IJAS Iranian Journal of Applied Animal Science

The Effect of Prebiotics and Isoleucine Addition to the Finisher Diet of Broilers on Their Performance and some Blood Parameters

Research Article

E. Ajdar[1], N. Eila[1*] and J. Yadi[2]

[1] Department of Animal Science, Faculty of Agriculture and Natural Resources, Karaj Branch, Islamic Azad University, Karaj, Iran
[2] Department of Animal Science, Faculty of Agriculture and Natural Resources, Saveh Branch, Islamic Azad University, Saveh, Iran

Received on: 23 Mar 2015
Revised on: 7 Jun 2015
Accepted on: 15 Jul 2015
Online Published on: Mar 2016

*Correspondence E-mail: nimaila@kiau.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

This study conducted to evaluate the effect of prebiotics (Fermacto) and isoleucine and their combination on performance and some blood parameters of broiler chickens during finisher period. At the first of experiment 300 one-day-old mixed Ross broiler chicks reared in same condition according to Ross manual guide up to 21 days old. Chicks at 21 day old were randomly allocated to 20 groups of 15 birds in a completely randomized design with 4 treatments and 5 replications. Control group were fed with the basal diet of corn-soya and wheat. In the experimental group, 0.1% of prebiotics and 0.04% isoleucine were added. Weight gain and feed intake of birds were measured and at the end of the experiment one bird from each pen were sampled and weighed and then killed. The results showed that prebiotics and isoleucine significantly ($P<0.05$) improved feed conversion and weight gain and serum cholesterol but it did not have any significant effect on traits such as feed, the serum amount of urea, uric acid, triglycerides, low density lipoprotein (LDL), high-density lipoprotein (HDL), very low-density lipoprotein(VLDL), LDL/HDL and serum total protein.

KEY WORDS blood parameters, broiler, isoleucine, performance, prebiotics.

INTRODUCTION

Prebiotics are non digestive substances that are used as additives in poultry diets in order to stimulate the activity of the endogenous choice or reproduction of bacteria that benefit the host. They also contain short-chain carbohydrates that are indigestible by the enzymes in gut of animals (Ashraf *et al*. 2013). Prebiotics effectively influence on bird health promotion through growth or activity of one or certain kinds of useful bacteria in the gut (Xu *et al*. 2003). Most of prebiotics are non digestible carbohydrates that include various types such as: fructo oligosaccharides (FOS), glucose oligosaccharides (GOS) and mannan oligosaccharides (MOS). Mannan oligosaccharides are mostly isolated from the outer wall of *Saccharomyces cerevisiae* yeast. These compounds mostly bind to the harmful bacterial cell wall or stimulate immune system against pathogen bacteria. Fructo oligosaccharides (FOS) are common prebiotic with special chemical structure can improve intestinal health. Some oligosaccharides such as inulin naturally non-digestible carbohydrates that easily fermented in cecum and colon (Awad *et al*. 2011).

Nowadays improved genetic strains of broilers increases studying the essential amino acids and as we know the main objective of crude protein of diets is providing essential amino acids. The results of the amino acid requirements studies show that three amino acid, methionine, lysine and threonine, that their synthetic compound is available can be

used in diets in order to achieve a balanced diet. Branched amino acid isoleucine is introduced as the limiting amino acid in the finisher period (22-42 days old) especially when wheat is included in diets (Kidd *et al*. 2004). Supplementing of this limiting amino acid in the final period minimizes the excess amount of amino acids and enhances biological value of protein of diets (Kidd *et al*. 2000). At the other hand isoleucine can increases some harmful clostridium species (Ikeda *et al*. 1998) which applying prebiotics may overcome the problem.

This study aimed to use prebiotics and isoleucine as the limiting amino acid in the final period to examine their impact on performance and blood parameters in broiler chickens.

MATERIALS AND METHODS

Animals and diets

At the first of experiment, 300 one-day-old mixed Ross broiler chicks reared in same condition according to Ross manual guide up to 21 days old. The 21 day-old chicks were randomly allocated to 20 groups of 15 birds in a completely randomized design with 4 treatments and 5 replications and experiment has been finished at 42 days old. Control group were fed with the basal diet of corn-soya and wheat. In the experimental group, 0.1% of prebiotics and 0.04% isoleucine were added. Weight gain and feed intake of birds were measured and at the end of the experiment one bird from each pen were sampled and weighed and then killed. Treatments were as follows: 1) treatment control: (A): basal diet; 2) treatment (B): basal diet with prebiotic Fermacto; 3) treatment (C): basal diet with isoleucine and prebiotic Fermacto and 4) treatment (D): basal diet with isoleucine. Diets and their analysis are shown in Tables 1 and 2.

Performance parameters

At the beginning and end of the experiment, body weight and feed intake were recorded for each experimental unit and functional traits were measured and recorded. These traits include body weight gain, feed intake and feed conversion ratio.

Experimental procedure

At the end of 42 days, one chick were randomly selected from each pen and serum is prepared from selected sample and sent to blood measure serum biochemical parameters with biochemical kit (Pars Azmoon kit, Pars Azmoon Inc., Tehran, Iran) for measurement of urea, uric acid, cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL), LDL to HDL ratio and total protein.

The data were organized by Excel and their analysis were performed with SPSS 20 statistical software (SPSS, 2011) following the normality test, then Duncan's test was used to compare the means.

RESULTS AND DISCUSSION

The results show that the effect of different treatments on feed intake was not significant ($P>0.05$). This result is compatible with the reported results of Sabuni *et al*. (1389) and Mejia *et al*. (2011). However other researchers found conflicting results (Midillim *et al*. 2008; De Castro Tavernari *et al*. 2012).

The variation in the effects of prebiotics may be due to various factors such as rearing conditions and intestine microbiology, kinds of feed additives and bird physiology that all factors can impact on the response of broiler chickens to prebiotics (Yang *et al*. 2009).

The results showed (Table 3) that weight gain of chicks fed prebiotics was significantly better than the control and isoleucine group. Reports show beneficial effects of probiotics and prebiotics on weight gain and feed efficiency of Ross 308 broiler chickens.

Khodadadi *et al*. (2013) reported that weight gain in chicks treated with prebiotics is more than the other groups and this feed additive caused feed efficiency improvement.

Some studies indicated that the different ratios of isoleucine have significant effect on gain weight in both periods of beginner (7-21 days) and finisher (30-43 days; $P<0.05$).

Chee *et al*. (2010) reported that dietary supplementation with mannan-oligosaccharides significantly improved the performance of broiler chicks. Despite of these results, Morales-Lopez *et al*. (2009) did not find any significant effect from prebiotics on performance of broiler and this discrepancy may be due to different levels of prebiotics in the diet and the type of prebiotic consumption. According to beneficial effects of prebiotics on development of intestine, which is resulted in most of reports, it seems better weight gain of chicks fed prebiotics in this experiment can be related to better absorption of nutrients due to increase absorption areas of gut.

Significant improvement of feed conversion ratio in chicks fed prebiotics comparing with control and chicks only fed isoleucine can emphasis this mechanism. Similar results have been seen by Sabuni *et al*. (1389).

Some studies reported that the different ratio of isoleucine to lysine increased feed conversion ratio in both beginner and finisher periods while many research showed conflicting results.

The results of evaluation of blood parameters show urea and uric acid levels and triglyceride and total protein did not have a significant difference ($P\geq0.05$).

Table 1 Ingredients contents of diets

Item	Starter (1-10 d)	Grower (11-24 d)	Finisher phase (21-42 d)			
			A	B	C	D
Corn	55.39	55.565	55.54	55.44	55.4	55.5
Wheat	-	-	12.9	12.9	12.9	12.9
Soybean meal	32.7	31.6	22.7	22.7	22.7	22.7
Fish meal	5.0	4.86	-	-	-	-
Oil	3.0	4.8	4.6	4.6	4.6	4.6
Di calcium phosphate	1.86	1.3	2.0	2.0	2.0	2.0
Calcium carbonate	0.86	0.85	0.76	0.76	0.76	0.76
DL-methionine	0.34	0.285	0.25	0.25	0.25	0.25
L-lysine HCl	0.1	-	0.25	0.25	0.25	0.25
L-threonine	-	-	0.1	0.1	0.1	0.1
Premixt*	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.15	0.12	0.15	0.15	0.15	0.15
Sodium bicarbonate	0.1	0.12	0.25	0.25	0.25	0.25
Fermacto	-	-	-	0.1	0.1	-
Isoleucine	-	-	-	-	0.04	0.04
Total	100	100	100	100	100	100

* Supplied per kilogram of diet: vitamin A (retinyl acetate+retinyl palmitate): 6050 µg; vitamin D_3: 55 µg; vitamin E (α-tocopheryl acetate): 22.05 µg; vitamin K_3: 2 mg; vitamin B_1: 5 mg; vitamin B_2: 6 mg; vitamin B_3: 60 mg; vitamin B_6: 4 mg; vitamin B_{12}: 0.02 mg; Pantothenic acid: 10 mg; Folic acid: 6 mg; Biotin: 0.15 mg; Ethoxyquin: 0.625 mg; CaCO: 500 mg; Fe: 80 mg; Zn: 80 mg; Mn: 80 mg; Cu: 10 mg; I: 0.8 mg and Se: 0.3 mg.

Table 2 Calculated analysis of diets

Ration	Starter (1-10 d)	Grower (11-24 d)	Finisher phase (21-42 d)			
			A	B	C	D
Metabolizable energy (kcal/kg)	3040	3190	3224	3221	3220	3223
Protein (%)	23.5	22.9	17	17	17	17
Digestible methionine (%)	0.68	0.61	0.5	0.5	0.5	0.5
Digestible methionine and cysteine (%)	0.97	0.9	0.74	0.74	0.74	0.74
Digestible lysine (%)	1.23	1.13	0.95	0.95	0.95	0.95
Digestible threonine (%)	0.75	0.73	0.64	0.64	0.64	0.64
Digestible tryptophan (%)	0.24	0.23	0.18	0.18	0.18	0.18
Digestible arginine (%)	1.47	1.42	1.05	1.05	1.05	1.05
Digestible isoleucine (%)	0.87	0.85	0.63	0.63	0.67	0.67
Digestible valine (%)	0.97	0.95	0.74	0.74	0.74	0.74
Calcium (%)	1	0.9	0.82	0.82	0.82	0.82
Available phosphorus (%)	0.5	0.45	0.42	0.42	0.42	0.42
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16

Table 3 Comparition the effect of treatment on performance traits

Treatments	Feed intake (g)	Weight gain (g)	Feed conversion ratio (g/g)
Control (A)	2662±123	1298±177^{b}	2.08±0.28^{a}
Prebiotic (B)	2770±206	1570±202^{a}	1. 80±0.17^{b}
Prebiotic + isoleucine (C)	2818±237	1422±100^{ab}	1.99±0.22^{ab}
Isoleucine (D)	2780±83	1306±109^{b}	2.13±0.10^{a}

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

As it can be seen in Table 4 parameters such as the amount of HDL, LDL, VLDL and the ratios of LDL/HDL have not significant differences but in cholesterol, it was observed that the difference between mean of treatments was significant (P<0.05) and due to the significantly lower value of chicks fed prebiotics and isoleucine compared to the control and chicks fed isoleucine.

We conclude that feeding prebiotics along with isoleucine has the priority probably due to the increasing popula tion of bacteria that produces esterase enzyme which can reduces the reabsorption of bile salts and destroys them and thus uses more cholesterol from the blood to produce bile salts and as a result decreases cholesterol in the blood.

Some studies reported in an experiment that in the finisher period, diets containing probiotics and prebiotics and different levels of mint has a significant effect on the concentration of total protein, cholesterol and HDL broilers' plasma (P≥0.05) but the concentration of cholesterol and

triglycerides in plasma of chickens fed with different levels of mint, probiotics and prebiotics decrease in comparison with the control (P≥0.05). Kannan *et al*. (2005) reported that the use of 0.5% mannan oligosaccharides in broiler diets caused significant reduction in serum cholesterol in 35th day compared with the control diet.

Table 4 Comparison the effect of treatment on blood parameters

Parameters (mmol/L)	Control (A)	Prebiotic (B)	Prebiotic + isoleucine (C)	Isoleucine (D)
Very low density lipoprotein	19.80±7.85	18.20±5.19	23.20±6.10	22.20±5.72
Triglycerides	98.60±17.09	90.60±22.65	115.40±31.29	111.40±17.31
Uric acid	4.40±1.04	4.38±1.03	4.32±0.82	4.64±0.95
Urea	3.80±1.92	4.00±1.41	3.80±0.83	4.00±1.58
Cholesterol	140.6±4.09^{a}	128.20±16.31^{ab}	123.40±3.43^{b}	137.40±6.30^{a}
Whole protein	2.94±0.32	2.96±0.50	2.92±0.26	3.24±0.35
Low density lipoprotein to high density lipoprotein ratio	0.28±0.08	0.26±0.05	0.22±0.08	0.20±0.07
Low density lipoprotein	22.60±5.98	21.80±5.26	18.40±5.31	19.00±7.10

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Fallah and Rezai (2013) stated that the use of Fermacto prebiotics reduced visceral fat and cholesterol and triglycerides in blood.

CONCLUSION

Based on the results obtained in this study, weight gain and feed conversion ratio was improved in chicks fed prebiotics, but feed intake was not significantly affected by prebiotics and isoleucine. Consumption of a combination of prebiotics and isoleucine in the diet reduced the cholesterol of blood serum but had no effect on total protein, urea, uric acid, triglycerides, LDL, HDL, VLDL and LDL/HDL. Although in this experiment, beneficial effects of prebiotics on weight gain and feed conversion ratio is clear but apparently the combination of prebiotic and isoleucine decreased this beneficial effect probably due to further growth of undesirable microorganisms in the intestine.

ACKNOWLEDGEMENT

Financial support for this study was provided by Islamic Azad University, Karaj Branch.

REFERENCES

Ashraf S., Zaneb H., Yousaf M.S., Ijaz A., Sohail M.U., Muti S., Usman M.M., Ijaz S. and Rehman H. (2013). Effect of dietary supplementation of prebiotics and probiotics on intestinal microarchitecture in broilers reared under cyclic heat stress. *J. Anim. Physiol. Anim. Nutr.* **97,** 68-73.

Awad W.A., Ghareeb K. and bohm J. (2011). Evalution of the chicory inulin efficacy on ameliorating the intestinal electrophysiological properties in broiler chickens. *J. Anim. Physiol. Anim. Nutr.* **95,** 65-72.

Chee S., Lji P., Choct M., Mikkelsen L. and Kocher A. (2010). Characterization and response of intestinal microflora and mucins to manno-oligosaccharide and antibiotic supplementation in broiler chickens. *Br. Poult. Sci.* **51(3),** 368-380.

De Castro Tavernari F., Lelis G.R., de Oliveira Carneiro P.R., Vieira R.A., Polveiro R.C., Luengas J.A.P., Rostagno H.S. and Albino L.F.T. (2012). Effect of different digestible isolucine/lysine ratios for broiler chickens. *Revist. Brasileira Zootec.* **41(7),** 1699-1705.

Fallah R. and Rezaei H. (2013). Effect of dietary prebiotic and acidifier supplementation on the growth performance, carcass characteristics and serum bio chemical parameters of broilers. *J. Cell. Anim. Biol.* **7(2),** 21-22.

Ikeda D., Karasawa T., Yamakawa K., Tanaka R., Namiki M. and Nakamura S. (1998). Effect of isoleucine on toxin production by Clostridium difficile in a defined medium. *Zentralbl. Bakteriol*. **287(4),** 375-386.

Kannan M., Karunakaran R. and Prabhakar T.G. (2005). Influence prebiotics supplementation on lipid profile of broilers. *Int. J. Poult. Sci*. **4(12),** 994-997.

Khodadadi A., Taghavi M., Nikpiran H. and Athari S. (2013). Influence of probiotic and prebiotic on broiler chickens performance and immune status. *J. Nov. Appl. Sci.* **28,** 256-259.

Kidd M.T., Burnham D.J. and Kerr B.J. (2004). Dietary isoleucine

responses in male broiler chickens. *Br. Poult. Sci.* **45(1),** 67-75.

Kidd M.T., Kerr B.J., Allard J.P., Rao S.K. and Halley J.T. (2000). Limiting amino acid responses in commercial broilers. *J. Appl. Poult. Res.* **9,** 223-233.

Mejia L., Zumwalt C.D., Kim E.J. Tillman P.B. and Corzo A. (2011). Digestible isoleucine-to-lysine ratio effects in diets for broilers from 4 to 6 weeks post hatch. *J. Appl. Poult. Res.* **20(4),** 485-490.

Midillim M., Kocabagli N., Muglali O.H., Turan N. and Yilmaz H. (2008). Effects of dietary probiotic and peribiotic supplementation on growth performance and serum IgG concentration of broilers. *South African J. Anim. Sci.* **38(1),** 235-248.

Morales-Lopez R., Auclair E., Garcia F., Esteve-Garcia E. and Brufau J. (2009). Use of yeast cell walls; 3-1, 3/1, 6-glucans and mannan proteins in broiler chicken diets. *Poult. Sci.* **88(3),** 601-607.

Sabuni S., Eila N. and Gholamhoseini B. (1389). Addition of probiotics and prebiotics in the diet on performance and small intestinal bacterial populations and morphology of ilium in broilers. *J. Anim. Sci. Res*. **6,** 11-23.

SPSS Inc. (2011). Statistical Package for Social Sciences Study. SPSS for Windows, Version 20. Chicago SPSS Inc.

Xu Z., Hu C., Xia M., Zhan X. and Wang M. (2003). Effects of dietary fructo oligosaccharide on digestive enzyme activities, Intestinal microflora and morphology of male broilers. *Poult. Sci.* **82(6),** 1030-1036.

Yang Y., Lji P. and Choct M. (2009). Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to antibiotics. *World's Poult. Sci. J.* **65,** 97-114.