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Impact of Probiotics and Acidifiers on Growth Performance and Blood Chemistry of Broiler Chickens

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Abstract: ***An experiment was carried out to study the effect of probiotics, acidifiers and their combination on broiler growth performance and blood chemistry. A total of 220 day old broiler chicks were randomly assigned to four experimental diets in a completely randomized design. Diet A basal diet as control, diet B basal diet supplemented with 500g/ton probiotic (bacillus subtilis), diet C supplemented with 2kg/ton organic acids (citric acid, fumaric acid, D-L malic acid, lactic acid, orthophosphoric acid), diet D supplemented with mixture of acidifier (2kg/ton) plus probiotic (500g/ton).Feed intake, body weight gain and feed conversion ratio were recorded on weekly basis throughout the experiment period. Blood serum parameters measured were glucose, albumin, total protein, cholesterol, triglycerides, calcium, phosphorus, sodium and potassium. The results showed that statistically there was no significant increase in body weight gain at weeks 1, 3, 4, 5 and 6 of age. The body weight of broiler chickens was significantly increased at weeks 2 of age when adding 0.05% bacillus subtilis. Dietary treatments had significant effect at second and third weeks on FCR. The data indicated that there were no significant effects of treatment on all blood parameters measured at day 21. However, at day 42 the cholesterol and triglyceride levels were numerically decreased as probiotic and acidifier were added. Birds fed diet contained 0.2% organic acid recorded the highest levels of serum glucose and lowest levels of serum calcium. While the highest value of serum calcium was obtained by birds received mixed diet (diet D). It concluded that inclusion of probiotic and probiotic plus acidifier to broiler chickens diets improved live body weight, blood serum parameters were not affected except blood calcium and glucose.***

Keywords: additives, broiler, promoter, serum analysis

1. Introduction

Feed additives were generally used to improve feed intake in broilers and to act as growth promoter (Scott et al 1982). Probiotic has been reported to protect the young chicks by competitive exclusion (La Ragione and Woodward, 2003). Probiotics improves the animal immune system and the hosts resistance to enteric pathogens, affects intestinal morphology as well as intestinal function, feed conversion ratio, weight gain and performance of birds (Ohh 2011). The addition of Bacillus subtilis into the diet increased weight gain and feed conversion (Fritts et al., 2000).Organic acids have made a great contribution to the profitability in the poultry production and also provided people with the healthy and nutritious poultry products (Ricke, 2003). They have beneficial effects on performance, some (e.g. butyric acid) also decrease the incidence of subclinical necrotic enteritis caused by C. perfringens, an additional beneficial effect which is highly relevant for the poultry industry (Timbermont, 2009). Acidification with various organic acids has been reported to reduce the production of toxic components by the bacteria and colonization of pathogens on the intestinal wall thus preventing the damage to epithelial cells also improve the digestibility of proteins, calcium, phosphorus, magnesium, zinc and serve as substrates in the intermediary metabolism (Langhout, 2000). Based on this background the main aim of the study was to investigate the effect of supplemental probiotic, acidifiers and their combination on performance and blood serum parameters of broiler chickens.

2. Materials and Methods

Experimental house

The house was constructed of iron post, reinforced bricks (one meter height) and wire netting sides. The roof was made of corrugated iron sheets. The heights of the roof were 2 and 3.5 meter for longitudinal sides and the ridge, respectively. The house floor was made of concrete covered with wood shavings. The house was divided into 20 pens, the dimensions of each pen was 1.5 meter length, 1 meter width and 0.5 meter height. Each pen was provided by one tubal feeder and plastic drinker and one bulb lamb (60 watts).

Experimental birds

Two hundred and twenty one-day old unsexed commercial broiler chicks (Ross 318) were purchased. On arrival birds were weighed and the average weights were recorded as initial weights.

Experimental diets

Four experimental diets were formulated iso-energetic and iso-nitrogenous to meet the broiler requirements according to NRC (1994). (A) basal diet as control, (B) basal diet supplementing with 500g/ ton probiotic (*bacillus substilis*), (C) basal diet supplementing with 2kg/ton organic acids (citric acid, fumaric acid, D-L malic acid, lactic acid, orthophosphoric acid)) and (D) basal diet supplementing with mixture of acidifiers (2kg/ton) plus probiotic (500g/ton). The composition and calculated analysis of experimental diets (starter and finisher) are shown in Tables (1,2,3 and 4), respectively.

Table 1: Composition of experimental starter diets

Ingredient %	*Experimental diets*			
	A	*B*	*C*	*D*
Sorghum	67.53	67.33	67.47	67.33
Groundnut meal	25.0	24.98	24.99	24.92
Super concentrate≈	5.0	5.0	5.0	5.0
Dicalcuim phosphate	0.50	0.50	0.50	0.50
Limestone	1.0	1.0	1.0	1.0
Salt	0.20	0.20	0.20	0.20
Lysine	0.20	0.20	0.20	0.20
Methionine	0.15	0.15	0.15	0.15
Antioxidant	0.20	0.20	0.20	0.20
Premix	0.25	0.25	0.25	0.25
Organic acid	0.00	0.20	0	0.20
Probiotics (*Bacillus subtilis*)	0.00	0.00	0.05	0.05

≈Premix per kg:vitamin A 800.000, vitamin D3 720.000 IU, vitamin E 4.100mg,Vitamin K3 300mg, Niacin 4.400mg, Folic acid 76mg,Choline chloride 57.800mg, Iron 5.700 mg, Zinc 16.200mg, Copper 4.500mg, Manganese 16.200mg, Iodine 540mg, Selenium 80mg, Calcium 250mg, Magnesium 11.610mg.

Table 2: Calculated analysis of experimental starter diets

Items	*Experimental Diets*			
	A	*B*	*C*	*D*
ME (Kcal/g)	3167.25	3160.65	3165.67	3159.19
Crude protein %	23.30	23.26	23.29	23.24
Crude fiber %	3.92	3.91	3.92	3.91
Lysine %	1.23	1.23	1.23	1.23
Methionine %	0.60	0.60	0.60	0.60
Calcium %	1.03	1.03	1.03	1.03
Phosphorus %	0.56	0.56	0.56	0.56

Table 3: Composition of experimental finisher diets

Items	*Experimental diets*			
	A	***B***	***C***	***D***
Sorghum	66.90	66.90	67.03	67.48
Groundnut meal	15.40	15.40	15.47	15.69
Wheat bran	8.540	8.500	8.200	7.470
Super concentrate	5.0	5.0	5.0	5.0
Vegetable oil	2.000	2.000	2.000	2.000
Dicalcuim phosphate	0.50	0.50	0.50	0.50
Limestone	0.800	0.800	0.800	0.800
Salt	0.20	0.20	0.150	0.150
Lysine	0.110	0.110	0.110	0.110
Methionine	0.10	0.100	0.100	0.100
Antioxidant	0.100	0.100	0.100	0.100
Premix≈	0.250	0.250	0.250	0.250
Organic acid	0.00	0.20	0.00	0.20
Probiotics (*Bacillus subtilis*)	0.00	0.00	0.05	0.05

Table 4: Calculated analysis of experimental finisher diets

Ingredients %	Experimental Diets			
	A	B	C	D
ME (Kcal/g)	3202.6	3202.6	3202.6	3202.6
Crude protein %	20.00	20.00	20.00	20.00
Crude fiber %	4.20	4.19	4.16	4.42
Lysine %	1.103	1.103	1.00	1.10
Methionine %	0.44	0.44	0.44	0.44
Calcium %	0.91	0.91	0.91	0.91
Phosphorus %	0.42	0.42	0.42	0.42

Management

Chicks were reared in deep litter with free access to feed and water *(ad-libitum)*. Drinkers and feeders were leveled using red brick cuboids. Continuous lighting was provided for 24 hrs. in the form of natural light during the day and artificial lighting during the night using an incandescent bulb. Birds were vaccinated against Newcastle (ND) disease and infectious bronchitis (IB) at the first week of age, Gumboro disease vaccine was administrated at the second week of age in drinking water. Vitamins offered as supportive dose in drinking water.

Collection of blood samples and analysis

At 21 and 42 days of age, 3ml of blood samples were collected from wing vein using sterile disposable syringes (5ml) from 2 birds of each treatment. Collected blood in the test tubes then after 3hr centrifuged at 3000 r.p.m for 15 minutes and the serum was separated then stored at -20°C. The investigated blood parameters were serum glucose, albumin, total protein, cholesterol, triglycerides, calcium, phosphorus, sodium and potassium.

Experimental design and statistical analysis

Four experimental treatments (A,B,C and D) were employed in completely randomized design and each treatment was replicated 5 times with eleven birds, the collected data was subjected to ANOVA using one–way analysis of variance (Steel and Torrie, 1980). Significant differences among treatments means were determined using Duncan's multiple range tests (Duncan, 1955).

3. Results and Discussion

Addition levels of probiotics (*bacillus subtilis*) 0.05%, acidifiers 0.20% and their combination in broiler feed had no adverse effects on broiler performance, although the feed intake (Table 5) was affected inconsistently at the second week. However, total feed intake was not affected by the dietary treatments. These results are in agreement with Hernandez et al, (2006) who found no difference in the cumulative feed consumption between the groups fed organic acids and the control group. Probiotcs and acidifiers were improved body weight gain at the 3rd week of age (Table 6). The improvement of body weight gain is probably due to the beneficial effect of organic acids on the gut flora. The organic acids may affect the integrity of microbial cell membrane or cell macromolecules or interfere with the nutrient transport and energy metabolism causing the bactericidal effect (Ricke, 2003). 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 (Fallah, and Rezaei 2013). The results of the present study regarding weight gains agree with the result of Owens et al. (2008), Sheikh et al., (2011) and Ghazalah, et al. (2011) who reported that the supplementation of organic acids in broiler chicken improve the body weight gain when compared with the unsupplemented group.

Table 5: Effect of Probiotics and Acidifiers on weekly feed intake (gm/bird) of broiler chickens

Weeks	*Treatments*				±SEM
	A	*B*	*C*	*D*	
1	71.70	71.11	66.55	75.43	3.13
2	302.49^{ab}	312.73^{a}	277.26^{b}	310.94^{a}	8.93
3	483.15	505.92	465.33	496.22	19.58
4	636.33^{ab}	674.51^{a}	596.17^{b}	676.62^{a}	18.43
5	851.26	878.48	845.84	889.49	54.57
6	1000.04	1000.11	987.88	1000.06	40.01

a , b are mean values within the same row with different superscripts letter are significantly different (P ≤ 0.05). SEM = standard error of means. A = Control, B = 0.05% Probiotics , C = 0.20% Acidifiers, D = 0.05% Probiotics plus 0.20% Acidifiers

Table 6: Effect of Probiotics and Acidifiers on weekly weight gain (gm/bird) of broiler chickens

Weeks	Treatments				±SEM
	A	B	C	D	
1	45.74	44.91	42.07	46.715	2.903
2	132.62^{b}	156. 24^{a}	140.41^{ab}	140.51^{ab}	6.124
3	279.38	296.38	292.39	319.40	13.177
4	271.20	273.48	270.19	278.03	12.238
5	408.04	406.69	398.03	428.60	17.352
6	420.01	455.09	455.22	458.79	32.072

A = Control, B = 0.05% Probiotics , C = 0.20% Acidifiers, D = 0.05% Probiotics plus 0.20% Acidifiers

Table 7 indicated that inclusion of probiotcs and acidifiers in broilers diet caused significant (P < 0.05) decreased in feed conversion ratio (FCR) in the 2nd and 3^{d} weeks compared to control treatment. The improvement in the feed conversion ratio could be possibly due to better utilization of nutrients resulting in increased body weight gain. Earlier researchers (Vogt et al., 1981; Runho et al., 1997) reported that the supplementation of organic acids improved the feed conversion ratio in broiler chicken. Zhang and Kim (2014) reported that dietary supplementation with multi strain probiotic significantly improved body weight gain and reduced feed conversion ratio FCR in broiler chickens compared with chickens from the control group. Likewise, Sinol et al. (2012) reported that inclusion of *Bacillus subtilis* C-3102 in broiler chicken diets resulted in improved feed conversion ratio (21–42 days) and weight gain (42 days).

Table 7: Effect of Probiotics and Acidifiers on weekly FCR (g feed/g gain) of broiler chickens

Weeks	Treatments				±SEM
	A	B	C	D	
1	1.59	1.59	1.58	1.64	0.61
2	2.30^{a}	2.01^{b}	1.98^{b}	2.22^{ab}	0.84
3	1.73^{a}	1.71^{ab}	1.60^{ab}	1.56^{b}	0.52
4	2.37	2.50	2.22	2.44	0.11
5	2.09	2.17	2.11	2.07	0.11
6	2.51	2.44	2.25	2.36	0.19

A = Control, B = 0.05% Probiotics, C = 0.20% Acidifiers, D = 0.05% Probiotics plus 0.20% Acidifiers

Blood serum parameters at day 21 of age (Tables 8) were not significantly affected by inclusion of probiotics, acidifiers and their combination compare to control. It was observed that total protein, albumin had numerical increased in the treated group compared to the control group. There was numerical decrease in cholesterol and triglyceride levels in the treated group compared to control group. The findings of serum lipid profile are in agreement with Abdo and Zeinb (2004) who reported that blood total lipids and cholesterol decreased significantly by dietary acidifiers. This results confirm with the report of Islam et al, (2004) who reported a reduced cholesterol and triglycerides in broilers diets containing probiotics in starter, grower and finisher phase. Blood serum parameters of broilers at 42 day of age (Tables 9) which include, glucose, total protein, albumin levels were increased. Cholesterol content triglyceride were decreased by consumption of probiotics and acidifier in the feed at 42 days of age, same result reported by Mahdi, (2015) who found that triglyceride and cholesterol were significantly (P < 0.05) decreased in both feed additive treatments probiotics 0.1% and acidifier 0.1% when compared with control group. A combination of both probiotics and acidifier increased the blood serum calcium. The increase of Ca and P levels in blood serum produced by addition of organic acids may be attributed to the lowering of PH in gastrointestinal-tract by using these acids, which increases the absorption of such minerals from the gut into the blood stream. Improving the utilization of calcium and phosphorus by organic acids supplementation was revealed by Boling et al, (2001). Abdo and Zeinb, (2004) observed an increase in blood calcium of broiler chicks fed on dietary acidifier. In this respect, Abdel-Azeem et al,. (2000) and Edwards and Baker (1999) found that the acidic anion has been shown to complex with Ca, P, Mg and Zn which results in an improved digestibility of these minerals. Furthermore, Kishi et al,(1999) reported that dietary acetic acid prevented osteoporosis through reducing the bone turnover, as it enhanced intestinal Ca absorption by improving Ca solubility.

Table 8: Effect of Probiotics and Acidifiers on serum parameters of broilers at 21 of age

Parameters	Treatments				±SEM
	A	B	C	D	
Glucose (mg/dl)	128.60	132.70	136.70	144.50	7.49
Total protein (g/dl)	7.18	8.13	7.86	7.96	0.65
Albumin (g/dl)	3.74	5.10	5.10	5.01	0.56
Cholesterol (mg/dl)	65.4	68.0	60.10	63.70	3.87
Triglyceride (mg/dl)	46.0	42.30	47.60	44.40	4.44
Calcium (mg/dl)	8.03	8.05	8.60	8.66	0.44
Phosphorus (mg/dl)	5.01	4.96	4.83	5.29	0.29
Sodium (mmol/l)	140.40	137.90	138.50	139.10	1.10
Potassium (mg/dl	4.87	4.63	4.74	4.82	0.91

A = Control, B = 0.05% Probiotics, C = 0.20% Acidifiers, D = 0.05% Probiotics plus 0.20% Acidifiers

Table 9: Effect of Probiotics and Acidifiers on serum parameters of broilers at 42 of age

Parameters	Treatments				±SEM
	A	B	C	D	
Glucose (mg/dl)	143.67^{ab}	154.89^{a}	117.11^{b}	144.00^{ab}	11.57
Total protein (g/dl)	7.22	8.50	7.34	8.04	1.06
Albumin (g/dl)	4.32	5.79	3.26	4.64	0.85
Cholesterol (mg/dl)	75.67	72.56	71.22	71.44	5.18
Triglyceride (mg/dl)	55.66	47.22	47.89	40.00	5.46
Calcium (mg/dl)	9.02^{ab}	7.82^{b}	8.89^{ab}	10.11^{a}	0.69
Phosphorus (mg/dl)	5.64	5.656	5.54	5.81	0.35
Sodium (mmol/l)	141.11	137.89	140.778	142.11	2.11
Potassium (mg/dl	5.03	4.73	4.97	5.04	0.16

A = Control, B = 0.05% Probiotics , C = 0.20% Acidifiers, D = 0.05% Probiotics plus 0.20% Acidifiers

4. Conclusions

Addition of probiotic and acidifiers (organic acid) into the broiler diets improved live body weight, feed conversion ratio. Feed intake was increased by adding probiotic and it combined with acidifiers and decreased when adding acidifiers alone. Better blood calcium level achieved by combination of probiotic and acidifiers diet.

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