



Effect of glycerol as an energy source with or without probiotic on carcass trait, litter moisture per cent, haematological and serum metabolites in broiler chicken

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ABSTRACT

An experiment was conducted to access glycerol feeding on the carcass traits, litter moisture %, haematological and serum metabolites of broiler chicken. The broiler chicks were randomly distributed in five groups, viz. T₀ (0% glycerol), T₁ (3% glycerol with probiotic), T₂ (3% glycerol without probiotic), T₃ (6% glycerol with probiotic) and T₄ (6% glycerol without probiotic) with four replicates of 15 chicks, each of 45.8±0.23 g body weight. Results indicated that, the dressed breast weight and its percent was significantly higher in T₁ followed by T₂ and almost comparable in T₃ and T₄ as compared to control. LDL was significantly lower in T₁ as compared to T₀, T₂, T₃ and T₄. The % litter moisture was significantly increased as glycerol inclusion level increased. From the results, it was concluded that 3% glycerol in diet with probiotic significantly improved dressed breast weight, lowered LDL and increased litter moisture with increase in glycerol level.

Keywords: Broiler chicken, Carcass traits, Glycerol, Haematological parameter, Serum metabolic parameter

India ranks 3rd in egg production and 4th in meat production but still there is scope to increase production of chicken meat for an ever increasing human population (Poultry Sector in India, 2017). Nutrition plays a crucial role in the production of poultry as the recurring expenditure on feed is about 70% of total production cost and energy is the most important major nutrient in the diet of poultry (Bertechini 2012). The broiler chickens are rapidly growing birds and require high energy for their rapid growth and metabolic activity. Cereals and oils are main energy sources used in poultry feed and their prices are too high and their availability is limited. So there is need to find some cheaper alternative energy source like crude glycerol which is easily available by-product of biodiesel industry. The production process of biodiesel produces approximately 10% of crude glycerol (Dasari *et al.* 2005). Glycerol being in important structural component of triglycerides and phospholipids which can be readily absorbed in the poultry and mammals gastrointestinal tract (Mandalawi *et al.* 2014). Glycerol is a precursor of glyceraldehyde 3-phosphate, which acts as an intermediate in lipogenesis process and gluconeogenesis pathways and can generate energy through the pathway of glycolytic and tricarboxylic acid (Lammers *et al.* 2008). Glycerol also acts as easily utilizable nutrient for probiotic

microbial growth because some of researchers observed that lactic acid bacteria based probiotic ferment glycerol and improve the growth rate as well as bacteriocin production (Ozdogan *et al.* 2014, Delgado *et al.* 2014). Now-a-days antibiotic resistance is the most serious problem throughout the world (Carter *et al.* 2009). Probiotics can enhance growth of animals, improves immune response and improve the action of intestinal microflora, hence can be used as an alternative to antibiotics (Isolauri *et al.* 2002, Ayasan *et al.* 2006, Ayasan 2013, Ayasan and Inci 2019). The supplementation of 5% glycerol combined with Flora Max B-11 (lactic acid bacteria base probiotic culture) to chickens did not found detectable *Salmonella* enteritis (Delgado *et al.* 2014). Therefore, the objective of this study was to determine the effect of glycerol with or without probiotic on carcass trait, litter moisture per cent, haematological and blood biochemical parameters in broiler chicken.

MATERIALS AND METHODS

Experimental design and feeding: The experiment was carried out on 300, day-old (Vencobb-400) broiler chicks each of 45.8±0.23 g body weight for a period of 42 days at experimental broiler shed at College of Veterinary and Animal Sciences, MAFSU, Parbhani, Maharashtra (India). The crude glycerol used in this study was procured from E-Shakti Binary Current Pvt. Ltd., Aurangabad, Maharashtra. Probiotic was added @ 250 g/tonne in the feed of experimental birds. Experimental chicks were randomly distributed on equal body weight basis into five groups, viz.

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Table 1. Percent composition of control and experimental ration

Ingredient	Standard broiler diet (SBD)			SBD with 3% Glycerol			SBD with 6% Glycerol		
	PS	S	F	PS	S	F	PS	S	F
Maize (CP : 8.92%)	52.26	53.22	57.36	48.68	49.755	53.81	45.100	46.210	50.21
Soybean (CP : 44.8%)	40.50	38.10	33.00	41.08	38.640	33.60	41.660	39.195	34.20
Oil	3.40	4.80	5.80	3.40	4.760	5.75	3.400	4.750	5.75
Glycerol	0	0	0	3.00	3.000	3.00	6.000	6.000	6.00
Limestone	1.36	1.40	1.36	1.36	1.360	1.36	1.360	1.360	1.36
DCP	1.65	1.65	1.65	1.65	1.650	1.65	1.650	1.650	1.65
Salt	0.50	0.50	0.50	0.50	0.500	0.50	0.500	0.500	0.50
DL-Methionine	0.13	0.13	0.13	0.13	0.135	0.13	0.130	0.135	0.13
Lysine	0.05	0.05	0.05	0.05	0.050	0.05	0.050	0.050	0.05
TM mixture	0.05	0.05	0.05	0.05	0.050	0.05	0.050	0.050	0.05
Vit. premix	0.05	0.05	0.05	0.05	0.050	0.05	0.050	0.050	0.05
Ch. chloride	0.05	0.05	0.05	0.05	0.050	0.05	0.050	0.050	0.05
Total	100.00	100.00	100.00	100.00	100.000	100.00	100.000	100.000	100.00
CP (%)	22.73	21.75	19.90	22.86	22.000	19.91	22.770	21.840	19.88
M. Energy	2992.38	3091.49	3198.42	2992.19	3090.680	3195.30	2992.004	3090.190	3194.91
Lysine (%)	1.30	1.24	1.11	1.31	1.250	1.12	1.310	1.250	1.13
Methionine (%)	0.50	0.49	0.46	0.50	0.490	0.46	0.530	0.500	0.45

PS, prestarter; S, Starter; F, Finisher.

Control (T_0) fed standard basal diet (SBD), T_1 (SBD with 3% glycerol + probiotic), T_2 (SBD with 3% glycerol without probiotic), T_3 (SBD with 6% glycerol + probiotic), T_4 (SBD with 6% glycerol without probiotic) with four replicates of 15 chicks in each having 45.8 ± 0.23 g of body weight. The uniform management practices were followed for all the groups throughout the experimental period. The ration for experimental birds were formulated as per BIS (2007) standard recommendation (Table 1). All experimental procedure on broiler chicken were carried out according to IAEC regulation guidelines for which approval had been taken from IAEC committee with resolution no. IAEC 46/19 dated on 02/03/2019. At the end of the experiment, two birds from each replicate of each treatment group were selected for sacrifice. Birds were sacrificed by using Islamic/Halal method. At the end of sixth week, blood samples of two birds from each replicate were collected in clean, dry and sterilized EDTA vials (for haematology) and in clot activator vials (for blood biochemical analysis) from wing vein. Chemical analysis of serum was carried out for the quantitative determination of serum total protein, albumin, globulin, albumin and globulin ratio, HDL, LDL, total cholesterol, triglyceride, BUN and blood glucose using commercial available kits (Ambica Diagnostics Pvt. Ltd., Parbhani, Maharashtra, India). Litter moisture was estimated by collecting random samples on 21, 28 and 42nd day of experimental period from each treatment group and mixed it homogeneously. Litter moisture of various treatment groups was determined as per the methods of AOAC (2000).

Statistical analysis: The data collected during the experiment were subjected to statistical analyses as per Completely Randomized Design (CRD) method with treatment as factor following statistical procedure of Snedecor and Cochran (1994). Means were compared as

per Duncan's multiple range test and data were processed for statistical analyses using SPSS Software package (23.0).

RESULTS AND DISCUSSION

The result of carcass traits like dressing percentage, weight of carcass, leg quarter, giblet weight, giblet meat percent, edible meat weight, edible meat percent, weight of spleen, bursa, breast and breast meat percentage of experimental birds fed standard basal diet (T_0), 3% glycerol with probiotic (T_1), 3% glycerol without probiotic (T_2), 6% glycerol with probiotic (T_3) and 6% glycerol without probiotic (T_4) is given in Table 2. The dressing percentage, weight of carcass, edible meat, leg quarter, spleen, bursa and edible meat percent did not differ significantly amongst the groups. The giblet weight and giblet % was numerically higher in T_1 as compared to other groups. Weight of breast meat and breast meat percent was significantly higher ($P < 0.05$) in T_1 group followed by T_2 and almost comparable in T_3 and T_4 as compared to control. Increase in breast yield might be due to improvement in protein deposition because of sparing effect of glycerol on gluconeogenic amino acid (Cerrate *et al.* 2006). Results of present experiment corroborated well with Cerrate *et al.* (2006) who noticed that birds fed with 2.5 and 5% glycerine had significantly higher breast yield and absolute weight of breast as compared to control. The results are consistent with findings of Abd-Elsamee *et al.* (2010) who found that as compared to control different levels of glycerol in the diet did not show significant difference in the dressing percentage, internal organs percentage or immune organ weights in broilers. Silva *et al.* (2012) found glycerol inclusion upto 10% in the broilers diet did not affect carcass yield. Mousa *et al.* (2018) also stated that there was no significant difference in the liver, heart and gizzard weight among the

Table 2. Carcass traits of experimental broiler chickens fed glycerol with or without probiotic

Parameter	Treatment					SEM	P value
	T0	T1	T2	T3	T4		
Dressing (%)	71.58	71.98	71.71	71.66	71.62	0.234	0.479
Carcass wt (g)	1725.50	1750.12	1738.50	1733.00	1728.62	10.852	0.227
Giblet wt (g)	95.02	97.61	97.04	96.04	95.63	1.776	0.602
Giblet (%)	3.94	4.01	4.00	3.97	3.96	0.066	0.801
Edible meat wt (g)	1630.47	1647.37	1641.36	1636.95	1632.99	10.140	0.497
Edible Meat (%)	67.64	67.76	67.71	67.69	67.65	0.243	0.990
Wt of Spleen (g)	2.24	2.14	2.20	2.17	2.21	0.093	0.859
Wt of Bursa (g)	1.22	1.17	1.19	1.18	1.20	0.027	0.098
Wt of Breast meat (g)	640.50 ^a	694.13 ^b	681.50 ^{bc}	672.63 ^{bc}	660.38 ^{ab}	17.286	0.021
Breast meat (%)	26.58 ^a	28.54 ^b	28.12 ^b	27.81 ^b	27.36 ^{ab}	0.633	0.023
Leg Quarter wt (g)	478.00	480.25	479.62	479.25	478.62	21.578	0.990

*Means bearing different superscript in a row a, b and c differ significantly (P<0.05).

Table 3. Haematological and blood biochemical profile of experimental broiler chickens fed glycerol with or without probiotic

Parameter	Treatment					SEM	P value
	T ₀	T ₁	T ₂	T ₃	T ₄		
Haemoglobin (g/dl)	8.63	8.71	8.69	8.65	8.60	0.164	0.964
Pack cell volume (%)	25.88	26.12	26.06	25.95	25.80	0.493	0.982
Total erythrocyte count (×10 ⁶ /ml)	2.38	2.42	242.00	2.41	2.40	0.088	0.964
Total protein (g/dl)	3.67	3.65	3.65	3.61	3.60	0.225	0.998
Albumin (g/dl)	1.65	1.58	1.58	1.58	1.60	0.074	0.850
Globulin (g/dl)	2.02	2.07	2.06	2.03	2.00	0.201	0.997
Albumin: Globulin ratio	0.83	0.77	0.79	0.80	0.82	0.079	0.923
Total cholesterol (mg/dl)	133.64 ^b	124.94 ^a	126.47 ^a	129.49 ^{ab}	130.98 ^{ab}	3.073	0.081
High density lipoprotein (mg/dl)	48.72	54.19	53.14	53.09	51.57	2.920	0.409
Low density lipoprotein (mg/dl)	57.69 ^c	45.98 ^a	48.43 ^{ab}	50.40 ^{ab}	52.93 ^{bc}	2.615	0.005
Triglyceride (mg/dl)	136.11	123.87	124.47	130.00	132.34	7.361	0.436
Blood glucose (mg/dl)	170.57	182.32	179.68	176.32	173.82	12.811	0.897
Blood urea nitrogen	0.54	0.52	0.53	0.54	0.54	0.014	0.375

*Means bearing different superscripts a, b and c in a row differ significantly (P<0.05).

treatment groups and control. Mousa *et al.* (2018) also observed that weight of bursa and spleen did not differ significantly in the glycerol supplemented groups and control. Similarly, Fortuoso *et al.* (2019) observe no significant differences among the treatment groups related to weight of carcass and weight of leg in the birds fed diet containing different levels of glycerol monolaurate.

The haematological and serum metabolic parameters of experimental birds are shown in Table 3. The effect of different level of glycerol with or without probiotic on haemoglobin [Hb (g/dl)], pack cell volume [PCV (%)] and total erythrocyte count [TEC (×10⁶/ml)] did not differ significantly among the all treatment and control group. Present study findings are in agreement with Fortuoso *et al.* (2019) who noticed nonsignificant differences on haemoglobin concentration, erythrocyte count and hematocrit level among the treatment groups in the broilers fed glycerol monolaurate. Likewise, Mohammed *et al.* (2018) also reported nonsignificant variations in the haematological indices (PCV, HB, RBC) of the broilers fed different levels of dietary cottonseed oil as an energy source.

Serum total protein, albumin, globulin, albumin: globulin (A: G) ratio, high density lipoprotein (HDL), blood glucose and blood urea nitrogen (BUN) did not differ significantly amongst all the treatment and control group. Triglyceride was numerically lower in T₁ followed by T₂ as compared to T₀, T₃ and T₄ which ultimately suggesting improved utilization of lipid and tissue storage. Serum total cholesterol was not significantly but numerically lower in T₁ and T₂ group and comparable in T₃ and T₄ whereas highest in T₀. LDL was significantly lower (P<0.05) in T₁ as compared to T₀, T₂, T₃ and T₄. Present study data is consistent with Mousa *et al.* (2018) who observed that crude glycerol supplementation in the broilers did not show any significant effect on serum total protein, albumin, globulin, serum glucose and HDL level. Pinto *et al.* (2019) who reported that blood glucose, total protein, albumin did not differ significantly among the treatment groups in the pigs fed diet containing mixed crude glycerine and ractopamine hydrochloride. Serum protein and uric acid concentration remain quiet similar between the groups (Erol *et al.* 2009). Yalçın *et al.* (2010) observed triglyceride level was not

affected by glycerol supplementation. Pinto *et al.* (2019) reported that serum cholesterol level did not affected by supplementation of glycerol. Mousa *et al.* (2018) reported that LDL level was significantly reduced in birds fed diet with 5% crude glycerol compared to broilers fed standard diet which recorded highest value. Present study is in disagreement with the findings of Mousa *et al.* (2018) who stated that serum cholesterol was significantly reduced in birds fed diet with 5% crude glycerol compared to broilers fed standard diet which recorded highest value.

The litter moisture (%) of various treatment groups fed with glycerol with or without probiotic is shown Table 4. On 21st day, litter moisture per cent was significantly ($P<0.01$) higher in T_4 , comparable in T_3 and T_2 , followed by T_1 as compared to T_0 . On 28th and 42nd day, moisture (%) of litter was significantly higher ($P<0.01$) in T_4 and T_3 , comparable in T_1 and T_2 as compared with T_0 . Increase in litter moisture might be due to low molecular weight hydrophilic glycerol compound which is easily excreted through the kidneys (Freitas *et al.* 2017). Present data is consistent with findings of Silva *et al.* (2012) who reported significant increase in litter moisture as inclusion level of glycerol increased in the diet of broilers. Cerrate *et al.* (2006) reported that litter moisture percent was significantly higher in the broilers fed 10% glycerine and stated that it might be due to 0.15% excess in potassium derived from the residue of catalyzer used in the transesterification reaction.

Table 4. Litter moisture (%) of experimental groups fed glycerol with or without probiotic

Age (days)	Treatment					SEM	P
	T_0	T_1	T_2	T_3	T_4		
21	27.04 ^a	29.46 ^b	29.64 ^{bc}	30.52 ^{bc}	30.73 ^c	0.506	0.001
28	31.77 ^a	33.67 ^b	34.08 ^b	36.46 ^c	36.85 ^c	0.754	0.001
42	37.64 ^a	40.06 ^b	40.72 ^b	42.71 ^c	43.40 ^c	0.742	0.001

**Means bearing different superscripts a, b and c in a row differ significantly ($P<0.01$).

Inclusion of 3% glycerol in diet with or without probiotic improved dressed breast weight and breast meat per cent while there was not any effect on other carcass traits like weight of carcass, dressing percentage, edible meat per cent and giblet weight. There were also no significant variations in the values of haematological parameters (Hb, PCV, TEC) among dietary treatments while blood biochemical parameters like serum total cholesterol, triglyceride numerically reduced and LDL was significantly lowered by supplementation of 3% glycerol with probiotic. Litter moisture per cent was increased as glycerol level increased in broiler chicken diet.

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