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Amalgamation effect of organic acid with prebiotics and probiotics on growth performance and carcass traits of Giriraja birds

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Abstract

The present investigation was conducted to study the amalgamation effects of formic acid with Fructooligosaccharide and *Saccharomyces cerevisiae* on Growth Performance and Carcass Traits of Giriraja birds. In this study, one-day-old broiler chicks (N=160) were divided into 4 groups with 5 replicates of 5 chicks each. The trial was conducted for a period of 42 days. The chicks in each treatment group (T_0 , T_1 , T_2 and T_3) were fed basal diet with organic acid @ 0, 1, 2 and 3 per cent respectively with constant levels of Fructo-oligosaccharides (0.05%) and *Saccharomyces cerevisiae* (0.01%). The results showed that amalgamation effects of formic acid with Fructo-oligosaccharide and *Saccharomyces cerevisiae* significantly increased body weight gain (P<0.05). All treatments were statistically significant (P<0.05) for body weight gain except first week. Better body weight gain was recorded in T_3 treatment as compared to other treatments. However, treatments T_2 and T_3 groups compared with the control one, and the highest was in T_3 . The amalgamation effects of formic acid with Fructo-oligosaccharide and *Saccharomyces cerevisiae* holds a promise as a growth promoter strategy for enhancing growth performance and carcass traits of Giriraja birds.

Keywords: Giriraja, formic acid, fructo-oligosaccharides, *saccharomyces cerevisiae*, body weight gain, carcass traits

Introduction

Poultry farming has made tremendous progress during the last decades from a meager backyard venture to a fully-fledged well-organized scientific techno commercial industry. However, with increase in production and demand, there is also an increase in consumer concerns over food safety due to the use of additives such as antimicrobial growth promoters, animal protein and genetically modified materials in feeds etc. to boost the intrinsic potential of poultry birds to perform better. Use of antibiotics as growth promoter had been banned in animal nutrition by the European Union in 2006 because of the development of bacterial resistance and potential consequences on the human health. Therefore, researchers had attempted other alternatives claiming to enhance the performance of broiler chicken. The positive effects of organic acids that can be used as suitable feed additives alternative to antibiotics was reported (Hyden, 2000; Gonzales et al. 2013; Armut and Filazi, 2012) ^[20, 17, 6]. Organic acids and their salts are generally regarded as safe and have been approved by most member states of EU to be used as the feed additives in animal production. In poultry, the use of organic acids had been reported to protect the young chicks by competitive exclusion, enhancement of nutrient utilization and growth and feed conversion efficiency (Thirumeigmanam et al., 2006) ^[33]. Virtually, organic acids including fatty acids and amino acids are carboxylic acids with short chain (C1-C7) and are associated with antimicrobial activity. They are either simple monocarboxylic acids such as formic, acetic, propionic and butyric acids or carboxylic acids with hydroxyl group such as lactic, malic, tartaric and citric acids or short chain carboxylic acids containing double bonds like fumaric and sorbic acids. The antibacterial action of organic acids depends on whether the bacteria are pH sensitive or not. Only certain types of bacteria are sensitive to pH viz. E. coli, Salmonella sp., L. monocytogenes and C. perfringens) while other types of bacteria are not sensitive (Bifidobacterium sps. and Lactobacillus sps). Dietary organic acids and their salts are able to inhibit microbial growth in the food and consequently to preserve the microbial balance in the

gastrointestinal tract. It has been shown that these acids have anti-microbial activity which results in modification of the gut micro flora profile (Chen *et al.*, 2013)^[9].

Saddeiy (2013) ^[29] and Pirgozliev et al. (2008) ^[27] reported that use of organic acids resulted in significant change in aerobic bacteria and coliform population, Escherichia coli and Lactobacillus in caecum. The decreased in secretions from the gastrointestinal tract in the presence of fumaric and sorbic acids may be a mechanism involved in the mode of action of dietary organic acids in the birds. Organic acids reduce production of toxic components by bacteria and a change in the morphology of the intestinal wall and reduce colonization of pathogens on the intestinal wall, thus preventing damage to the epithelial cells and enhance growth performance and carcass quality of broiler chicks. As the uses of organic acids are becoming more acceptable to feed manufacturers, poultry producers and consumers, there is a growing interest in substituting them for antibiotic as growth promoters. The effects of organic acids as substitute of antibiotic have not yet been evaluated.

Feed organic acids suppress the growth of certain species of bacteria, particularly acid-intolerant species such as *E. coli*, *Salmonella* spp. and *Campylobacter* ssp. (Lückstadt, 2005)^[24]. Their principal role is to lower and stabilize the pH in the stomach and intestines so that the gut environment is too acidic for normal bacterial growth. Additionally, they improve protein digestion in young animals by stimulating pancreatic enzyme secretion. Thus, dietary organic acids suppress the growth of pathogenic bacteria, encourage the growth of beneficial microflora and ensure that the digestive enzymes function at maximal capacity (Dibner, 2004)^[11].

Prebiotics are non-digestive feed ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the digestive tract. The prebiotic, Fructooligosaccharide (FOS), is a carbohydrate, derived from yeast cell walls, and can block pathogenic bacterial proliferation and stimulate the non-specific immune system; thus tending to improve the health and growth performance of birds. Probiotics are pure cultures of one or more live microorganisms given orally. They proliferate in the gastrointestinal tract (GI) of the host and ensure that the bird maintains a beneficial microbial population in the GI tract by limiting the damage caused by pathogenic bacteria, reinforcing intestinal mucosal integrity and creating a positive balance of digestive microflora. Improved epithelial cell integrity, increased immune response, well balanced gut microflora, better utilization and digestion of diet are also additive beneficial effects of dietary probiotics.

The beneficial effects of the dietary supplementation of organic acids (Denli *et al.*, 2003) ^[10], prebiotics (Bozkurt *et al.*, 2005) ^[8] and probiotics (Molnár *et al.*, 2005) ^[26] on broiler performance are well documented. However, there is lack of information on the collective supplementation of prebiotics and organic acids and probiotics as performance enhancer feed additives. A prebiotic preparation (FOS) has been shown to interfere with the use of antibiotics in diets of broilers (Waldroup *et al.*, 2003) ^[35], whereas no benefit has been found relating response of broiler live performance to dietary added FOS in the presence of a probiotic (Hofacre *et al.*, 2003) ^[18].

Due to increasing demand for poultry meat, short supply of mutton and limited acceptability of beef and pork in some countries as considering religious and cultural points like India, the poultry production is under rapid expansion in the world. The importance of backyard poultry is well recognized by Government of India and special programs are formulated for its promotion. Hence, efforts have been diverted into producing dual purpose native hybrids with improved production profiles. These hybrids are readily accepted by the rural farmers and consumers owing to their phenotypic appearance of the local birds. Hence, the introduction of Giriraja has generated new opportunities for poultry production in rural areas. These breeds grow fast and require low input like feed, management, health care, housing etc. and sustain different vagaries of climatic and environmental changes. Moreover, these breed are in high demand with consumer preference owing to their local breed, which the consumers and farmers are exploiting under the name of 'Gavran' breed. However, major issues with these breeds are low FCR, low growth rate, and high feed intake. Therefore, any feed supplement that can take care of these factors will be beneficial to Poultry farmers in economic terms. Therefore, the objective of the present study was to examine the performance and some slaughter characteristics of Giriraja birds fed an experimental diet containing an formic acid with constant level of a prebiotic and a probiotic.

Material and Method

The present piece of research was carried out at Poultry Unit of Department of Animal Husbandry and Dairy Science, Rajashree Chhatrapati Shahu Maharaj College of Agriculture, Kolhapur, Maharashtra, India. In the present study, day old broiler chicks (N=160) of Giriraja breed were procured from The Regional Egg Incubator Center, Kolhapur, Maharashtra. On arrival, chicks were weighed and distributed randomly into four treatment groups *viz.*, T_0 , T_1 , T_2 and T_3 with 40 chicks in each treatment as replicates, on equal weight basis. The chicks were reared for 42 days. The chicks were housed in separate compartments. The chicks were fed experimental diets with different levels of supplementation of Formic Acid with constant level of prebiotics and probiotics during the experimental period of six weeks of age. Treatment details are as under-

Treatment details

The dietary treatments are as follows,

- $T_1: BSM/BFM with + prebiotics (0.5g per Kg mash) + Probiotics (0.1g per Kg mash) + Organic Acid (1.0\% of mesh).$
- T₂: BSM/BFM with + prebiotics (0.5g per Kg mash) + Probiotics (0.1g per Kg mash) + Organic Acid (2.0% of mesh).
- $T_{3}: BSM/BFM with + Prebiotics (0.5g per Kg mash) + Probiotics (0.1g per 100 Kg mash) + Organic Acid (3.0% of mesh).$

(BSM-Broiler starter mash, BFM-Broiler finisher mash, Prebiotics-Fructo oligosaccharides, Probiotics-Saccharomyces cerevisiae, Organic acid-Formic Acid)

Housing and Management

All the experimental chicks were reared in deep litter system with use of paddy husk as a litter material in a well-ventilated house with identical management and environmental conditions. Proper brooding of chicks was done by providing sufficient heat and light by using electric bulbs in each group for first three weeks of age. Afterwards, sufficient artificial light was provided during night hours throughout the experimental period. All the precautionary measures for controlling diseases were taken throughout the experimental period of six weeks. The standard and uniform management practices like brooding, lightening etc. were followed for all the groups. The chicks were provided 23 h light and one dark hour, 95°F temperature during first week that was reduced by 5°F during every successive week. The relative humidity of the shed was maintained to $60\pm5\%$.

Calculations and chemical analysis of different diets were performed according to AOAC (2005)^[5]. Diet composition and chemical analysis are shown Table 1. Birds in different experimental groups were weighted initially then weekly till the end of the experimental period. Body weight development, body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) were calculated. Chicks were vaccinated against Infectious Bursal Disease, New Castle Diseases and Lasota at days 14, 21 and 28, respectively, via the drinking water.

Table 1: Diet c	composition	and chemical	analysis (as fed basis)
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Items	Starter	Grower	Finisher		
Feed ingredient, %					
Yellow corn	55.44	60.63	62.83		
Soybean meal (45.5%)	33.30	27.80	24.35		
Corn gluten meal	3.00	3.20	4.20		
DL-Met	0.24	0.24	0.20		
L-Lys	018	0.24	0.16		
Soy oil	3.66	3.83	4.33		
Mono calcium phosphate	1.64	1.58	1.49		
Broiler premix1	0.30	0.30	0.30		
Choline chloride	0.10	0.10	0.10		
Lime stone	1.66	1.61	1.59		
Sodium chloride	0.35	0.30	0.30		
Sodium bicarbonate	0.08	0.12	0.10		
Anticoccidial drug	0.05	0.05	0.05		
Total	100	100	100		
Calculated analysis, %					
ME, kcal/kg	3033	3108	3180		
СР	21.50	19.50	18.70		
EE	2.65	2.70	2.77		
CF	3.02	2.94	2.80		
Lysine	1.30	1.20	1.30		
Methionine	0.61	0.59	0.55		
Threonine	0.85	0.78	0.75		
Ca	1.00	1.00	1.00		
Total P	0.75	0.72	0.69		
Av. P	0.50	0.48	0.45		
Na	0.17	0.16	0.16		
Cl	0.19	0.17	0.17		
Chemical analysis, %					
СР	21.4	19.6	18.9		
EE	2.85	2.50	2.90		
Ca	1.10	1.05	1.03		
Total P	0.73	0.71	0.68		

Carcass traits

At the end of the experiment (at 42 d), 5 birds of similar body weight to the group average were selected from each treatment group 5 birds per replicate, weighted and killed by severing of the brachial vein. After evisceration, hot carcasses were weighted immediately to determine the hot carcass yield. The weights of the Breast, Giblet, Drumstick, Thigh pancreas, were recorded individually.

Statistical analysis

The data were analyzed using one way analysis of statistical package for social sciences (SPSS) and comparison of means tested using Duncan's multiple range test (1997) and significance was considered at (P<0.05).

Result and Discussion

Growth parameters

The supplemental effects of formic acid with constant level of FOS and *Saccharomyces cerevisiae* on live performance of broiler chickens are shown in Table 2. All dietary supplements improved (21 d, P<0.01; 42 d, P<0.05) body weight to a similar extent compared with the control. These results clearly show that the formic acid (3%), with constant levels of FOS and *Saccharomyces cerevisiae* stimulated the growth of broilers during the entire experimental period. Compared to the control, improved growth rates of 4.2-5.1 per cent were measured during starter period and 1.9-2.5 per cent for the entire experimental period. These results confirm the growth promoting effect of supplemental organic acids, that of prebiotics reported by Bozkurt *et al.* (2005) ^[8] and probiotics observed by Molnár *et al.* (2005) ^[26].

The beneficial effect of organic acids in pig diets has been well documented, but similar responses were inconsistent in studies on broilers. While Ramana et al. (2017)^[28] reported that fumaric acid supplementation into diet at the level of 0.125 per cent increased final weight of broiler chickens (P < 0.05), Fascina *et al.* $(2012)^{[15]}$ recorded dietary supplementation of organic acids increased the body weight. The above results are in accordance with those obtained by Houshmand *et al.*, (2012) ^[19] and Azza *et al.*, (2014) ^[7]. They found that body weight at 6th week of age was higher in organic acid group than control group. Similar results were obtained by Abdel Raheem and Abd Allah, (2011)^[1] who reported that body weight at 42th day of age was the highest in synbiotic group compared to control, probiotic and prebiotic groups. However, organic acids, fed either individually or combined, offer a chemical alternative for growth promoting antibiotics (AGP) as used in poultry diets. This was accepted as a participial AGP alternative, with propionic acid, formic acid and lactic acid as the most effective and universally accepted products (Kamel et al., 2016) [22]. Strong bactericidal and bacteriostatic effects have been demonstrated for formic acid, the shortest chain organic acid. In fact, apart from their antimicrobial properties, organic acids make a significant contribution to feed hygiene, since they suppress the growth of mould and thus restrict the potentially harmful effects of mycotoxins (Lückstädt et al., 2005)^[24].

These results indicate that broilers fed with organic acid, prebiotic and probiotic (T_3) were more efficient at converting feed to body mass during the rearing period. To stimulate the growth of beneficial bacteria in the gut using treatment T_3 was slightly more effective than the other additive programmes in this study. In general, improvements in feed efficiency were attributed to an encouraged growth of the beneficial microflora in the GIT induced by dietary supplementation of organic acid, prebiotic and probiotic.

Weeks	Treatments				Moon SE (+)	CD @ 5%
	T ₀ (gm)	$T_1(gm)$	T ₂ (gm)	T ₃ (gm)	Mean SE (±)	CD @ 5%
Initial Weight	37.42 ± 0.24	36.85 ± 0.55	36.89 ± 0.44	37.19 ± 0.14	37.10 ± 0.38	NS
First	69.16 ± 0.32	69.76 ± 0.31	71.24 ± 0.27	72.25 ± 0.35	68.53 ± 0.32	NS
Second	129.18 ± 0.89^d	133.09 ± 0.44^{c}	$144.36\pm0.61^{\text{b}}$	148.81 ± 0.92^a	139.85 ± 0.74	2.24
Third	219.19 ± 1.08^{d}	234.82 ± 0.97^{c}	$266.08 \pm 1.6^{\ b}$	282.55 ± 1.92^{a}	250.66 ± 1.44	4.38
Fourth	325.56 ± 1.78^d	357.43 ± 2.74^{c}	416.35 ± 3.13^{b}	447.71 ± 2.15^{a}	386.02 ± 2.51	6.59
Fifth	457.91 ± 3.28^d	502.23 ± 2.73^{c}	583.52 ± 2.18^{b}	631.82 ± 2.78^{a}	543.87 ± 2.77	8.39
Sixth (Final Weight)	670.39 ± 2.05^{d}	$744.65 \pm 1.85^{\circ}$	768.31 ± 5.48^{b}	804.50 ± 3.28^{a}	705.72 ± 3.48	10.53

 Table 2: Amalgamation effect of formic acid with constant levels of Fructo-oligosacharide (0.05%) and Saccharomyces cerevisiae (0.01%) supplementation on cumulative body weight changes of Giriraja birds

Carcass parameters

The effects of the different dietary supplements on relative weight of some internal organs and carcass yield are summarized in Table 3. These results suggest that Carcass traits of birds were not affected in control treatments (P>0.05), while the final body weights of birds in treatment groups were affected (P<0.05). Feeding formic acid with constant level of FOS and Saccharomyces cerevisiae supplemented diets decreased (P<0.05) non edible weights compared with the control. The average dressed weight was found to be highest in T₃ group. The dressing percentage of birds of different experimental groups from T₀ to T₃ was 63.06, 64.01, 64.38 and 67.12, respectively. The highest dressing percentage was found in T₃ followed by T₂, T₁ and the least in T₀. Weight of carcass also followed the same trend. Similar finding had also been reported by Aksu et al. (2007)^[3] and Fascina et al. (2012)^[15] who had observed higher dressing percentage and carcass yield when organic acid was supplemented in the diet of broiler. Well established evidence by Engberg et al. (2000) ^[14] indicated that dietary inclusion of feed grade antibiotics, given as growth promoters, reduced intestine weight by thinning the intestinal wall evoked particularly by antimicrobial activity in gut lumen. However, a series of reports suggested similar antimicrobial mode of action for prebiotics (Ferket, 2004)^[16] and organic acids (Dibner, 2004) [11]. This is in agreement with the findings of Kim et al. (2015) ^[23] who reported that the dietary addition of probiotics lowered the small intestine weight.

Likewise, dietary treatments had significant effect on edible weight of birds in the present study. Similarly breast percentage and giblet percentage were statistically significant in treatment group T_3 supplemented with 3 per cent formic acid, 0.05 per cent Fructo-oligosaccharide and 0.01 per cent *Saccharomyces cerevisiae*. Similar results were observed by researchers who studied supplementation of prebiotics (Bozkurt *et al*, 2005) ^[8], organic acids (Vidyarthi *et al.*, 2019) ^[34] and probiotics (Egbal *et al.*, 2017) ^[12] to broiler diets. Drumstick percentage and thigh percentage had significant

increase in treatment group T₃ over other treatments. Also dressing percentage showed significant differences in all treatments. Giblet per cent of the carcass weight was slightly higher in T_3 as compared to T_2 and T_1 and had more when compared to control group. Similar outcome were also observed by Kabir et al. (2004) ^[21] who recorded that the supplementation of probiotics to broiler chickens increased thigh and breast yield as compared to control treatment. Results identical to our findings were also reported by Saiyed et al. (2015) [31] who observed the effect of probiotic, prebiotic and its combination in broiler diet and their effect on carcass characteristics and economics of commercial broilers. Among all carcass traits, dressing percentage, abdominal fat weight and abdominal fat percentage (as a percentage of dressed weight) were recorded significant (p < 0.05) difference among different treatment groups.

However, definitive data are lacking with respect to effects of dietary organic acids, probiotics and fructo oligosaccharide on the intestinal tissue of poultry in comparison to the welldocumented effects of antibiotics. The effects of feed additives used in this study were associated with growth stimulation, enhanced nutrient digestion and absorption, though this enhancement was not converted to carcass yield. Similar observations were reported by Seyedi et al. (2015) ^[32]; Ramana et al. (2017) ^[28] and Sakineh et al. (2018) ^[30] for organic acids, and by Eman and Mohammed (2016) [13] and Alçiçek et al. (2004)^[4] for probiotics and by Bozkurt et al. (2005)^[8], Eman and Mohammed (2016)^[13] for prebiotics. Similarly, Abdel-Raheem and Abd-Allah (2012) investigated the effects of dietary supplementation of prebiotic (MOS), probiotic (Saccharomyces cerevisiae) and their combination (synbiotic) on feed intake and some carcass traits in broilers. The final carcass yield percentage and organ weights were significantly (P < 0.05) increased in probiotic and synbiotic supplemented broilers in comparison with the control. Also Mehr et al. (2007) [25] observed higher body and carcass weights and breast percentage with higher level of probiotic supplementation compared with a lower level and the control treatment control and prebiotic groups.

 Table 3: Amalgamation effect of formic acid with constant levels of Fructo-oligosaccharides (0.05%) and Saccharomyces cerevisiae (0.01%) supplementation on carcass traits of Giriraja birds

Carcass traits (%)	Treatments					CD @ 50/
	T ₀ (gm)	$T_1(gm)$	T ₂ (gm)	T ₃ (gm)	Mean SE (\pm)	CD @ 5%
Live body weight	670.39 ± 2.05^{d}	744.65 ± 1.85 °	768.31 ± 5.48^{b}	804.50 ± 3.28 a	705.72 ± 3.48	10.53
Dressed Weight	382.14 ± 0.23^{d}	$478.84 \pm 0.21^{\circ}$	491.59 ± 0.24^{b}	539.84 ± 0.27^a	455.60 ± 0.24	0.74
Dressing (%)	63.06 ± 0.18^{d}	64.38 ± 0.16^{c}	64.01 ± 0.33^{b}	67.12 ± 0.13^a	64.64 ± 0.21	0.64
Breast (%)	63.28 ± 0.34^d	64.32 ± 0.67^{c}	64.75 ± 0.30^b	65.36 ± 0.21^{a}	64.43 ± 0.42	1.27
Giblet (%)	4.62 ± 0.18^{d}	4.99 ± 0.19^{c}	5.32 ± 0.28^{b}	5.64 ± 0.09^{a}	5.14 ± 0.18	0.584
Drumstick (%)	11.33 ± 0.14 ^d	11.54 ± 0.21 ^c	$11.16 \pm 0.05^{\ b}$	$11.96^a\pm0.22^a$	11.5 ± 0.16	0.503
Thigh (%)	10.72 ± 0.25^d	10.88 ± 0.27^{c}	11.34 ± 0.23^{b}	12.16 ± 0.18^{a}	11.28 ± 0.23	0.69
Edible (%)	63.16 ± 0.29^d	64.32 ± 0.33^{c}	64.81 ± 0.25^{b}	65.29 ± 0.24^a	64.39 ± 0.28	0.85
Non-Edible (%)	36.82 ± 0.26^d	$35.67 \pm 0.31^{\circ}$	35.18 ± 0.28^b	34.72 ± 0.08^a	35.60 ± 0.25	0.76

Conclusion

Amalgamation effect of organic acid with constant levels of prebiotics and probiotics supplementation on Giriraja birds showed significant increase in the body weight gain. Dietary supplementation of formic acid (3.0%), Fructooligosaccharides (0.05%) and *Saccharomyces cerevisiae* (0.01%) significantly increased breast per cent, giblet per cent, thigh per cent and drumstick per cent with better dressing percentage and low non edible parts. The study concludes that combination of formic acid (3.0%), Fructooligosaccharides (0.05%) and *Saccharomyces cerevisiae* (0.01%) holds a promise for alternative to antibiotic as growth promoter.

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