IJAIR

International Journal of Agriculture Innovations and Research
Volume 3, Issue 4, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received : 01/02/2015 | Accepted on : 05/02/2015 | Published : 12/02/2015

Combination Effect of Probiotic and Organic Acids on Blood Biochemistry and Immunity Parameters of Broilers

Dr. Mahdi Hedayati
Assistant Professor, Dept. of Animal Science, College of Agricultural Sciences, Malayer University, Malayer, Iran. Postal Code: 65719-95863

Dr. Milad Manafi
Assistant Professor, Dept. of Animal Science, College of Agricultural Sciences, Malayer University, Malayer, Iran. Postal Code: 65719-95863

Dr. Saeed Khalaji
Assistant Professor, Dept. of Animal Science, College of Agricultural Sciences, Malayer University, Malayer, Iran. Postal Code: 65719-95863

Dr. Mojtaba Yari
Assistant Professor, Dept. of Animal Science, College of Agricultural Sciences, Malayer University, Malayer, Iran. Postal Code: 65719-95863

Mr. Ali Esapour
Post Graduate Student, Dept. of Animal Science, College of Agricultural Sciences, Malayer University, Malayer, Iran. Postal Code: 65719-95863

Mr. Essa Nazari
Post Graduate Student, Dept. of Animal Science, College of Agricultural Sciences, Malayer University, Malayer, Iran. Postal Code: 65719-95863

Ms. Fatemeh Mohebi
Post Graduate Student, Dept. of Animal Science, College of Agricultural Sciences, Malayer University, Malayer, Iran. Postal Code: 65719-95863

Abstract: **A total of 180 day-old unsexed Ross 308 broiler chicks were randomly distributed in a completely randomized experimental design with three treatments and four replications of fifteen chicks each. Diets prepared without additive as Control; Probiotic(containing live yeast from mannan oligosaccharides (MOS) for 8x10^7 in each gram of product, vitamins and antioxidants) @ 0.1% and Acidifier (containing citric acid, acetic acid, propionic acid, lactic acid)@0.1%.The results of cholesterol, LDL, HDL, triglyceride, AST, total protein, globulin, Alkaline phosphatase (Alk. Phos.)and glucose showed improvement by addition of probiotic and acidifier in to the basal diet, whereas the findings in albumin and ALT levels were remained non-significant. Also significant improvement for antibody titers again Newcastle Disease (ND) and Infectious Bursal Disease (IBD) were noticed in acidifier feed additive treatment while in Avian Influenza (AI) titer values, were significantly altered by addition of both probiotic and acidifier. It can be concluded that dietary probiotic and acidifier could have a clear positive effect on immune response and could partially improve the biochemical parameters studied in current experiment**

Keywords: **Probiotic, Organic Acids, Biochemical, Immunit y, Broilers.**

1. Introduction

The vast demand in poultry production across the World has initiated the search for suitable replacer to antibiotic growth promoters in poultry industry. Probiotics and organic acids have the potentiality to be used in broiler industry for this concept. Literature suggests that dietary administration of bioactive feed additives such as probiotics and organic acids could, in principle, benefit poultry performance and health [1]-[2]-[3]. Probiotics have recently introduced into the poultry industry, means “for life” In Greek language and are a compound of live microorganisms(for instance,Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida and Saccharomyces) which promote the host natural intestinal microflora [4] and reported to be beneficial to poultry performance and immune system [5]. The effect of probiotics can be summarized as: turnover of efficient microflora in digestive system [6]-[7]-[8] changes in bacteria metabolisms[9] neutralization of entertoxins[8] and stimulation of immune system [10]-[8]. Mannan oligosaccharide (MOS) is one of the most important ingredients of many probiotics. There are several studies on the effect of this substance on the performance and immune system of poultry. There is extensive information on the effect of probiotics on immune system [11]. Bailey et al. [12] conducted a study on the effect of MOS on turnover of Salmonella in intestine mucous and immunity of broilers and reported that these compounds were really effective in prohibiting turnover of deleterious bacteria like Salmonella. Havenaar and Spanhaak [13] have reported that probiotics stimulate the immunity of the chickens through microflora of the gut wall. Numerous studies have demonstrated three different pathways of immune system enhancement: (I) enhanced macrophage activity which increases the ability to phagocytose microorganism or of the intestinal microbiota; (II) increased production of antibodies like IgG&IgM and; (III) increased resistance to infection [14].

Many other studies suggesting the daily poultry gut conditioning with probiotic and acidifier is a beneficial tool for poultry performance and health [15]. Acidifier shave been used for more than 30 years toreduce bacterial growth and load in feedstuffs and thus preserve hygienic quality. In feed legislation, they are registered as preservatives, but their positive effects on animal health and performance are reported by their addition in feed in sufficient quantities. Acidifiers used as feed additives are predominantly compounds thatnaturally occur in cell metabolism, thus they are natural products with low toxicity [16].Health and performance promoting effects reported for a number of acidifiers, including formic, fumaric, citric and lactic acid and their salts.Besides improvement in hygiene and a corresponding reduction of pathogen intake,effects on feed digestion and absorption and on stabilization of gut flora eubiosis have been

International Journal of Agriculture Innovations and Research
Volume 3, Issue 4, ISSN (Online) 2319-1473

demonstrated in a number of investigations.Acidifiers have been used in feed preservation, protecting feed from microbial and fungal destruction and increase the preservation effect of fermented feed. It has also been used as antimicrobial feed additives for long times. The objective of dietary acidification is the inhibition of intestinal bacteria of those both pathogenic and competing with the host for available nutrients and reduction of possibly toxic bacterial metabolites. Acidification increases gastric proteolysis, protein and amino acid digestibility and utilization of minerals and thus improving performance of the animal[17]. When acidifiers are included in the feed, they can modify the pH of both the feed and the GIT of the host animal. Also, the acidifiers in their un-dissociated form are able to pass through the bacterial cell membrane inside the cell, where they dissociate in H+ ions which lower the pH of the cell and RCOO- ions that can disrupt the normal cell function and protein synthesis. As a result, the affected microorganisms are unable to replicate efficiently and the microflora of the digestive tract is modified [18]. The potential of single organic acids in feed preservation lies in their ability to protect feed from microbial and fungal destruction, and its effect on stomach pH and gut flora, and has been known for decades and proven in many laboratory and field trials [19]-[20].In animal husbandry the final goals are classified as improved feed conversion rates and daily weight gain, as well as reduced incidence of diarrhoea, enhances economic return by lower feed costs and shorter time to market.The positive effects of acidifiers as feed additive can be explained by several working mechanisms. These acids can break down the DNA (deoxyribonucleic acid) structure in the bacterial cell nucleus and as a result the bacterial cell can no longer divide or may even die. Certain bacteria are sensitive to pH, e.g. *E. coli*, *Salmonela spp.*, *Listeria monocytogenes*, *Clostridium perfringens*, while others are not, e.g. Bifidobacteria, Lactobacillus spp. The aim of the present investigate was to study the comparison effect of probiotic and mixture of organic acids on blood biochemistry and immunity parameters of broilers.

2. Materials and Methods

The present study was conducted in the Department of Animal Science, Faculty of Agricultural Sciences, Malayer University, Malayer, Iran with an objective of assessing the blood biochemical parameters and immunity of commercial broilers fed with probiotic and acidifiers.

Experimental Design, Housing, Management and Testing Diet

A total number of 180 day-old unsexed Ross 308 broiler chicks were wing banded, weighed and distributed in a completely randomized experimental design with three treatments and fourreplications of fifteen chicks each. Chicks in all the replicates were reared up to six weeks of age under uniform standard conditions throughout the study. Brooding was done for 10 days using incandescent bulbs. The temperature was maintained at 30±1°C in the first week and reduced by 2.5°C per week to 21°C. From day one until day 4 the lighting schedule was 24 h continuously. At days 5-42 the dark time was increased to 1 h. Each pen was fitted with an automatic bell type drinker and a hanging tubular feeder. Chicks were provided with feed and water ad libitum throughout the study. Feeding of test diets began at first day of age and continued till the termination of experiment. Basal diet was formulated and compounded to meet the nutrient requirements of commercial broilers (NRC, 1994) during the starter (0-2 wks), grower (2-4 wks) and finisher (4-6 wks) feed. The composition of experimental diets is shown in Table 1. Diets prepared without additive as Control (group1); 0.1% probiotic (group2) and 0.1% acidifier(group 3). The feed additives for probiotic and acidifier used in this study were Provital (containing live yeast from mannan oligosaccharides (MOS) for $8x10^7$ in each gram of product), vitamins and antioxidants)Totacid (containing citric acid, acetic acid, propionic acid, lactic acid), respectively, provided by a commercial company (Tehran Dane Limited, Tehran, Iran).

Vaccination Schedule

Vaccination schedule was as follow: Vaccination against Newcastle Disease (ND) virus happened three times: first spray at the commencement of experiment, second on the 12th day as B1 (CEVA SANTE ANIMALE®, Libourne, France) in drinking water and booster of them on 20th day as clone-30 (HIPRAVIAR® CLON, Amer, Spain) in drinking water. Vaccination against Infectious bronchitis happened twice as the following: first spray at commencement of the experiment and the booster in drinking water on the 10th day, both as H-120 (CEVA SANTE ANIMALE®, Libourne, France). Vaccination against Infection Bursal Disease (IBD) happened twice: first on day 15 and the second on the 24th day, both as Gambo-l (CEVA SANTE ANIMALE®, Libourne, France) in drinking water. The sera were applied to HI test in 28 the day, to determine antibodies to NDV. In titers lower that 5, the booster B1 (CEVA SANTE ANIMALE®, Libourne, France) was administrated in drinking water to broilers.

Studied Parameters

*Biochemical parameters*The collected blood samples were analyzed for cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride, Alanine transaminase (ALT), Aspartate transaminase (AST), total protein, serum albumin and globulin, Alkaline phosphatase (Alk. Phos.)and glucose using automatic analyzer (Boehringer Mannheim Hitachi 704 automatic analyzer, Japan). The methodology and the set of reagents used in respect of each parameter were as recommended by the manufacturer of the analyzer system. Data are presented as mean±SEM treatment wise.

Immunity parameters

At the end of the trials, upon obtaining the permission of Ethical Committee of the University, six birds from each replicate were bled by jugular veinpuncture and blood samples were individually collected in 10-mL heparinized tubes and stored on ice for hematological analysis. Serum was separated after 8 to 10 hours as per the standard procedures [21] and was stored at –20ºC for subsequent analysis. The individual serum samples were analyzed for antibody titers against Newcastle disease (ND), Infectious bursal disease (IBD) and Avian Influenza (AI) by ELISA technique. Treatment-wise means of titers were computed and reported.

Statistical analysis

The experimental data were analyzed statistically by using the General Linear Model procedure of the Statistical Analysis System (SAS®) software [22]. Overall data were analyzed using one way ANOVA test. Duncan multiple range test at 0.05 probability level was employed for comparison of the means [23].

3.Results and Discussion

The findings of current experiment on blood biochemical parameters of broilers fed probiotic and acidifier are shown in Tables 2 and 3. Cholesterol content (mg/dL) is found to be decreased significantly ($P \leq 0.0001$) by consumption of probiotic and acidifier in the feed. The level of LDL (mg/dL) was significantly ($P \leq 0.0001$) increased in both groups of probiotic and acidifier. The values for HDL (mg/dL) were found to be significantly ($P \leq 0.0006$) enhanced in probiotic and acidifier fed groups. The triglyceride (g/dL) indexes were significantly ($P \leq 0.05$) decreased in both feed additive treatments, when compared with control group. The ALT (IU/L) was remained non-significant in studied treatments of current study. The AST (IU/L) levels in the blood plasma of broilers fed probiotic was significantly ($P \leq 0.0057$) increased, where other treatment showed no significant change, when compared with control group. The total protein values (g%) were decreased significantly ($P \leq 0.0001$)in all groups, with more emphasis on the groups having acidifier supplemented in addition to the basal diet. No significant changes in the albumin content (g%) of broilers fed different dietary treatments were observed. On the contrary, the globulin indexes (g%) were enhanced significantly ($P \leq 0.0001$), with more emphasis in acidifier group, when compared with control treatment. In case of Alk. Phos. (IU/L), interestingly, significant ($P \leq 0.0001$) increase in probiotic group and significant ($P \leq 0.0001$) decrease in acidifier group were noticed, when compared with control group. The glucose levels (mg/dL) in different dietary treatments were lessened significantly ($P \leq 0.0001$) in both studied treatments, compared with control group.

The results of current study on the immune response of broilers fed dietary probiotic and acidifier at 42 days of age are shown in Table 4. It has been found that by incorporation of acidifier into the diet, the antibody titer values against ND and IBD were significantly ($P \leq 0.0001$) decreased and remained unchanged in probiotic fed group.In case of antibody titer values against AI, it is revealed that dietary inclusion of probiotic and acidifier significantly ($P \leq 0.0001$) enhanced the titer values of broilers, with much more emphasis in group 3 (acidifier).

Probiotics, which are products made from living microorganisms or their L-forms (without cell wall). The micro-organisms included as probiotics are usually assumed to be non-pathogenic components of the normal microflora, such as the lactic acid bacteria. However, there is goodevidence that non-pathogenic variants of pathogenic species can operate in much the same way as traditional probiotics. In poultry, the early use of probiotics was [24]. In their experiments, the authors observed that the intestinal contents of normal adult birds, orally administered to chicks with one day of age, altered their sensitivity to infection by Salmonella spp. From there, several studies have been made and continue being developed with the use of probiotics. Inconsistent results from the use of probiotics in animal production have been a constraint for the promotion of their use. Variations in the efficacy of probiotics can be due to the difference in microbial species or micro-organism strains used, or with the additive preparation methods [25]. However, other factors can justify the variations in the results of probiotic use in poultry, such as origin species, probiotic preparation method, survival of colonizing micro-organisms to the gastrointestinal tract conditions, environment where the birds are raised, management (including the application time and application route of the probiotic), the immunologic status of the animals, the lineage of the poultry evaluated, as well as age and concomitant use or not of antibiotics.

Feed Acidifiers are acids included in feeds in order to lower the pH of the feed, gut, and microbial cytoplasm there by inhibiting the growth of pathogenic intestinal microflora. This inhibition reduces the microflora competing for the host nutrients and results in better growth and performance of the chicken. They also act as mold inhibitors. They are added upto0.25% of the diet. Most acids are efficacious and their effect remains as long as the acid is not volatilized.

Acidifiers have been used extensively for more than 25 years in swine production and more recently in poultry. The antimicrobial effect of organic acid ions in controlling bacterial populations in the upper intestinal tract leads to beneficial effects. Inorganic acids such as HCl and H3PO4though pH reducing are ineffective. The anti bacterial action of organic acids depends on whether the bacteria are pH sensitive or not. Only certain types of bacteria are sensitive to pH (ex.: *E. coli*, *Salmonella spp.*, *L. monocytogenes*, *C. perfringens*) while other types of bacteria are not sensitive (*Bifidobacterium spp.*, *Lactobacillus spp.*).

More over, acidifiers improved growth performance through establishment of low gastro intestinal pH condition by supporting endogenous digestive enzymes and reducing undesired gut microorganisms [26], Acidification of diets with weak organic acids such as formic, fumaric, propionic, lactic and sorbic have been reported to decrease colonization of pathogen and production of toxic metabolites, improved digestibility of protein, Ca, P, Mg, Zn and served as substrate in the intermediary metabolism [16]-[27].

Acidifiers act as performance promoters by lowering the pH of gut (mainly upper intestinal tract), reducing potential proliferation of unfavorable microorganisms. Acidification of gut stimulates enzyme activity and optimizes digestion and the absorption of nutrients and minerals. Un-dissociated forms of organic acids penetrate the lipid membrane of bacterial cells and dissociate into anions and protons. After entering the neutral pH of the cell's cytoplasm, organic acids inhibit bacterial growth by interrupting oxidative phosphorylation and inhibiting adenosine triphosphate in organic phosphate interactions. Improved broiler performance by supplementation with single acids was noticed for formic acid [28] and fumaric acid [29]-[20] and Izat et al., [30] found significantly reduced levels of Salmonella spp. In carcass and caecal samples after including calcium formate to broiler diets. In another trial from [31] buffered propionic acid was used to counteract pathogenic microflora in the intestine and carcass of broiler chickens, and resulted in a significant reduction in *E. coli* and *Salmonella spp.*,The use of pure formic acid in breeder feed reduced the contamination of tray liners and hatchery waste with *S. enteritidis* drastically [32]. Kirchgessner et al., [19] found significantly better feed utilization in laying hens after adding fumaric acid, but only when the feed was low in protein and methionine and cysteine. Performance enhancement was influenced by both quantity and quality of the protein, although these trials were performed either with single organic acids or with the corresponding salt of a single acid. Hinton and Linton [33] examined controlling salmonella infections in broiler chickens by using a mixture of formic and propionic acid. They demonstrated that under experimental conditions 6 kg/t of that organic acid blend was effective in preventing intestinal colonization with *Salmonella spp.* from naturally or artificially contaminated feed.

The effect of feed additive on the immune response of broilers may have plenty reasons. The environmental condition may have the prime reason, because this experiment was performed in an almost entirely aseptic condition. Probiotics in particular seems to be cited as positive for immune effects. Immune function would be enhanced as a consequence of a more stable intestinal health favored by feed additives containing vitamins or by animals being less exposed to microbial toxins or other undesired metabolites, for example ammonia and biogenic amines. Consequently, additives like aromatic herbs or volatile oils may relieve the animals from immune defense stress during critical situations, raising the intestinal availability of essential nutrients for absorption and thus, assist the animal to grow better within its genetic potential. Increased immune responses have been reported with the use of probiotic containing EO and MOS [34]-[35] and herbal extracts [36] in diets, which is in agreement with the results of current study. Researchers have shown that diets containing MOS and vitamins increase immune response via enhancement of the formulating bacteria on an acquired immune response exerted by T and B lymphocytes [37], Immune system stimulation by probiotics may be due to increase of T cells, phagositosic cells and serum protein levels [38]. Christensen et al., [39] suggested that these effects were mediated by cytokines secreted by immune system cells stimulated with probiotic bacteria. Indirect effect of probiotics may occur via changing the microbial population of the lumen of gastrointestinal tract. Probiotics increase in gram positive bacteria such as, lactobacillus and bifidobacteria that improve immune response [38]. Cook and Samman [40] noted that herbal extracts stimulate immune response by increasing vitamin C activity. It has been reported that probiotics stimulates phagocyte activity and thus promotes host protective responses [41] Studies have proved that adding organic acids to broiler diets increase immunity response [42]. It has also been suggested that this increase is due to stimulation or activation of immune cells by these feed additives. Organic acids can decrease intestinal pH, and cause enhancement of gut characteristics and immune response. Khovidhunkit et al., [43] showed that antibiotics restrain gram-positive bacteria that stimulate the immune system. Therefore, antibody titer was diminished by use of antibiotic which was confirmed by the results of this study. Furthermore, probiotics increase short-chain fatty acids [44], decrease intestinal pH [45], and improve intestinal morphology and immune response. It has been suggested that probiotic cause reduce pathogenic bacteria in digestive tract of broiler chickens which can help to improve intestinal health of these birds. However, the above mentioned feed additives could induce broilers immune response. Another beneficial effect of such additives could be due to better utilization of feed and improve the microflora and reduce harmful bacteria of the gut.

Many researchers indicated that organic acid could stimulate immune response and increase resistance to microbial pathogens as they are utilized in broilers diet. Acidifiers inhibit joining pathogenic bacteria to intestinal mucosa and creating acidic environment in intestine [46]. Other important mechanisms which can be used in order to improve the immune level and the intestinal microfloras are changing acidity of intestine through increasing concentration of lactic acid in intestine and reducing activity of deleterious bacteria of intestine (E. Coli, Salmonella and Clostridium) and increasing activity of lactobacillus. According to Savage et al. [10], it was found that the rate of IgA that comes into intestine from bile duct and also the rate of plasma IgA have increased numerically, when fed with acidifier [10]. As a result, addition of a balanced acid blend, such as combinations of lactic acid, formic and propionic acid based on a sequential release medium, increases the performance of broiler chicken and is an option for maintaining or improving broiler growth and efficiency results without resorting to supplementation with an AGP.

Table 1.Ingredientsand composition of the basal diets (NRC, 1994) (as-fed basis, %)

Ingredients (%)	Starting diet (0-2wk)	Growing diet (2-4wk)	Finishing diet (4-6wk)
Corm	59.00	67.36	72.01
Soybean meal	33.74	28.63	24.46
Soybean oil	1.56	0.65	0.56
Calcium carbonate	0.60	0.67	0.63
Dicalcium phosphate	1.41	1.02	0.84
Oyster shell	0.66	0.66	0.63
Common salt	0.30	0.30	0.30
Vit. And Min. Permix[1]	0.50	0.50	0.50
DL-Methionine	0.13	0.06	0.02
Lysine – HCL	0.09	0.14	0.05
Calculated analysis			
ME (Kcal/kg)	2900	2950	3000
Crude protein (%)	20.84	18.43	16.87

1The vitamin and mineral premix provide the following quantities per kilogram of diet: vitamin A, 10,000IU (all transretinal); Vit.D3(cholecalciferol),2,000IU;vitaminE,20 IU (α-tocopherol); vitaminK3,3.0mg; riboflavin, 18.0mg; niacin, 50mg; D calcium pantothenic acid,24mg;chol-inechloride,450mg;vitaminB12,0.02mg;folicacid,3.0mg;manganese,110mg;zinc,100mg;iron,60mg;copper,10mg;iodine,100mg; selenium,0.2mgan dantioxidant,250m.

Table 2. Effect of different dietary treatments on selected biochemical parameters of broilers at 42 days of age (mean±SEM)

Treatments	Cholesterol (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	Triglyceride(g/dL)	ALT (IU/L)	AST (IU/L)
Control	183.07[a]	75.45[a]	56.21[b]	126.70[a]	4.84[a]	170.25[b]
Probiotic	168.00[b]	58.13[b]	60.62[a]	118.65[b]	4.94[a]	178.78[a]
Acidifier	156.59[c]	69.74[b]	59.61[a]	121.30[b]	4.51[a]	167.69[b]
SEM	2.10	0.89	0.54	1.00	0.10	1.56
P-Value	<.0001	0.0006	0.0008	0.0015	0.1922	0.0057

CON: Control; Probiotic @ 0.1% and Acidifier @0.1%. SEM: Standard Error of the Means. SEM: Standard Error of the Means.LDL: low-density lipoprotein; HDL: high-density lipoprotein; ALT: alanine transaminase; AST: aspartatetransaminase.[a–d] Different lowercase superscripts in the same row indicate a significant difference between treatments at (P≤0.05).

Table 3. Effect of different dietary treatments on blood parameters of broilers at 42 days of age (mean±SEM)

Treatments	Total protein (g%)	Albumin (g%)	Globulin (g%)	Alk. Phos. (IU/L)	Glucose (mg/dL)
Control	3.70[a]	1.44[a]	1.90[c]	153.78[b]	285.44[a]
Probiotic	3.60[b]	1.43[a]	2.15[b]	160.11[a]	250.53[c]
Acidifier	3.50[c]	1.45[a]	2.30[a]	148.05[c]	260.00[b]
SEM	0.02	0.00	0.03	1.16	2.86
P-Value	<.0001	0.3646	<.0001	<.0001	<.0001

CON: Control; Probiotic @ 0.1% and Acidifier @0.1%.SEM: Standard Error of the Means. [a–d] Different lowercase superscripts in the same row indicate a significant difference between treatments at (P≤0.05).Alk. Phos.: Alkaline phosphatase.

Table 4: Effect of different dietary treatments on immune response of broilers at 42 days of age

Treatments	ND	IBD	AI
Control	5.04[a]	339.02[a]	2.66[c]
Probiotic	4.93[ab]	334.02[ab]	2.92[a]
Acidifier	4.88[b]	332.26[b]	2.80[b]
SEM	0.02	1.18	0.02
P-Value	0.0461	0.0469	<.0001

CON: Control; Probiotic @ 0.1% and Acidifier @0.1%. SEM: Standard Error of the Means. [a–d]Different lowercase superscripts in the same row indicate a significant difference between treatments at (P≤0.05). ND: Newcastle Disease; IBD; Infectious BursalDisease; AI: Avian Influenza.

4. Conclusion

From the findings of current study, it could be concluded that addition of probiotic and mixture of organic acid individually into the broiler diets at 42 days of age could improve the blood biochemistry and enhanced the immunity of broilers at 42 d of age. However, when probiotic and organic acids were included into the basal diet of broilers, it is revealed that the best improvement in biochemistry parameters and immunity were noticed.

Acknowledgements

This study was funded by Directorate of Research, Malayer University, Malayer, Iran. Authors wish to thank Tehran Dane Feed Manufacturing Company Ltd. for supplying the feed additives.

References

[1] J. D. Patten, and P. W. Waldroup, Use of organic acids in broiler diets. Poult. Sci., 67(11), 1988, pp. 78-1182.
[2] Y. Yang, P. A. Lji, A. Kocher, E. Thomson, L. L. Mikkelsen, and M. Choct, Effects of mannan oligosaccharide in broiler chicken diets on growth performance, energy utilization, nutrient digestibility and intestinal microflora. Birit. Poult. Sci., 49, 2008, pp. 186-194.
[3] T. J. Applegate, V. Klose, T. Steiner, A. Ganner, and G. Schatzmayr, Probiotics and phytogenics for poultry: Myth or reality? J. Appl. Poult. Res., 19, 2010, pp.194–210.
[4] G. R. Gibson, and R. Fuller, Aspects of in vitro and in vivo research approaches directed toward identifying probiotics and prebiotics for human use, J. Nutr. 2000, pp. 391-395.
[5] S. M. LutfulKabir, The role of probiotics in the poultry industry, Int. J. Mol. Sci., 10, 2009, pp. 3531-3546.
[6] F. D. Bello, J. Walter, C. Hertel, and W. P. Hammes, In vitro study

of prebiotic properties of levan-type exopolysac-charides from Lactobacilli and non-digestible carbohydrates using denaturing gradient gel electrophoresis, Syst. Appl. Microbiol, 24, 2001, pp. 232-237.

[7] F.M. Lemieux, L. L. Southern, and T. D. Binder, Effect of mannan-oligosaccharides on growth performance of weanling pigs, J. Anim. Sci., 81, 2003, pp. 2482-2487.

[8] J. L. Vegad, Prebiotics, probiotic, acidfires and antibiotic growth promoters, in Poultry Diseases a Guide for Farmers and Poultry Professional, Int. Book Dist. Co. USA, 2nd ed. vol. 3, 2004, pp. 339-342, 343-346.

[9] G. Zoppi, Probiotics, prebiotics, synbiotics and eubiotics, Pediatr, Med. Chir., 20, 1998, pp. 13-16.

[10] T. F. Savage, P. F. Cotter, and E. I. Zakrzewska, The effect of feeding mannan oligosaccharide on immune-globulins, plasma IgG and bile IgA, of Wrolstad MW male turkeys, Poult. Sci., 75, 1996, pp. 143-148.

[11] B. Deplancke, and H. R. Gaskins, Microbial modulation of innate defense: goblet cells and the intestinal mucus layer, Americ. J. Clin. Nutr., 73, 2001, pp.1131-1141.

[12] J. S. Bailey, L. C. Blankenship, and N. A. Cox, Effect of fructooligosaccharides on Salmonella colonization of the chicken intestine, Poult. Sci., 70, 1991, pp. 2433-2438.

[13] R. Havenaar, and S. Spanhaak, Probiotics from animmuno logical point of view, Curr. Opin. Biotechnol., 5, 1994, pp. 320-5.

[14] R. D. Rolfe, The role of probiotic cultures in the control of gastrointestinal health, J. Nutr., 130, 2000, pp. 396-402.

[15] K. C. Mountzouris, P. Tsitrsikos, I. Palamidi, A. Arvaniti, M.Mohnl, G. Schatzmayr, and K. Fegeros, Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecalmicroflora composition, Poult. Sci., 89(1), 2010, pp. 58–67.

[16] M.Kirchgessner, and F. X. Roth, Ergotrope Effect edurch organische Säuren in der Ferkelaufzucht und Schweinemast. [Nutritive effects of organic acids in piglet rearing and pig fattening] Übersichtenzur Tierernährung, 16, 1988, pp. 93-108.

[17]M. Hedayati, M. Manafi, M. Yari and P. Vafaei, Effects of supplementing diets with an acidifier on performance parameters and visceral organ weights of broilers, Euro. J. Zoo. Res., 2 (6), 2013, pp. 49-55.

[18] C. Lückstädt, N. Senköylü, H. Akyürek, and A. Agma, Acidifier – a modern alternative for anti-biotic free feeding in livestock production, with special focus on broiler production, VeterinarijairZootechnika, 27, 2004, pp. 91–93.

[19]M.Kirchegessner, B. Eckel, F. X. Roth, and U. Eidelsburger, Influence of formic acid on carcass composition and retention of nutrients: 2. Nutritive value of organic acids in piglet rearing, J. Anim. Physiol. Nutr. 67, 1992, pp. 101-110.

[20]U. Eidelsburger, F. X. Roth, and M. Kirchgessner, ZumEinflußvon Ameisensäure, Calciumformiat und Natriumhydrogencarbonat auf täglicheZunahmen, Futteraufnahme, Futterverwertung und Verdaulichkeit. 7th communication:[Studies on nutritive effects of organic acids in piglet rearing], J. of Anim. Physio. and Anim. Nutrit., 67, 1992, pp. 258-267.

[21] B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W.Yoldre, J. Dis. Poult., Iowa State University Press, Ames, Iowa, USA, 9th edition, 1991, pp. 198–212.

[22] SAS Institute, SAS® User 's Guide, Statistics. Version & Edition.SAS Institute Inc., Cary, NC, USA, 2000.

[23] D. B. Duncan, Multiple range and multiple F tests. Biometrics, 11, 1995, pp. 1-42.

[24] E. Nurmi, and M. Rantala, New aspects of Salmonella infection in broiler production, Nature, 241(111), 1973, pp. 210-211.

[25] L. Z.Jin, Y. W. HO, N. Abdullah, and S. Jalaludin, Effects of adherent Lactobacillus cultures on growth, weight of organs and intestinal microflora and volatile fatty acids in broilers, Anim. F. Sci. Techno., 70, 1999, pp. 197-209.

[26] J. D. Richards, J. Gong and C. E. M. Delange, The gastrointestinal microbial and its role in monogastric nutrition and health with an emphasison pigs; current understanding possible modulations and New Technologies studies, Canad. J. Anim. Sci., 85, 2005, pp. 421-435.

[27] R. Fallah, and H. Rezaei, Effect of dietary prebiotic and acidifier supplementation on the growth performance, carcass characteristics and serum biochemical parameters of broilers, J. of Cell and Anim. Bio., 7(2), 2013, pp. 21-24.

[28] H. Vogt, S. Matthes, and S. Harnisch, Der Einfluß organischer Säuren auf die Leistungen von Broilern und Legehennen, ArchivfürGeflügelkunde, 45, 1981, pp. 221-232.

[29] M.Hedayati, M. Manafi, M. Yari, and A. Avara, The Influence of an Acidifier Feed Additive on Biochemical Parameters and Immune Response of Broilers, Annu. Rese.& Rev. in Bio., 4(10), 2014, pp. 1637-1645.

[30] A. L. Izat, N. M. Tidwell, R. A. Thomas, M. A. Reiber, M. H. Adams, M. Colberg, and P. W. Waldroup, Effects of a buffered propionic acid in diets on the performance of broiler chickens and on the microflora of the intestine and carcass, Poult. Sci., 69, 1990, pp.818-826.

[31] A. L. Izat, M. H. Adams, M. C. Cabel, M. Colberg, M. A. Reiber, J. T. Skinner, and P. W. Waldroup, Effect of formic acid or calcium formate in feed on performance and microbiological characteristics of broilers. Poult. Sci., 69, 1990, pp. 1876-1882.

[32] T. J. Humphrey, and D.G. Lanning, The vertical transmission of salmonellas and formic acid treatment of chicken feed. A possible strategy for control, Epidem.and Infec., 100, 1988, pp. 43-49.

[33] M. Hinton, and A. H. Linton, Control of Salmonella infections in broiler chickens by the acid treatment of their feed, Vet. Rese., 123, 1998, pp. 416–421.

[34] A. K. Panda, M. R. Reddy, S. V. Ramarao, and N. K. Praharaj, Effect of Dietary Supplementation of Probiotic on Performance and Immune Response of Layers in Decline Phase of Production, Indi. J. Poult. Sci., 35, 2000, pp. 102–104.

[35] M. E. Koenen, J. Karmer, R. Van der Hulst, S. H. Heres, T. Jeurissen, and W. J. Boerma, Immuno modulation by probiotic lactobacill in layer and meat type chickens, Brit. Poult. Sci., 45, 2004, pp. 355 - 66.

[36] R.Mathivanan, and K.Kalaiarasi, Panchagavya and Andrographis-panculata as Alternatives to Antibiotic Growth Promoters on Hematological, Serum Biochemical Parameters and Immune Status of Broilers, Poult. Sci., 44, 2007, pp. 198 - 204.

[37] S. M. L. Kabir, M. M. Rahman, M. B. Rahman, and S. U. Ahmed, The dynamic of probiotics on growth performance and immune response in broiler, Int. J. of Poult. Sci., 3(5), 2004, pp. 361-364.

[38] R. Fuller, The importance of Lactobacilli in maintaining normal microbial balance in the crop, Brit. Poult. Sci., 1, 1977, pp. 85-94.

[39] H. R. Christensen, H. Frokiaer, and J. J. Pestka, Lactobacilli Differentially Modulate Expression of Cytokines and Maturation Surface Markers in Murine Dendritic Cells, J. Immunol., 168, 2002, pp. 171-188.

[40] N. C. Cook, and S. Samman, Metabolism, Cardioprotective Effects, and Dietary Sources, J. Nutr. Biochem., 7, 1996, pp. 66 - 76.

[41] D. Gudev, S. Popova- Ralcheva, P. Moneval, M. Bonovska, G. Valchev, and A. Valcheva, Effect of supplemental Sangrovit on some biochemical indices and leukocytes phagocytic activity in growing pigs, Arch. Zootec., 7, 2004, pp. 123 - 134.

[42] U. Roser, Effects of organic acids in liquid and solid forms on the survival rate of Salmonella in pelleted compound feed after recontamination, J. Immunol., 82, 2006, pp. 12 - 9.

[43] W. Khovidhunkit, M. Kim, R. A. Memon, J. K. Shigenaga, A. H. Moser, K. R. Feinfold, and C. Grunfeld, Thematic Review Series; the Pathogenesis of Atherosclerosis. Effects of Infection and Inflammation on Lipid and Lipoprotein Metabolism Mechanism, J. Lipid. Res., 49, 2004, pp. 788–795.

[44] T. Sako, K. Matsuloto, T. Ryuichiro, Recent progress on research and application of non-digestible galactooligosaccharides, Int. Dair. J., 9, 1999, pp. 69 – 80.

[45] M. K. Huang, Y. J. Choi, R. J. Houde, W. Lee, B. H. Lee, and X. Zhao, Effects of Lactobacilli and an acidophilic fungus on the production performance and immune responses in broiler chickens, Poult. Sci., 83, 2004, pp. 788 – 95.

[46] Y. C. Chen, C. Nakthong, and T. C. Chen, Improvement of laying hen performance by dietary prebiotic chicory oligofructose and inulin, Int. J. Poult. Sci., 4, 2005, pp. 170-178.