Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.5, No.20, 2015

The Role of Early Feeding on the Effects of Alternative Antibiotics on Blood Picture, Immune Competency and Gut Health in Broilers

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Abstract
This experiment was designed to evaluate the effects of early feeding programs (FP) (early access to vitagel® and versus delayed access to feed post hatch for 24 hours) and growth promoters (GP) as well as investigate the synergistic effects of and GP on blood picture , immune competency , and intestinal microbiology of broiler. A total of 1200 male chicks were allotted into two groups. In hatchery : Fed groups (received nutritional supplement (vitagel®) during transport until 24 hours post hatch and deprived groups restricted within transport box for 24 hours post hatch without nutritional supplement . In farm, the birds divided into negative control (basal diet) and positive control (antibiotic (10mg /kg feed)*; Bacillus subtillis* (200mg /kg feed) and medicinal plant blend (1g /kg feed).The immune response against NDV(Newcastle disease) was significantly increased ($p<0.05$) , while antibody titers(Ab) against SRBCs (sheep red blood cells) were numerically high by vitagel®. Ab against SRBCs significantly enhanced ($p<0.001$) with natural growth promoter(*Bacillus subtillis* and Medicinal plants blend).Vitagel®reduces the pathogenic bacteria($p<0.05$),also it maintains on beneficial bacteria .Growth promoters showed significant reduced ($p<0.001$) of pathogenic bacteria(*Clostrodium Spp* and *E.Coli*) and increased ($P<0.05$) of beneficial bacteria (*Lactobacilli* and *Bifidobacteria*).The Lymphocyte(L) was higher with natural GP or interaction effect of vitagel® and GP. ($p<0.05$) , the Heterophils(H) to Lymphocyte ratio (H/L ratio) and Heterophil were lower ($P<0.01$) with interaction effect or natural GP.Results concerning the early feeding supplement confirmed the fact that early feeding post hatch with growth promoters may affect the gut health and immune competence.
Keywords :early feeding supplement, immunity, bacteriology, growth promoters, blood picture

Introduction

The first days of life is very critical for gastrointestinal (GI) maturation and immunity development, especially for high yield breeds. However , in commercial hatchery practice the birds within this period exposures for many stressors , such as newly hatched chicks remain for up to 36 hours after hatching before they are pulled from hatching cabinet and then it followed by hatchery processes (sexing , counting , packaging). After these processing, newly hatched chicks are transported to brooder farms where they final have access to feed and water (VandeVan , 2012 ; Fanguy *etal*. 1980) . As observed by Dibner *etal*. (1998) the yolk content post hatch are not always enough to satisfy nutritional requirement of newly hatched chicks during the first few days post hatch ,as result of this malnutrition the newly hatched chicks resort to gluconeogensis to cope of this deficit .This metabolic disorder obligates the chicks to use their maternal antibodies reserves in yolk content (Bata and Person , 2002) and using of fatty acid within yolk content for nutritional purpose. Within this period, intestinal maturation needs to considerable amount of energy to reach of its full development. Use of yolk sac as the sole source of energy for prolonged time before placement is very damaging of immune system because it deprives the chicks from innate immunity and from the fatty acid. Fatty acids in yolk sac are precursor to metabolite that potent mediator of inflammation (Biloni *etal*. 2013). Therefore, in order not to exposure the chicks to jeopardize the delayed access to feed , the best approaches to absolute these problem is early feeding supplementation (Noy and sklan 1998). Previous experiments show how delayed access to feed has been associated with chick losing body weight , decrease development of immune organs ; irregular morphology in small intestine ; delayed gut bacteria population and slower develop of gastrassociated lymphoid tissue (Mead Adams , 1975 ; Uni and Ferket , 2004; Yadav *etal*. 2010) and how early access to feed post hatch have a rapid development of cell activity that increase skeletal growth and development of lymphoid organ and immune response (Dibner and Knight , 1999 ; Bhanja *etal.* 2010). In some cases, delayed access to feed may be performed to prevent some bacteria outbreak like *E Coli* or concerning of vaccination responses (some research referred that better immunity response was with fasted bids), and other cases, early feeding after hatching is not enough alone to alleviate environmental , pathogenical , oxidative and nutritional stressors. Therefore, there is need for attenuating stressors after hatching of broiler chickens by other approaches. In the

last years, application studies have been revealed that natural growth promoters especially after banned of antibiotic could be a possible strategy to attenuate of immunological and nutritional stressors . In this sense the use of probiotic and medicinal plant blend have been shown to stimulate mucosal immunity , manipulation of gut function and enhanced microbial ecosystem (Fuller 1989 ; Christensen *etal*.2002 ;Gudev, *etal*. 2004; Mathivanan *etal*. 2007 ; Rahimi *etal*. 2011) of young or stressed broiler chicks. Biloni *etal* (2013) who referred that providing early feeding supplement during shipping resulted in increased performance,although early feeding supplementation does not completely compensate for the delayed exposure to nutrients , also concluded that combination of probiotic and early feeding supplementation improved gut morphology , reduced pathogenic bacteria as well as improved growth performances compared with control and each product individually. In present study, we have evaluated the efficacy of vitagel$^{(R)}$ alone or combination between vitagel$^{(R)}$ followed it by GP on immune competency, blood pictures and gut microbiology under commercial conditions.

Materials and Methods

Experimental design: The experimental procedure was approved by the animal research committee of the University of Tehran. A total of 1200 newly hatched male Ross 308 broiler chicks (41 ± 1.5 g mean body weight) purchased from a local hatchery, and were randomly allotted into 2 equal groups. One of this groups was received vitgel® within transport boxes and continued during transport till brought to the delivery hall of farm until completion of 24 hours of post hatch (Fed birds).The vitagel® was inserted directly into the transportation boxes during the chicks counting just before the transport loading .Each bird proximately utilized 10 g /chick /24 hours. While second group restricted in transport boxes and deprived from (vitgel®) for 24 hours post hatch (Deprived birds).In farm after 24 hours, they weighed and divided into eight treatments according to randomized completely block design with a 2 × 4 factorial arrangement. The first factor was early feeding (with or without Vitagel®) and the second was the kind of growth promoters (none, antibiotic, probiotic, Herbal mixture). Each treatment was consisted of 5 repetitions of 30 birds. The chicks were kept in floor pens with 1.2 × 3 m in dimensions, under standard management practices and environmental condition for 40 days. 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^{-1} CP, 12.55 MJ ME Kg^{-1}) from day 15-28, and a finisher diet (18.0 g Kg^{-1} CP, 12.76 MJ ME Kg^{-1}) from day 29-40, respectively (Table 1,Calculated basis). Nutrients in the diets were formulated using UFFDA feed formulation package (User-friendly Feed Formulation, Done Again, programmed by J. Hargrave, University of Georgia, Athenes, GA,USA) and balanced to be iso-caloric and iso-nitrogenous and to meet nutrient requirements of the birds according to the Ross 308 recommendations for all nutrients(Broiler Nutrition Specifications). 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*)(PRO), and 4&8) Basal diet+2 gr kg^{-1} BioHerbal 10% (a commercial herbal mixture supplement, Pars Imen Darou Co., Tehran, Iran)(HM). The temperature was regulated at 32 ± 1 ℃ in the first week and reduced by 3 ℃ per week to receive 21 ℃ in the third week. Feed and water were provided *ad-libitum* and a continuous lighting schedule were used throughout the experimental period.

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: 12000 IU Vit. A, 2500 IU Vit. D_3, 11 IU Vit. E, 1.5 mg Vit. B_1, 4mg Vit B_2, 10 mg calcium pantothenate, 35 mg Niacin, 2.5 mg Vit. B_6, 10 µg Vit. 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 minerals.

Data Collection:-

Lymphoid organs : On day 40 of experimental , two randomly selected bird from replicate were killed by cervical dislocation and the abdominal cavity was opened , the lymphoid organs (Bursa of fabricius and spleen) were separated and weighed then weight (%) relative to body weight were determined .

Cell Mediated immune response Evaluation : The cell mediate immune response was assessed using the, cutaneous basophils hypersensitivity response to phytohemagglutinin (PHA-P)(Pahar –Azmoon Co., Tehran . Iran). Ten birds from each treatment were intradermaly injected between the 3rd and 4th digits of right foot with 100µg of PHA in 0.1 ml of sterile phosphate buffer saline (PBS) at day 35. The thickness of the skin was measured with a pressure sensitive micrometer before the injection and 24 and 48 hours after the injection (Corrier and Deloach , 1990).

Evalation of Humoral immunity

Antibody titers against SRBCs : At 22 and 29 days of age, 0.1 ml SRBC suspension (5%) in sterile PBS was injected in pectoral muscle of 10 birds per treatment. Blood samples (2ml) were collected from the wing vein before; and 7 days after each SRBC injection. Antibody titers were expressed as log 2 of the reciprocal of heist dilution giving complete agglutination (Wegman and Smithies (1966).

Antibody against NDV vaccine : NDV vaccine (Losta) was administrated intra eye (dropping)at day 10 of life . Blood samples were collected on 7 and 14 days after vaccination then antibody response was determined by using hamoagglutination inhibition method according to Anon (1971).

Blood pictures : on day 40 of rearing , blood from the sub cutaneous elbow vein was withdrawn once from 10 birds /treatment . Leukocytes(WBC) were carried out with a cell- counter (Nihon Cohden Cenltax –MEK-6450K) Japan .(without division into fraction) .WBC were assessed calculating their numbers by the chamber method. The prepared blood smears were stained using Giemiza stain (Coles , 1989).

Methodology of Bacteria enumeration

Conventional microbiological techniques using selective agar media were used for analysis. Ileum sample 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).

Statistical method:-

Data was collected, arranged, summarized and then analyzed using the computer programs SPSS/17 (2001). The statistical Method was ANOVA test (two way analysis of variance) to test the differences among treatments of broilers according to feeding programs and different experimental feed additives.

Result and Discussion

Humoral immunity response against SRBCs : Although the antibody level in the vitagel(R) were numerically higher after 7 day of each SRBC inoculation, Table 2 .This result attributed to that vitagel® containing some vitamins like C and E which take part in the synthesis of leukocytes , especially phagocytes and neutrophils which enhance immunity in the broiler (Null , 2001) or may be attributed to alter cytokine production (Erf *etal*. 1998) . Also this findings referred that deprivation of feed for 24 hours post hatch led to depress humoral immune response measured as Ab titer response to NDV and SRBC response. Dibner (1999) ; Bhanja *etal.*(2010) they reported that restricting the availability of nutrients during early life (24 hours post hatch) affected the development of immune system measured as Ab titer against SRBC and NDV response . Also this result reinforced Panda *etal.* (2010) who pointed out that deprivation for 24 hours had adverse affected development of immune competence during effect on lymphoid organs and cell mediated immune measured as response to PHA injection and humeral immunity (measured as AB titer against SRBC ; NDV response) , but this effect did not alter the growth performances compared to early access to feed and water chicks . In contrast with This findings , Juul –Madsen *etal.*(2004) ; Engber *etal.* (2013) ; Shinde *etal.* (2015) they suggested that food deprivation for short period (approximately 24 hours post hatch) appears to be acceptable for growth and normal immunological performance . The highest antibody titer against SRBC were observed in natural GP compared to control in primary response ($p<0.001$) and ($p<0.01$) in secondary response. While lowest antibody titer appeared in basal diet and antibiotic respectively ($P<0.05$) .This result confirmed by many research, they reported significant effect of medicinal plant blend and probiotic on antibody titer response to SRBC (Rahmi *etal*. 2011 ; Yakheshi *etal*. , 2011 Ghasemi *etal*. (2014).The interaction effect were detected between early feeding (vitagel®)and GP . Also interaction effect of vitagel® then followed it by GP caused significant increased on antibody titers at primary stage ($p<0.01$) and secondary stage ($p<0.05$) compared to Deprived birds received same diets .It appears that birds received vitagel® then followed it by GP could play a complementary role on immune response , in which vitagel® may enhance the efficacy of immune competency (Ao *etal*. 2012).

Humeral immunity response agaist NDV response:-

The antibody titer against NDV was recorded as higher in vitagel® at primary stage ($p<0.05$) and numerically increased at secondary stage ,table 2. One of the most profound effects of delayed access to feed and water is its ability to retard the immune system functions (Savino , 2002 ; Hangalapura *etal.* 2005). Nnadi *etal* (2010) who explained despite to non change in lymphoid organ , but the early fed chicks appeared" immunological wise" than their fasted counterparts for 72 hours post hatch . As observed with Bhanja *etal*. (2010) they reported that restriction the availability of nutrients during early life (24 hours post hatch) affected the development of immune system measured as Ab titer against SRBC and NDV response . The GP did not alter in antibody titers compared to control ($p<0.05$) at all stages .The result of GP was similar with those Al –Ankari *etal*. 2004 ; Sadeghi *etal* 2012 ; Hahn, (2014) who referred no significant effect of medicinal plant blend (garlic ; cinnamove , anise ; thymus turmeric) on Ab titers against NDV compared with control . This finding contrast with Mahmoodibadzardi *etal*. (2014). Who explained role of essential oil of herbal plant on improved of Ab titers against NDV in broiler chicken. Also unfit with Nikpiran *etal*. (2013) Who found that probiotic showed significant influence on immune response to NDV vaccine in broiler, also they explained higher titer of Ab against SRBC with probiotic compared with control and prebiotic. However combination effect of vitagel® and

GP improved efficacy of the medicinal plant blend, it showed increased of antibody at primary stage ($p<0.05$) and numerically at secondary stage . This result agreed with Ao *etal*. (2012) who suggested that when the chicks are exposed to cetain stressors like delayed access to nutrients post hatch or transport , early feeding and certain dietary additives might have a greater ability to modulate the development of lymphoidv organs and enhance the immune compentence of the bird after deprivation .Also Cengiz *etal*. (2012) who referred that the addition of organic acid solely to broiler diet may not be a protective feeding practice in preventing stress caused from delayed access to feed post hatch of broiler chick.

Cell mediate immunity(CMI)response:-

Our study the cell mediated immune response to PHA-P did not support by vitgel$^{(R)}$for 24 hours post hatch or GPs ($p<0.05$). This data approximately fit with Friedman and Bar-Shir (2005) found that the retard of immunity response is reduced after two week of age. Also Sottosanti ,(2009) who explained that adaptive immune response at a later post hatch age remain relatively strong despite previous stress exposure . Engberg *etal*. (2013) who suggested that the early nutrition or delayed access to feed post hatch have not affect on immunity response .This finding may be attributed to increase of body weight , there are negative correlation between BW and immune response (Martin *etal*. 1990). That mean, increased of body mass lead to minimize of hypersensitivity response. Also this result fit with Rahimi *etal*. (2011) in regard of herbal plant , who explained that garlic did not alter hypersensitivity cutaneous basophiles response . Yakheshi *etal*. (2011) found that probiotic; antiobiotic and herbal plant were not altered the immunity response in primary immune response in broiler chicken under normal condition .

Table 2: Effects of early feeding and growth promoters on immune system function Ross 308 Broilers

Treatments		Antibody- NDV response (log $_2$)		Antibody- SRBC response (log $_2$)		Web toe thickness (mm) following PHA injection		Increasing of web toe thickness (%) following PHA injection	
Main Effects						24h PI	48h PI	24h PI	48h PI
Early feeding	+	4.20	3.52	2.37	3.47	1.52	4.20	27.4	75.5
	-	3.55	3.29	2.28	3.28	1.31	4.21	24.7	80.1
Pooled SEM		0.18	0.22	0.12	0.17	.15	. 32	2.8	5.6
Growth Promoters	-	3.95	3.68	1.83^{C}	2.83^{C}	1.55	4.30	29.4	81.3
	Antibiotic	3.55	3.55	2.25^{BC}	3.10^{BC}	1.50	4.10	28.2	75.7
	Probiotic	4.35	3.55	2.80^{A}	3.67^{AB}	1.40	4.25	24.6	79.2
	Herbal	3.65	2.85	2.43^{AB}	3.90^{A}	1.21	4.16	21.9	75.2
Pooled SEM		0.26	0.32	0.17	0.24	.21	. 46	4.0	7.9
Interaction Effects									
Early Feeding (+)	-	4.30^{ab}	3.70	1.67^{c}	3.00^{ab}	1.60	4.40	38.3	79.7
	Antibiotic	3.90^{abc}	4.10	2.43^{ab}	3.20^{ab}	1.62	4.39	29.2	79.6
	Probiotic	4.40^{a}	3.40	2.93^{a}	3.60^{ab}	1.59	3.80	27.3	64.2
	Herbal	4.20^{abc}	2.90	2.47^{ab}	4.07^{a}	1.32	4.20	24.8	78.6
Early Feeding (-)	-	3.60^{abc}	3.67	2.00^{bc}	2.67^{b}	1.50	4.21	30.5	82.9
	Antibiotic	3.20^{bc}	3.00	2.07^{bc}	3.00^{ab}	1.40	3.79	27.1	71.8
	Probiotic	4.30^{ab}	3.70	2.67^{ab}	3.73^{ab}	1.20	4.70	22.0	94.1
	Herbal	3.10^{c}	2.80	2.40^{ab}	3.73^{ab}	1.11	4.11	19.1	71.7
Pooled SEM		0.35	0.44	0.23	0.33	.28	1.25	5.2	10.7
Probability									
Early Feeding		*	NS	NS	NS	NS	NS	NS	NS
Growth Promoters		NS	NS	***	**	NS	NS	NS	NS
Early Feeding × Growth promoters		*	NS	**	*	NS	NS	NS	NS

$^{A\text{-}C,\ a\text{-}e}$ means within the same columns with no common superscripts have significant differences. NDV = Newcastle disease virus, SRBC = Sheep red blood cell, BI = Before- injection, PI = Post-injection, SEM = Standard error of means, NS = not statistically significant,* = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Lymphoid organ

Shape and development of lymphoid organs has been known to be as mirror for their function .Dibner etal (1998) found that early fed chicks showed a higher bursa weight as a percentage of body weight , and better disease resistance than their held hatch mates .This adverse effect of delayed access to feed was attributed to stress hormones(corticosteron), it may lead to lymphoid tissue involution as well as to retard of humoral and cell mediate immune response (Lesson and Summer 2001). But in this result corticosteron was not enough to suppress of lymphoid organ**.**($p<0.05$) (not recorded). This finding in this experiment explained that when broiler chicks are exposed to certain environmental stressors , here early feeding post hatch and some dietary additives might assist in modulating the development of these lymphoid organ and enhance the immune competence of the bird . In current study, the stress factors may be for short periods. Pall *etal.* (2013) who 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. In our experiment we did not find any affect of GP on relative weight of spleen . This finding fit with Rahimi *etal*(2011) who referred that herbal plant and antibiotic did not affect spleen relative weight in broiler chicken . In generally the relative size of burs of fabricius improved with GP ($P<0.03$). However, Fathi *etal.* (2003); Nnadi *etal* (2010) they reported that shape of bursa and thymus did not affect the cell mediate immune response . In other hand , synergistic effect of FP and GP appeared through the complementary role of vitagel® with herbal plant blend in improving ($p<0.05$) of relative weight of bursa of fabracius in broiler chicken. This result contrast with Cengiz *etal.* (2012) who concluded that no synergistic effect of GP(organic effect) and delayed access to feed post hatch for 36 hours on internal organs and lymphoid organ .

Blood pictures

No significant difference was noticed in all types of WBC count and H/L ratio with FP or GP ($P<0.05$) table (3)... Ferket and Kellems (2007) have referred that under the influence of malnutrition that most lymphoid organs , thymus , bursa , spleen and lymphoid nodes will become damaged with reduced circulating lymphocytes ,this effect was not observed in this study ,table (3). This result fit with Nnadi etal (2010) who explained that value of total leukocyte count , lymphocyte , momocyte and heterophils showed comparable values for bird received feed and water post hatch chicks and delayed for 72 hours. The present results revealed that Lymphocte count of chicks fed diet containing probiotic and medicinal plant blend had recorded significant increase($p<0.01$) compared with control . While reduced of hetrophils ($P<0.05$)and L/H ratio ($P<0.01$) with natural growth promoters. Data of this experiment fit with those of Golian *etal.* 2009). Also with Ali, (2011) Who concluded that herbal plant (digestarom) did not alter WBC count in broiler chicken . Rahimi *etal* (2006) evaluated the effected of dietary supplementation of antibiotic and probiotic on bird under heat stress condition .It revealed that probiotic supplementation at 0.1 % decrease heterophil to lymphocytes compared to control .The interaction effect showed that GP especially medicinal plant blend with (vitgel®) caused significantly increased of lymphocyte and reduced of Hetrophil and H/L ratio ($p<0.05$) compared to deprived birds received same diets. This means, the GP with/ without vitagel® enhanced of lymphocyte cells and reduced hetrophil and H/L ratio .

Table 3: Effects of early feeding and growth promoters on hematological variables in Ross 308 Broilers

Treatments		White blood Cell ($\times 10^4$)	Lymphocytes (%)	Heterophil (%)	Heterophil/Lymphocyte
Main Effects					
Early feeding	+	1.384	61.23	34.06	0.57
	-	1.467	62.21	32.81	0.55
Pooled SEM		0.064	0.89	0.96	0.02
Growth Promoters	-	1.450	58.43^{B}	36.48^{A}	0.63^{A}
	Antibiotic	1.407	61.58^{AB}	34.19^{AB}	0.58^{AB}
	Probiotic	1.357	62.20^{A}	32.37^{B}	0.53^{BC}
	Herbal	1.488	64.69^{A}	30.75^{B}	0.49^{C}
Pooled SEM		0.091	1.26	1.35	0.03
Interaction Effects					
Early Feeding (+)	-	1.487	57.93^{b}	37.21^{a}	0.65^{a}
	Antibiotic	1.414	61.00^{ab}	35.14^{ab}	0.59^{ab}
	Probiotic	1.260	61.33^{ab}	32.40^{ab}	0.54^{ab}
	Herbal	1.375	64.67^{a}	31.50^{ab}	$o.50^{ab}$
Early Feeding (-)	-	1.414	58.92^{b}	35.69^{ab}	0.61^{ab}
	Antibiotic	1.400	62.15^{ab}	33.23^{ab}	$o.57^{ab}$
	Probiotic	1.453	63.07^{ab}	32.33^{ab}	0.52^{ab}
	Herbal	1.600	64.71^{a}	30.00^{b}	0.48^{b}
Pooled SEM		0.124	1.72	1.88	0.04
Probability					
Early Feeding		**NS**	**NS**	**NS**	**NS**
Growth Promoters		**NS**	**	*	**
Early Feeding × Growth promoters		**NS**	*	*	*

$^{A-C, a-b}$ 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

Gut Bacteriology

In current study, the early feeding with vitagel$^{(R)}$ reduced the pathogenic bacteria count (*E.coli* and *Clostridium*) ($p<0.05$) and total bacteria ($p<0.001$) , table 4 . This would imply that early nutrition post hatch lead to accelerate ealy colonization of beneficial intestinal bacteria and prevent colonization pathogenic bacteria(Maioka and Dahlke 2006).Also this finding may be attributed to well distribute of Band T lymphocyte in GALT and maturation of GALT caused from essential elements presented in early feeding which stimulate the lymphocyte and macrophage in intestinal lumen (Fridman and Bar-Shira 2005).This observations are in accordance with the result obtained by Enberg *etal*. (2013) found the early nutrition during shipping for 24 hours causes lower number of Ecoli in the ileum content in broiler chicken . Also with Potturi *etal* (2005), who pointedout that the increased presence of aerobic bacteria within the ileum in poult with delayed access to feed . Our result , inconsistent with (Alhotan , (2011)who explained that early nutrition supplement or holding chick for 24 h did not impact pathogenic bacteria in broiler chicken .

Growth promoters reduced ($p<0.05$) the total bacteria count in addition the pathogenic bacteria was lower ($p<0.01$) with GP compared to control . Beneficial bacteria significantly reduced ($p<0.05$) with availmycin . The role of GP against pathogenic bacteria have been proved . Probiotic inhibits colonization pathogens bacteria and prevent their proliferation the bacteria in gut . Probiotic produced the short chain fatty acids which are helped in providing nutrients of intestinal epithelial cells not only , but also are control factor on living of intestinal bacteria (DanKowiaKowska *etal* 2013). Gue *etal* (2004) who concluded that some herbal plants stimulated *Bifidobacterium* and *Lactobacillus* but reduced the number of *Bacteroides Spp* and *Ecoli* in ceca . The combination effect of FP and GP was significant reduced with respect to total bacteria count ($p<0.01$) for birds received (vitgel®)followed by GP. Also same tendency with pathogenic bacteria ($p<0.05$), especially with probiotic. However the *Lactobacillus* did not affect with combination effect ($p<0.05$). *Bifidiobacterium* positively response($p<0.05$) to (vitgel®) then followed it by medicinal plant blend .This finding agreed with Ao *etal*. (2012) who suggested that early nutrition may only or in incombination with dietary feed additives be

considererd as approach to alleviate disease risks in the post antibiotic broiler industry during decrease *Clostridium Spp* and *Ecoli* . Finally, it can be concluded that , early feeding with vita gel offered a degree of protection against *clostridium* and *coliform* bacteria and maintain on beneficial microflora .

Table4: Effects of early feeding and growth promoters 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**

$^{A\text{-}B,\ a\text{-}e}$ 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

Conclusion

Under normal hatchery processes,chicks with vitagel$^{(R)}$ alone or with natural growth promoter showed 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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