



Effect of Bakery Waste as Maize Replacer on Blood Biochemical and Immune Parameters of Broiler Chicken

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ABSTRACT

Present study was conducted to evaluate the effect of bakery waste incorporation on the blood biochemical and immune parameters of broiler chicken. Two hundred and forty day-old chicks were randomly divided into five dietary treatments: Control, T₁, T₂, T₃ and T₄ having 0, 5, 10, 15 and 20% bakery waste as maize replacement respectively. Blood biochemical parameters and immune parameters were analysed during the trial. The blood biochemical parameters viz. total protein, albumin, globulin, albumin-globulin ratio, cholesterol, triglycerides, Aspartate aminotransferase (AST or SGOT) and Alanine aminotransferase (ALT or SGPT) were estimated in serum using "ERBA Diagnostic Kits" and were statistically similar (p>0.05) as well in the normal physiological range indicating no adverse effect of bakery waste utilisation on the health of broiler chicken. Likewise, the cell mediated immune response and the humoral immune response did not show any significant (p>0.05) difference. Thus, this study shows that the use of bakery waste as maize replacer upto 20% in broiler ration supports the physiological growth of broiler chicken.

HIGHLIGHTS

- Bakery waste can be used to replace maize in broiler ration.
- Successful utilisation of bakery waste is indicated by absence of adverse effects on blood and immune parameters.

Keywords: Bakery waste, broiler, blood, immunity

Poultry sector is one of the fastest growing sector owing to the increasing demand for poultry products for human consumption. The increasing demand has resulted in higher prices of the various conventional feed ingredients. Since, feed costs represent about 60-70% of the poultry rearing costs (Olugbenga *et al.*, 2015), there is an urgent need to search for unconventional alternatives for several conventional feed ingredients. The ICAR- Indian Grassland and Fodder Research Institute (IGFRI), Jhansi has estimated that there is deficit of 11.24%, 23.4 % and 28.9% in green fodder, dry fodder and concentrates respectively, in the country (Roy *et al.*, 2019). Hence, lot of stress is being laid on search of feeds as replacer of maize because there is reliance on maize as feed source for many

meat/dairy animals as well as for human consumption. In this context, there is urgency for poultry raisers, animal nutrition researchers and the feed industry to look for alternative feed ingredients to keep down the costs. As an alternative feed, bakery waste seems to be a potential option to replace maize because of its availability and high energy content (Yadav *et al.*, 2014). Bakery waste is available in commercial quantities at many places in India for animal feeding. As per the estimates, the total volume

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of cakes, pastries and muffins production in India in 2017 was 16.6 thousand metric tons, whereas the total volume of production of breads, buns and croissant during the same time in India was 257 thousand metric tons (Statista, 2021).

Newer feed resources like bakery waste can be incorporated in rations of broiler chicken, but some of these might result in negative impact on the health of the host. Blood carries dietary nutrients to different parts of the body and thus, plays an essential role in the physiological state of the animal (Etim *et al.*, 2014). Analysis of blood biochemical parameters is a rapid method of understanding the health state of livestock and poultry especially during feeding trials, as the nutrition has quantifiable effects on blood composition (Etim *et al.*, 2014). Immune response measurement is also crucial because wastes may contain chemical or biological stress agents and trigger the cell or humoral mediated immune system. Normal physiological values of all these parameters indicate no adverse effects of the use of unconventional feed ingredients.

Therefore, this study was undertaken to scrutinise the effect of substituting maize with 20% bakery waste on the haematological and biochemical indices of broiler chickens.

MATERIALS AND METHODS

This study was conducted in the Division of Animal Nutrition, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, R.S. Pura, Jammu. Bakery waste was procured from a local bread factory while other feed ingredients were bought from local market. The experimental trial was conducted on two hundred and forty day old chicks of commercial strain Cobb-400 randomly divided into five treatment groups *viz.* control, T₁, T₂, T₃ and T₄. The control group birds were offered feed containing maize with no replacement with bakery waste and the treatment groups T₁, T₂, T₃ and T₄ were provided feed containing 5%, 10%, 15% and 20% of maize as bakery waste respectively. The birds were reared in deep litter system and the trial was conducted up to six weeks. All the dietary treatments were supplemented with soybean meal, meat bone meal, mineral mixture, vitamin supplements and common salt etc. formulated to satisfy the nutrient requirements of chicks as per ICAR

(2013). All diets were isonitrogenous and isocaloric. Table 1 presents the composition of broiler ration for different treatment groups.

MORTALITY

No mortality was observed during the trial period.

BLOOD BIOCHEMICAL ANALYSIS

Blood was collected from eight birds per group at the end of feeding trial (day 42) for analysis of blood biochemistry. About 2 ml of blood was collected in sterile centrifuge tube for serum collection for evaluation of blood biochemical profile. All the serum samples were stored in deep freezer at -20° C until processed for biochemical profile analysis.

All the biochemical parameters *viz.* total protein, albumin, globulin, albumin-globulin ratio, cholesterol, triglycerides, Aspartate aminotransferase (AST or SGOT) and Alanine aminotransferase (ALT or SGPT) were estimated in serum using “ERBA Diagnostic Kits” manufactured by Transasia Bio- Medicals Limited in technical collaboration with ERBA diagnostics Mannheim GmbH. Estimation of total serum protein was done as per Direct Biuret method (Gomal *et al.*, 1949). Estimation of Albumin was done as per Bromocresol green method (Dumas *et al.*, 1971). Serum globulin concentration was estimated by subtracting albumin concentration (g/dl) from the total protein concentration (g/dl). A: G ratio was then calculated using albumin (g/dl) and globulin (g/dl) values. The total serum cholesterol was determined in the units as mg/dl as per methodology given by Roeschlau's *et al.* (1974). Triglycerides in the serum were determined as per the method described by Cole *et al.* (1997). Estimation of AST was done as per the method described by Bergmeyer *et al.* (1986a). Estimation of ALT was done as per the method of Bergmeyer (1986b).

Immune response assessment

The effect on cell mediated immunity (CMI) in broiler chicks on cell mediated immune response or foot pad thickness was assessed through *in vivo* cutaneous delayed type hypersensitivity (DTH) reaction against phytohaemagglutinin-P (PHA-P) (Corrier and Deloach, 1990). At the age of 5 weeks, the cell mediated immune status was assessed at the age of 5 weeks on eight birds

Table 1: Ingredient composition (%) of experimental diets of broiler chicks

Attributes	C	T ₁	T ₂	T ₃	T ₄	C	T ₁	T ₂	T ₃	T ₄	C	T ₁	T ₂	T ₃	T ₄
	Pre- Starter Phase (0-14 Days)					Starter Phase (15-21 Days)					Finisher Phase (22-42 Days)				
Maize	60.50	57.47	54.45	51.43	48.40	60.88	57.94	54.89	51.85	48.80	67.50	64.13	60.75	57.38	54.00
Bakery Waste	0.00	3.03	6.05	9.07	12.10	0.00	3.04	6.09	9.13	12.18	0.00	3.38	6.75	10.13	13.50
Meat Bone Meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soyabean Meal	31.08	31.08	31.08	31.08	31.08	30.34	30.34	30.34	30.34	30.34	24.51	24.51	24.51	24.51	24.51
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sodium Bicarbonate	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Soyabean Oil	2.00	2.00	2.00	2.00	2.00	2.70	2.70	2.70	2.70	2.70	2.20	2.20	2.20	2.20	2.20
Methionine	0.17	0.17	0.17	0.17	0.17	0.13	0.13	0.13	0.13	0.13	0.09	0.09	0.09	0.09	0.09
Lysine	0.12	0.12	0.12	0.12	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dicalcium Phosphate	0.13	0.13	0.13	0.13	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Limestone Powder	0.59	0.59	0.59	0.59	0.59	0.54	0.54	0.54	0.54	0.54	0.29	0.29	0.29	0.29	0.29
Vitamin Supplement	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Total Mineral Mixture	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10

per group and this was evaluated through measurement of foot pad thickness.

Humoral immune response was evaluated at the beginning of 32 days through antibody titre (HA titre log₂ values) on randomly selected 40 experimental chicks (two birds from each replicate) (Hay and Hudson, 1989). Venous blood was collected aseptically from sheep in equal volume of Alsever's solution and a suspension of 0.5 % SRBC was prepared with PBS. For immunization, 100 µl of this suspension was injected intravenously in wing vein of each tested bird. The blood from the immunized birds was collected 10 days after post immunization. The blood was allowed to clot for 2 hours at 37 °C for 30 minutes to inactivate the complement fraction of the serum and Haemagglutination assay was performed. The same procedure (for preparation of 0.5% SRBC) was used to collect SRBC from the same sheep and was diluted to 0.5% solution. Total SRBC antibody titers were assayed in the serum of each bird. Anti-body titer was reported as log₂ of the highest dilution of serum that agglutinated an equal volume of a 0.5% SRBC in a normal saline solution (NSS).

STATISTICAL ANALYSIS

Data generated were subjected to a one way analysis of variance (ANOVA) and means were compared for

significant differences using the Duncan's Multiple Range Test SPSS package (Snedecor *et al.*, 1994).

RESULTS AND DISCUSSION

Blood biochemical parameters

Table 2 presents the effect of dietary inclusion of bakery waste on blood biochemical parameters of broiler chicken. The level of plasma glucose (mg/dl) was statistically similar and within the normal physiological range as suggested for broiler chicken (Dacie and Lewis, 1991). This indicates no adverse effect of bakery waste inclusion as maize substitute on the glucose levels. The results are in similar line with the findings of Ahaotu *et al.* (2018) who too reported similar glucose levels while replacing maize with Bakery waste at different inclusion levels. Contrary to our results, Adekunle and Omoh, (2014) reported lower glucose levels on supplementing bakery waste in broiler finisher ration. The differences found in the above two studies may be attributed to different feed ingredients used for feed preparation and different sources of bakery waste.

The level of total serum protein (g/dl), albumin (g/dl) and globulin (g/dl) were found to be statistically similar. Also, no significant difference was seen in the A: G ratio irrespective of different levels of dietary inclusion of bakery waste as maize substitute. These values are in the

Table 2: Effect of bakery waste inclusion on haemato-biochemical profile of broiler chicken

Particulars	Groups				
	C	T ₁	T ₂	T ₃	T ₄
Glucose (mg/dl)	153.42 ± 5.87	160.15 ± 6.04	160.53 ± 8.02	160.21 ± 7.27	162.93 ± 8.12
Cholesterol (mg/dl)	151.81 ± 1.83	151.21 ± 1.99	148.66 ± 2.38	150.84 ± 2.09	151.66 ± 1.29
Triglyceride (mg/dl)	60.55 ± 0.48	59.72 ± 2.26	59.44 ± 1.62	61.93 ± 1.12	60.17 ± 2.06
Total protein (g/dl)	4.11 ± 0.09	3.79 ± 0.06	3.83 ± 0.11	3.90 ± 0.20	4.00 ± 0.12
Albumin (g/dl)	2.43 ± 0.13	2.13 ± 0.09	2.14 ± 0.10	2.22 ± 0.19	2.11 ± 0.13
Globulin (g/dl)	1.68 ± 0.18	1.67 ± 0.08	1.69 ± 0.10	1.68 ± 0.12	1.89 ± 0.05
A/G ratio	1.52 ± 0.23	1.29 ± 0.11	1.28 ± 0.12	1.36 ± 0.19	1.12 ± 0.08
SGOT (IU/L)	146.68 ± 4.02	148.37 ± 1.90	147.66 ± 3.6	150.68 ± 1.52	153.43 ± 2.51
SGPT (IU/L)	13.58 ± 0.91	12.93 ± 1.13	12.86 ± 0.91	13.62 ± 1.14	14.82 ± 0.98

normal physiological range value for chickens (Suchint *et al.*, 2004) which indicated that the bakery waste based diets are identical with the control diet and have no adverse effect on these parameters. Hoffenberg and Block (1996) had reported that serum protein and albumin synthesis depends on the availability of protein and with the increase in protein intake, the rate of synthesis increases where as catabolic rates don't change easily. The rations were prepared iso-nitrogenous in all the dietary groups which resulted in similar protein intake and similar blood profile of proteins. This result was in accordance with the observations of Shittu *et al.* (2016), Epao *et al.* (2017) and Ahaotu *et al.* (2018) who too reported similar values for total protein, albumin and globulin while replacing maize with bakery waste in broiler ration. However, Adekunle and Omoh (2014) reported similar protein but higher albumin (1.80 vs. 2.10 g/dl) and lower globulin (3.70 vs. 2.90 g/dl) on replacing maize with 50% bakery waste. The cholesterol levels (mg/dl) were found to be statistically similar in all five treatment groups which imply no adverse effect on the health of the broiler chickens. Similar to our results, earlier researchers too reported no significant difference in the cholesterol levels in the birds fed bakery waste in comparison to the birds fed maize-soya based basal diet (Adekunle and Omoh, 2014; Shittu *et al.*, 2016, Epao *et al.*, 2017 and Prabhale *et al.*, 2019). However, Ahaotu *et al.* (2018) reported significantly higher cholesterol levels in the birds fed 100% bakery waste as maize replacer in the broiler ration. This differences found may be explained in the light of fact that higher inclusion of bakery waste (50 and 100%) were used in the diets as well as their Bakery waste source contains higher EE content (17.14%)

in comparison to the present study. The values of serum triglycerides (mg/dl) are statistically similar in all the treatment groups indicating no adverse effect of bakery waste incorporation in treatment groups.

Similar to our findings, Epao *et al.* (2017) and Prabhale *et al.* (2019) too reported comparable values for triglycerides in the broiler birds fed on bakery waste and maize- soya based ration. Serum enzymes (SGOT and SGPT, IU/L) were not affected by dietary incorporation of bakery waste at graded levels while replacing maize. But normal values indicate no adverse effect of bakery waste inclusion on the liver parenchyma of the broiler chicken. These results were in accordance with the findings of Shittu *et al.* (2016) who reported no significant difference in the levels of SGOT and SGPT in birds fed on bakery waste (Biscuit dough) incorporated ration compared to those fed maize-soya based basal diet. The literature pertaining to the above parameter is not available to the best of our knowledge, so the results cannot be discussed in the light of above facts. All the above blood biochemical parameters were in the normal physiological range, it may be inferred that bakery waste may safely replace maize up to 20%. It also ruled out possibility of harmful substances in the bakery waste and suggests that bakery waste has the required nutrients to support stable physiological activities of blood parameters.

Cell mediated and humoral immunity

The cell mediated immune response or foot pad thickness against the phytohaemagglutinin-p (PHA-P) as antigen injected intradermally in the foot pad of broiler chicks

Table 3: Effect of bakery waste inclusion on cell mediated immune (CMI) response of broiler chicken

Groups	Periods				MEAN \pm S.E.
	0	24	48	72	
C	100.00	109.15 \pm 2.12	120.26 \pm 3.19	108.37 \pm 1.32	109.45 \pm 1.52
T ₁	100.00	113.66 \pm 3.98	125.81 \pm 3.26	108.15 \pm 2.09	111.90 \pm 2.31
T ₂	100.00	110.90 \pm 2.63	123.51 \pm 2.89	111.31 \pm 1.84	111.43 \pm 1.58
T ₃	100.00	106.98 \pm 1.10	120.38 \pm 1.78	112.40 \pm 2.54	109.94 \pm 0.96
T ₄	100.00	107.60 \pm 0.83	119.99 \pm 1.96	109.01 \pm 2.19	109.15 \pm 1.19

is calculated as percentile (zero hour values are taken as 100% and other values are calculated accordingly) and presented in Table 3. The average CMI response of broiler birds was found to be statistically similar ($p > 0.05$) in all the treatment groups throughout the 72 hours. Similarly, humoral immune response (HA titer log₂ value) presented in Table 4 was also statistically similar ($p > 0.05$) in all the treatment groups, which indicates no impaired effect of bakery waste inclusion on the immune response of birds.

The physiologically normal and statistically similar values of blood and immune parameters indicate no adverse effect of bakery waste inclusion in the poultry diets.

Table 4: Effect of bakery waste inclusion on humoral immune response of broiler chicken

Treatment	HA Titre (log ₂ value)
C	2.11 \pm 0.20
T ₁	2.11 \pm 0.44
T ₂	1.96 \pm 0.45
T ₃	1.81 \pm 0.23
T ₄	2.26 \pm 0.42

CONCLUSION

The results of this study indicate that the bakery waste can serve as a replacement of maize upto 20% in broiler ration, without any adverse effects on the blood biochemistry and immune status of the broilers.

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