing unit. The study was conducted according to Tehran Home Office regulations under the Home Office project license Project No. 7509010/6/8).

Experimental design: A total of 1200 broiler chickens (male) were divided into eight treatment randomly in the hatchery. The half of these treatments were given vitagel® that were inserted within transport boxes 10 grams/bird for 24 hours (early feeding group (EF), whereas the other treatments were restricted in transport boxes for 24 hours post hatching (delayed feeding group (DF), after 24 hours The chicks were weighed and reared in floor pens with 1.2 × 3 m in dimensions, under standard management practices and environmental condition for 40 days. Dietary treatments were as follow: 1 & 5) Basal diet (as controls), 2 & 6) Basal diet+10 mg kg^{-1} Avilamycin (AB), 3 & 7) basal diet+20 mg kg^{-1} Galipro® (a commercial probiotic including *Bacillus subtilis* 4×10^{9} cfu/g) (PRO), and 4 & 8) Basal diet+2 gr kg^{-1} BioHerbal® (a commercial herbal mixture supplement, Pars Imen Darou Co., Tehran, Iran)(HM). The birds were fed on a starter diet (22.0 g Kg^{-1} CP, 12.13 MJ ME Kg^{-1} from day 1-14, a grower diet (20.5 g /Kg CP, 12.55 MJ ME/ Kg) from day 15-28, and a finisher diet (18.0 g/Kg CP, 12.76 MJ ME/ Kg) from day 29-40, respectively (Table 1, Calculated basis). Nutrients in the diets were formulated using NRC 1994. Feed and water were provided *ad-libitum* and a continuous lighting schedule were used throughout the experimental period.

Data Collection:-

Lymphoid organs: On day 40 of experimental, two birds from replicate were slaughtered by cervical dislocation and the abdominal cavity was opened, the lymphoid organs (intestine, Bursa of Fabricius and spleen) were separated and weighed then weight (g/kg body weight) relative to body weight were determined.

Small intestines of selected birds were cleaned from mesenteric tissues, and their lengths measured with non-stretchable thread and scale.

Methodology of Bacteria enumeration

At the end of the experiment (day 25), 10 birds from each treatments with weight of treatment mean ± 5% were selected and killed by cervical dislocation. Samples of ileum were obtained from the slaughtered birds Laboratory microbiological techniques using selective agar media were used for analysis. Ileum samples were homogenized in buffered peptone water and serial of decimal dilution were prepared (10^{-3} to 10^{-7} used). Selective agar media were used for numeration of target bacterial groups. Selective media were: plate count agar (Merk Company, Germany); De Man Rogosa Agar (MRC) (Biolife Company, Italia); MacConkey Agar (Merk Company, Germany) and SPS Agar (Darmstadt, Germany) were used for total bacteria count; *Lacto bacillus; Bifido bacterium; Coliform and Clostridium Spp* respectively. Anaerobic milieu in the anaerobic jar for *Bifidiobacterium and Clostridium Spp* for 48 hours. in addition, microbial populations for total bacteria and *coliform* were counted after aerobic incubation at 37 C for 24 hours and *Lactobacillus* after aerobic incubation at 37C for 48 hours. Results were expressed as log10 colony forming units per gram of ileum (log cfu/g).

Oxidative stability of thigh meat from broiler chickens

At 40 days of age two broilers from each replicate pen, 10 birds per treatment were killed by cervical dislocation. After 24 hours of chilling (4°C), carcasses were trimmed for thigh meat by removing bones, and connective tissue. The left thigh from the respective treatments were sliced longitudinally into two equal parts, equating to 20 thigh muscle samples per treatment. Each sample was weighed, five grams from each fillet was sampled, wrapped in tinfoil, vacuum sealed and stored at -18 °C for testing lipid oxidation on day one (fresh sample) and day 30, respectively.

Lipid oxidation was determined as the thiobarbituric acid assay (TBARs), TBARs value is common method for measuring Malondialdehyde (MDA) (MDA is major degradation product of lipid hydroperoxidase) by using the method described by Nikos *et al*. (1994). The percentage of lipid oxidation was calculated by following equation:{ (absorbance of control – absorbance of sample) ÷ absorbance of control × 100} Thiobarbituric acid-reacting substances (TBARS) were expressed as micrograms of malonaldehyde (MDA) per gram of meat after stored at -18°C for lipid oxidation studies On day one (fresh sample) and 30, respectively.

Table 1: Formulation of the diet and estimated composition of experimental basal diets

	Starter (1-14 d)	Grower (15-28 d)	Finisher (29-40 d)
Ingredients (g Kg^{-1} as fed basis)			
Corn	537.2	572.1	640.0
Soybean Meal	393.0	360.0	290.0
Vegetable Oil	25.0	28.0	30.4
Calcium Di-phosphate	19.0	16.5	15.5
Calcium Carbonate	12.5	11.0	11.5
Salt	3.9	3.9	3.7
DL-methionine	2.4	1.9	2.0
Lysine HCl	2.0	1.6	1.9
Vit. + Min. Premix*	5.0	5.0	5.0
Chemical Composition (g Kg^{-1})			
Metabolisable energy (MJ Kg^{-1})	2900	3000	3050
Crude protein	219	207	182
Total arginine	15.02	14.18	12.30
Total lysine	13.35	12.25	10.75
Total methionine	5.86	5.24	5.03
Total methionine + cystine	9.25	8.48	7.87
Total threonine	8.85	8.43	7.90
Calcium	10.3	9.1	8.9
Available phosphorus	5.0	4.5	4.2
Sodium	1.7	1.7	1.6

*Each kg of the vitamin and mineral supplied: Vit. Retinol, (A) 12000 IU Vit. Cholecalciferol (D_3), 2500 IU, Vit. Tecopherol (E) 11 IU, 1 Vit. Thiamine (B_1) .5 mg, Vit Riboflavin (B_2) 4mg, 10 mg calcium pantothenate, 35 mg Niacin, 2.5 mg Vit. Pyridoxine (B_6), 10 µg Vit. cobalamin B_{12}, 0.15 mg biotin, 2.2 mg Vit K, 75 mg iron, 75 mg manganese, 6 mg Copper, 64.8 mg zinc, 0.87mg Iodine, 0.2 mg Selenium, 500 mg Choline chloride. * Vitagel® Supplement contains: energy sources, vitamins and mineral

3.Statistical Analysis

The experiment had a randomized complete block design with a 2 × 4 factorial arrangement (analysis of variance, ANOVA), SPSS/17 statistical package[14]: $y_{ijk} = \mu + a_i + b_j + a_ib_j + e_{ijk}$, where y_{ijk} is the response measured, μ is mean of the observation, a_i is the effect of early feeding (+/-), b_j the effect of growth promoter, a_ib_j is the interaction between early feeding and type of growth promoter, and e_{ijk} is the error term. Duncan's multiple range test method was used as a follow up to compare sets of means and general linear model was used to determine the main effects of factors and any possible interactions between factors. Significance was accepted at the $P < 0.05$ level.

4.Results

Lymphoid organ: There were no effects of early feeding (Vitagel®) on the Lymphoid organ of broiler chicks. chicks that fed on antibiotic tended to have greater bursa of fabricius (P>0.03) than chicks on the control feeding (Table 2). The effect of early feeding and feed additives were not observed on relative weight of spleen. There were no interactive effects of early feeding and feed additives on spleen. Although chicks that fed antibiotic and herbal plant tended to have lower bursa of fabricius with held for 24 hours post hatching than chicks that were fed vitagel during 24 hours.

Intestinal weight and measures: The effect of interaction between EF and GPs on the Intestinal weight and measures in broiler chicken is presented in table 2.

Length of intestine.

By day 40 post hatch, the intestinal length was not affected by EFs (p≥0.05). The antibiotic treatment had shorter intestine (p≤0.05) when compared with other treatments, while natural GPs (*Bacillus subtilis* (232.5cm); herbal blend (230.9) treatments showed numerically increased (p≥0.05) when compared with control. There were no interactive effects of FPs and GPs on the length of intestine (P≥0.05) of broiler chicks at 42 days of age

Relative weight of Intestine

There was a significant main effect of early feeding on the intestinal weight as percentage carcass weight in which the Fed group had greater (p≤ 0.0 3) intestinal weight than the Deprived group.

At the sixth week, the recorded results showed a significant increase at (P < 0.001) in average relative weight of intestine of control(6.16 ± 0.13) and herbal mixture treated group(6.42± 0.13) when compared

with Antibiotic (5.52± 0.13) and probiotic group (6.31± 0.13).

The interactive effect of FPs and GPs were not observed on the relative weight of intestine (P>0.05) at 40 days of age.

Table 2: Effects of nutritional; supplement and feed additivs on relative weights of lymphoids organs and intestine of Ross 308 Broilers

Treatments					
Main Effects		Bursa of Fabricius	Spleen	Relative Weights of Carcass traits (%)	Length of Intestine (cm)
Early feeding	+	0.101	0.111	6.34	227.0
	-	0.102	0.135	5.88	225.2
Pooled SEM		0.007	0.008	0.03	2.3
Growth Promoters	-	0.084^{B}	0.105	6.16^{A}	223.6^{AB}
	Antibiotic	0.123^{A}	0.113	5.52^{B}	219.8^{B}
	Probiotic	0.099^{AB}	0.122	6.31^{A}	232.5^{A}
	Herbal	0.098^{AB}	0.154	6.42^{A}	228.6^{AB}
Pooled SEM		0.008	0.022	0.13	3.2
Interaction Effects					
Early Feeding (+)	-	0.081^{b}	0.098	6.45^{ab}	224.9
	Antibiotic	0.134^{a}	0.114	5.69^{cd}	220.3
	Probiotic	0.084^{b}	0.121	6.58^{a}	232.1
	Herbal	0.105^{ab}	0.112	6.62^{a}	230.9
Early Feeding (-)	-	0.087^{b}	0.112	5.88^{bcd}	222.3
	Antibiotic	0.114^{ab}	0.112	5.39^{d}	219.3
	Probiotic	0.115^{ab}	0.123	6.05^{abc}	232.9
	Herbal	0.091^{b}	0.195	6.21^{abc}	226.4
Pooled SEM		0.012	0.032	0.19	4.3
Early Feeding		NS	NS	**	NS
Growth Promoters		*	NS	***	*
Early Feeding × Growth promoters		*	NS	***	NS

$^{A\text{-}B,\ a\text{-}c}$ means within the same columns with no common superscripts have significant differences. SEM = Standard error of means, NS = not statistically significant, * = P ≤ 0.05, ** = P ≤ 0.01, *** = P ≤ 0.001

Bacteriology:

Table 3 shows the effects of administration time of early feeding and feed additive on the bacteriology in the ileum digests chicks at 25 days of age.

The contents of total intestinal bacteria were changed and lower in immediate access to feed post hatch birds (p>0.001) compared to the delayed access to feed birds. The early feeding with Vitagel® reduced the detrimental bacteria count (E.coli and *Clostridium*) (p<0.05).

Feed additives reduced (p<0.05) the total bacteria count in addition the detrimental bacteria was lower (p<0.01) with feed additives compared to control. Friendly bacteria significantly reduced (p<0.05) with Availmycin.

The interactive effect of early feeding and feed additives was significant reduced with respect to total bacteria count (p<0.01) for birds received (Vitgel®) followed by feed additives. Also same tendency with detrimental bacteria (p<0.05), especially with probiotic. However the *Lactobacillus* wasnot affect with interactive effect (p<0.05) while *Bifidiobacterium* was positively responsed (p<0.05) to Vitgel® then followed by herbal plant blend.

Meat peroxidation

Table 4 shows the thiobarbituric acid-reacting substances (TBARS) as a measure of lipid oxidation in broiler Thigh meat.

In current study, MDA level was higher in early feeding of Fresh sample (one days) (p<0.05) and (p<0.001) in stored samples than the birds that were deprived from early feeding for 24 hour post hatching. The TBARS were higher in fresh sample in Antibiotic (0.222), herbal plant (0.190) and probiotic (0.188) respectively and were lower in control treatment (0.148), while in stored samples, the meat from birds fed feed additives had the highest TBARS (P< 0.05) and were the lowest in meat from antibiotic birds (0.280 v. 0.366). The interactive effect of administration time of early feeding and feed additives showed a significant increase at (P < 0.05) in average TBARS of the early feeding group that are treated with feed additives (p<0.05) in fresh samples and (p<0.01) in stored samples when compared with the DF group that were fed same diet.

Table 3: Effects of nutritional; supplement and feed additives on microflora population of intestine in Ross 308 Broilers

Treatments		Total Count ($\times 10^{7}$)	*E. coli* ($\times 10^{7}$)	Colestridia ($\times 10^{8}$)	Bifidobacteria ($\times 10^{8}$)	Lactobacilli ($\times 10^{8}$)
Main Effects						
Early feeding	+	1.140	3.17	7.96	3.93	2.69
	-	11.54	8.87	30.39	4.61	3.10
Pooled SEM		2.06	1.86	7.67	1.15	0.78
Growth Promoters	-	12.60^{A}	14.1^{A}	70.84^{A}	4.20^{AB}	4.81^{A}
	Antibiotic	7.97^{AB}	4.87^{B}	3.29^{B}	1.91^{B}	0.97^{B}
	Probiotic	3.23^{B}	2.06^{B}	1.91^{B}	6.83^{A}	2.17^{AB}
	Herbal	1.57^{B}	2.98^{B}	0.65^{B}	4.11^{AB}	3.65^{AB}
Pooled SEM		2.91	2.64	10.84	1.63	1.11
Interaction Effects						
Early Feeding (+)	-	0.64^{c}	8.67^{ab}	28.07^{b}	5.14^{ab}	3.27
	Antibiotic	0.73^{c}	0.89^{b}	2.5^{b}	3.35^{b}	1.67
	Probiotic	1.31^{c}	1.17^{b}	1.49^{b}	1.94^{b}	1.15
	Herbal	1.88^{c}	1.96^{b}	0.25^{b}	5.27^{ab}	4.66
Early Feeding (-)	-	24.6^{a}	19.7^{a}	113.6^{a}	3.26^{b}	6.34
	Antibiotic	15.2^{ab}	8.85^{ab}	4.58^{b}	0.48^{b}	0.27
	Probiotic	5.13^{bc}	2.95^{b}	2.32^{b}	11.7^{a}	3.18
	Herbal	1.25^{c}	4.00^{b}	1.06^{b}	2.96^{b}	2.63
Pooled SEM		2.30	2.54	5.98	1.39	1.12
Probability						
Early Feeding		***	*	*	NS	NS
Growth Promoters		*	**	***	*	*
Early Feeding × Growth promoters		**	**	**	*	NS

$^{A-B,\ a-e}$ means within the same columns with no common superscripts have significant differences. SEM = Standard error of means, NS = not statistically significant, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 4: Effects of nutritional supplement and feed additives on lipid proxidation of thigh muscle in Ross 308 Broilers

Treatments		MDA (µg/g)	
Main Effects		Fresh meat	After 1 month keeping in -18°^{C}
Early feeding	+	0.198	0.382
	-	0.176	0.332
Pooled SEM		0.007	0.009
Growth Promoters	-	0.148^{C}	0.366^{B}
	Antibiotic	0.222^{A}	0.280^{A}
	Probiotic	0.188^{B}	0.378^{B}
	Herbal	0.190 B	0.385^{B}
Pooled SEM		0.010	0.013
Interaction Effects			
Early Feeding (+)	-	0.140^{c}	0.369^{bc}
	Antibiotic	0.262^{a}	0.334^{c}
	Probiotic	0.199^{b}	0.432^{a}
	Herbal	0.194^{b}	0.393^{ab}
Early Feeding (-)	-	0.157^{bc}	0.364^{bc}
	Antibiotic	0.183^{b}	0.225^{d}
	Probiotic	0.178^{bc}	0.323^{c}
	Herbal	0.187^{b}	378^{abc}
Pooled SEM		0.011	0.014
Probability			
Early Feeding		***	***
Growth Promoters		***	***
Early Feeding × Growth promoters		***	***

$^{A-C,\ a-b}$ means within the same columns with no common superscripts have significant differences. SEM = Standard error of, NS = not statistically significant, * = $P \leq 0.05$, ** = $P \leq 0.01$*** = $P \leq 0.001$

5.Discussion

Lymphoid organs

The morphometeric measurement of intestine, bursa of fabricius and spleen gave a reliable indication of immunity status and stress (Fahimeh Alipour, *et al.* 2015).

Main effect of feeding programs

In the broiler chickens, delayed access to feed and water post hatch has been associated with poor development of bursa of fabricius and spleen (Friedman & Bar-Shira 2005; Pires *et al.* 2007). This effect is due to stress factors which stimulate corticosteron production which may lead to lymphoid tissue involution as well as suppression of humoral and cell mediate immune response (Lesson & Summer 2001). At high concentration of corticosteroid atrophy bursa might occur by apoptosis. Dibner *et al*. (1998) found that chicks that had early access to feed and water showed a higher bursa weight as a percentage of body weight, and better disease resistance than their held hatch mates.

This finding in this experiment shows that when broiler chicks are exposed to certain environmental stressors, here early feeding post hatch and some feed additives, it might assist in modulating the development of these lymphoid organs and enhance the immune competence of the bird.

In current study, the stress factors may be for short periods. Pall *et al*. (2013) explained that the corticosteron hormone did not increase with delayed access to nutrient for 12 and 48 hours post hatch and they pointed out that nutrient supplied after starvation had positive effect on this neonatal stress response.

Main effects of antibiotic alternatives regardless of FPs

The feed additives (especially antibiotic) increased significantly bursa as a percentage of BW while relative weight of spleen was not appear any different between treatments.

This finding was consistent with Rahimi *et al.* (2011) who reported that herbal plant and antibiotic did not affect spleen relative weight in broiler chicken. In general, the relative size of bursa of fabricius was improved with feed additives. The reason of this finding is attributed to enhanced health status with feed additives. This result is in agreement with Zahid *et al.* (2015) who pointed out that chicken fed herbal plant (Livol) showed significant increase in bursa to BW ratio. Also, Eucalyptus and peppermint oils increase bursa to body weight ratio as compared with untreated (control) (Awaad *et al.* 2010).

In contrast to these findings regarding bursa of fabricius, AL-Ankari *et al.* (2004) explained there was no significant effect of mix herbal plant (thyme and turmeric) on relative weight and lymphoid organs.

Interactive effects of Feeding programs and antibiotic alternatives

The interactive effect of feeding programs and feed additives showed that the Vitagel® with herbal mixture in improvement of relative weight of bursa of fabracius in broiler chicken is slight. This result is in contrast with Cengiz *et al.* (2012) who concluded that there was no synergistic effect of GP (organic effect) and delayed access to feed post hatch for 36 hours on internal organs and lymphoid organ. It is therefore clear that Fed birds that received antibiotic and herbal mixture showed increase in relative weight of bursa of fabricius compared to Deprived birds that received the same diets.

Relative intestinal weight % and intestinal length(cm):

Main effect of Feeding programs

The development of gut is slow with delayed access to feed post hatch (Noy & Skan 1999). This study is in concomitance to those of Yadav *et al.* (2010) who reported that supplementation of early nutrition supplements immediately post hatch helps in increasing weight and length of small intestine. Gonzales *et al.* (2003) found absolute and relative weight and length of intestine were depressed by fasting and depression was more pronounced when fasting time was at least 36 hours.

Results of the present study indicated that there was no correlation between the length and weight of intestine and growth performances.

Previous studies concluded that adverse effect of delayed access to feed for 24, 48, 36, and 72 after hatching may be removed during first weeks of age by re almentation (Abed, *et al.* 2011; Koksal, *et al*. 2013; Wang, 2014 and Tossaporn *et al.* 2015). However, the current study that is in contrast with mentioned studies. It concluded that adverse effect of delayed access to feed for 24-post hatch on intestinal morphology might be continued for long life.

This finding fit with Jackson (2005) found the highest weight in small intestine when were supplemented with oasis® fed (hydrate feeding) concurrently with starved bird post hatch.

The decreased relative weight of intestine with delayed access to feed due to deprive this organ from energy and protein which it is necessity for maturation and development. While early feeding lead to supply of intestine with enough energy and protein which it is necessary for development and cell hyper plasia (Simon Pophal *et al*. 2004).

These results may be attributed to the stimulatory effect of Vitagel® on intestinal lymphoid tissue leading to more development of this tissue. This may explain the slight thickening (increase the weight) of whole intestine in Vitagel® treated broiler chickens in the current study. This thickening may be attributed to the

increase of the length of intestinal villi showed by the histopathological pictures which obtained by our findings. The obtained data revealed that, broiler chickens received early feeding post hatch for 24 hours had significantly increased of the relative intestinal segments (duodenum, jejunum, ileum, caecum and colon) weight (g /kg body weight) and numerically increased in lengths (cm), but these increased did not alter the growth performances of broiler chicken at experimental periods. The increase of the relative weight of intestine (gm/kg body weight) with Vitagel® was attributed to minimize adverse effect of nutritional stress caused by delayed access to feeding within incubator.

Main effects of antibiotic alternatives regardless of FPs

The increase intestinal length with natural GPs numerically may be attribute to mode of action of feed additives are effective in changing the intestinal microflora, it may often affect water consumption by affecting water absorption and retention (constant relationship between feed intake and water intake) in the intestinal tract (for example, by prevention of diarrhea). The large intestine of growth promoter –fed chicken usually are larger and filled with a greater quantity of moist excreta than the large intestine of chicken fed same diet without GPS (Lesson & Summer, 2001). This finding is similar to those previously reported by who used different doses of natural growth promoters (Diarra *et al*. 2014; Hossain *et al*. 2015).

The obtained data revealed that, Avilamycin for six successive weeks was reduced the intestinal (duodenum, jejunum, ileum, and colon) lengths (cm). This findings may be due to reduced intestinal damage through reducing bacterial load in GIT and reduced of turnover in the intestinal epithelium.

In the current study, natural GPs did not influence the weight of the gut. This finding is similar to those previously reported by Ahmed, (2007), Durrani *et al.* (2006), Gowda *et al.* (2008), and Mehala & Moorthy (2008) who reported that dietary supplementation of herbal plants did not affect the relative weight of gastrointestinal tract of broiler chickens.

Interactive effects of Feeding programs and antibiotic alternatives

Regarding, the interactive effect of early nutrition versus delayed access and growth promoters did not appear on relative intestinal weight and its length. This result agreed with; Daskiran *et al.* (2012); Koksal *et al.* (2013) who noted no interactive effect of delayed access to feed for at least 36 hours and growth promoters on intestine weight and length during experimental period.

Gut Bacteriology

Main effect of feeding programs

In current study, the early feed with Vitagel® reduced of detrimental bacteria count (E Coli and *Clostridium*) and total bacteria may be attributed to role of antibodies presented in yolk sac, which are a source of intestinal mucosal protection against pathogenic bacteria especially during first week, it is noticeable that the number of detrimental groups of tested bacteria increased rapidly after the chicks were exposed to starvation period. This would imply that early nutrition post hatch facilitates immunological development by faster formation of a microbial communal population and accelerating early colonization of friendly intestinal bacteria. It lead to prevent colonization pathogenic bacteria (Maioka & Dahlke 2006). Also this finding may be due to good distribution of B and T lymphocyte in intestine and maturation of GALT caused by essential elements that were presented in Vitagel® that induced of lymphocyte and macrophage in intestinal lumen (Fridman & Bar-Shira 2005). These observations are in accordance with the result obtained by Enberg *et al.* (2013) who found the early nutrition during shipping for 24 hours had lower number of E coli in the ileum content in broiler chicken. Also with Potturi *et al.* (2005) reported the increased presence of aerobic bacteria within the ileum in poult with delayed access to feed.

Main effects of antibiotic alternatives regardless of FPs

Probiotic protects the GIT against colonization by potential pathogens and prevent their proliferation in gut through producing short chain fatty acids which in the reflux process are not only nutrient for intestinal epithelium, but also are the control factor living in these microflora (Dan Kowia Kowska *et al.* 2013). Also the effect of herbal plant is attributed to some compounds like thymol which causes paralyses bacteria by inducing cell lysis and subsequent leakage of cell contents (Burt, 2004).

The current study findings suggest that pathogenic bacteria are decreased by the dietary supplementation of natural growth promoters. This result is confirmed by Guo *et al.* (2003; 2004) who concluded that some herbal plants stimulated *Bifidobacterium* and *Lactobacillus* and reduced the number of *Bacteroides spp* and E.coli in ceca.

Interactive effects of Feeding programs and antibiotic alternatives

The interactive response between administration time of early access to feed and antibiotic alternatives was significant with respect to total bacteria count, E.coli; *Clostridium spp* and *Bifidiobacterium spp*. The data showed that synergistic effect of Vitagel® and antibiotic alternatives reduced pathogenic bacteria and total bacteria count in addition increased of beneficial bacteria (*Bifidiobacterium*).

Meat Peroxidation

Main effect of Feeding programs

Malandialdehyde (MAD) level is endogenous reflection of lipid peroxidation (PX).

In our findings, Malandialdehyde concentration was high in early feeding and growth promoters, this is a direct reflection of PX activity with early feeding and antibiotic alternatives. This is may be attributed to be early feeding or antibiotic alternatives inability to confer adequate antioxidant protection against lipid oxidation. This result attributed to increased polyunsaturated fatty acid in poultry meat, which is considered function ingredients to prevent coronary heart disease and other chronic disease (Jung *et al*. 2010). As observed by Mountney (1976) rancidity of broiler meat occurs because of high unsaturated fatty acid content. That means, under high yield breed was necessary to increase natural or synthetic antioxidan in broiler diet. Since the lipid oxidation is influenced by the addition of antioxidant substances, it is much better for natural or synthetic antioxidants to be incorporated in feed mix (Kusevelt 1996).

This finding is in agreement with Marcincak *et al*. (2005) who reported MDA value depended on the degree of phospholipids in sub cellular membranes and oxidative potential was correlated with degree of unsaturated fatty acid in meat (Coetzee & Hoffman, 2001). Moreover Cengiz *et al*. (2012) stated that early access to feed and delayed access to feed did not change MDA concentration in muscle and MDA tended to increase with increased time of storage.

This result may be interpreted according to Sung *et al*. (2013) who explained that fluctuation in temperature during transport and handling may affect MDA ratio in meat.

Main effects of antibiotic alternatives regardless of FPs

The increased MDA concentration in fresh muscle sample may attribute to increase of unsaturated fatty acid in meat. Also the reason may attribute to increase of (ROS), because ROS have an important function to perform in cell, for example cell of the thyroid gland must make hydrogen peroxide in order to attach iodine atom to thyroglobulin in the synthesis of thyroxine. Also macrophage and neutrophils must generate ROS in order to destroy some species of bacteria that engulfed by phyagocytosis, since, the antibiotic alternatives especially probiotic and herbal plants increased of activation of macrophage and immunity system particularly in GIT, thus may causes increased in ROS. This result is agreement with Aluwong *et al*. (2013) who reported that probiotic increased lipid oxidation in broiler chicken. Also this result partially agreed with Bidarnamani *et al*. (2015) who explained that prolonged time of preserving meat in the fridge lead to increase of MDA in chicken during the first month after preservation regardless on presented of probiotic or herbal plant in broiler diet, but probiotic during the first day of slaughter reduced the MDA. This result is in contrast with Sarker *et al*. (2010) who explained that addition of probiotic and antibiotic reduced the lipid oxidation.

Interactive effects of Feeding programs and antibiotic alternatives

Interactive effect of this experiment indicates that feeding program with antibiotic alternatives did not reduce MDA under high body weight. This finding is in contrast with Cengiz *et al*. (2012) who explained there are no interaction effect between delayed access to feed and growth promoter (organic acid) on MDA concentration in meat after the slaughter.

Also concluded from this result, the administration of high level of natural or synthetic antioxidant with probiotic or high dose of herbal plant containing antioxidant in their constitute like phenol is necessary to prevent lipid oxidation in high body weight. Under normal hatchery condition, chicks with Vitagel® exhibit superior immune competency and enhanced gut health through reduced pathogenic bacteria.

Acknowledgments

This study was financially supported by Research Council of University of Tehran (Project No. 7509010/6/8).

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7/11/2016