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Effect of Feeding Fermented Rapeseed meal on the Serum Biochemical Constituents and Immune Response of Commercial Broiler Chicken

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ABSTRACT

The study evaluated the impact of raw and fermented rapeseed meal (RSM and FRSM) on serum biochemical parameters and immune response of commercial broiler chicken. Experiment was conducted on day old chicks for 42 days and birds were allotted randomly. Dietary treatments consisted of a control standard corn-soya bean meal diet and 6 test diets where raw or fermented rapeseed meal included at 5, 10 and 15% levels. Blood samples taken at day/ 37 measured total protein, albumin, globulin, creatinine, aspartate amino transferase (AST), alanine transaminase (ALT), and New castle Disease antibody titer, while cell-mediated immunity was assessed via a phyto haemagglutinin phosphate (PHA-P)induced skin response on day/ 40.Results showed no effects on albumin, globulin, creatinine, or AST/ALT ratio across treatments. However, total protein was significantly higher in the 10% FRSM group ($P<0.05$), with other groups comparable to the control. Neither antibody titers nor cellular immunity were affected by the inclusion of RSM or FRSM at any level. The study concludes that incorporating up to 15% of either raw or fermented rapeseed meal in broiler diets has no adverse effect on serum biochemical parameters or immune responses, making these feed components safe for commercial use.

KEYWORDS: Cell mediated immunity, Fermented rape seed meal, Humoral immunity, Serum biochemical parameters.

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INTRODUCTION

Rising soybean meal prices and demand have sparked interest in cost-effective, protein-rich alternative feed resources that support nutritionally sound, least cost rationing (Supriya et al., 2025; Balaji et al.,2025).Rapeseed meal is one such alternative, it is a by product of oil extraction, rich in proteins, but its high content of anti-nutritional factors such as glucosinolates limits its use in animal diets (Elangovan et al., 2001; Tripathi and Mishra, 2006).To overcome the ill effects of RSM various processing techniques such as solvent extraction, heat treatment, use of chemicals like copper sulphate, ferrous sulphate, iodine and enzyme treatment were tried which can remove the anti-nutritional factors. Fermentation process is an effective technique to eliminate the anti-nutritional factors and to improve the nutritional value of vegetable protein supplements (Ashayerizadeh et al., 2018).Fermentation, a biological process involving microorganisms, can degrade these anti-nutritional compounds, increases its nutritional quality, making fermented rapeseed meal (FRSM) a more viable and beneficial ingredient (Supriya et al., 2025; Cheng et al., 2022; Olukomaiya et al., 2019).FRSM has gained attention as a potential feed ingredient in livestock nutrition due to its promising effects on animal health and performance (Shi et al., 2015; Wlaz³o et al., 2022; Fazhi et al., 2011). Fermentation of RSM with Aspergillus niger improved growth and nutrient digestibility in pigs (Shi et al., 2016) and similarly fermentation of RSM with *Saccharomyces cerevisiae* improved antioxidant status and decreased lipid peroxidation in post-weaned piglets (Taranu et al., 2022). Solid-state fermentation (SSF) may be the most promising way to effectively reduce anti-nutritional factors and improve nutritional value of RSM (Van Winsen et al., 2001). Besides, SSF can effectively reduce the glucosinolate level of RSM (Vig and Walia, 2001) so SSF improve the nutritional value of RSM when it is fed to broilers.

The impact of FRSM on serum biochemical parameters has been of particular interest, as it may influence various metabolic and physiological

functions in birds. Additionally, the immune response of birds could be enhanced by fermented products, which are thought to boost immune system efficiency and disease resistance (Zhu et al., 2020; Guo et al., 2021). Several studies have shown that fermented feeds may improve gut health, nutrient absorption, and reduce inflammation, all of which contribute to better overall immune function (Guo et al., 2021; Lian et al., 2024).The studies on inclusion of fermented rape seed meal on different species around the world are available, however work on inclusion of FRSM at higher levels in broilers in India is scarce. Hence, the present study was conducted to explore how Raw and fermented rapeseed meal at level of 5%, 10% and 15% affects both serum biochemical parameters and immune responses in commercial broilers.

MATERIALS AND METHODS

A study was undertaken for 42 days to assess the effect of feeding fermented rapeseed meal on the serum biochemical parameters and immune response of commercial broilers. The experiment was conducted at the Poultry Experimental Station, Rajendranagar, Hyderabad. The laboratory analysis of the biological collected samples was done at Department of Animal Nutrition, College of Veterinary Science, Rajendranagar, Hyderabad and Indian Council of Agricultural Research-Directorate of Poultry Research, Rajendranagar, Hyderabad. The experiment was conducted following the guidelines of Institutional Animal Ethics Committee.

Fermentation of Rapeseed meal

Solid state fermentation of rapeseed meal was done by following the protocol given byRS-L HEALTH.(M/S Loonshot Ventures Private Limited, India). The starter culture contains *Enterococcus faecium* ($1x10^8$CFU/g) and *Lactobacillus plantarum* ($1x10^8$CFU/g). The culture was activated by mixing with lukewarm water (30-35 °C) in bin and incubated at 30-35 °C for 36 hr culture turned from light to bright yellow, which indicates the activation of bacteria. Solid state fermentation of rapeseed meal was done by following the protocol (75ml culture in 75L water for 200kg meal) given by RS-L HEALTH and was standardized for RSM by mixing different levels of water (1, 1.5 and 2X) and culture (1, 1.5 and 2X) in order to achieve the pH of fermented RSM (FRSM) below 4.5 and lactic acid content > 25 g/kg. The RSM was hand mixed with inoculum and water and fermented in air tight vacuum bags and incubated at 33-35! for 84 h and later sampled for pH and lactic acid. Based on pH and LA content in FRSM, the best combination for fermentation of RSM was selected for the broiler experiment. The chemical composition of soyabean meal, raw and fermented rapeseed meal is given in Table 1.

Table 1. Chemical composition (% DM basis) of raw and fermented rapeseed meal

Constituent	Soyabean meal	Rapeseed meal	Fermented[1] rapeseed meal
Dry Matter	88.1	89.3	88.4
Crude Protein	46.2	34.6	35.8
Crude Fibre	7.5	12.4	9.34
Ether Extract	1.2	1.49	2.91
Calcium	0.34	0.63	0.61
Phosphorous	0.23	0.39	0.34
Gross energy (kcal/Kg)	4710	4155	4202
Metabolizable energy (kcal/Kg)	2704	1770	1790

Note**:** ME (Kcal/kg) = 0.57 × GE (Kcal/kg)

[1]Fermented with starter culture containing *Enterococcus faecium* and *Lactobacillus plantarum*.

Serum biochemical constituents

Blood was collected from one bird per replicate on 37th day of age. Blood samples were collected aseptically from brachial vein of birds with the help of sterilized needles and blood was collected in clean sterilized glass tubes and kept in slanted position at

room temperature for serum collection. The collected serum samples were then centrifuged at 3000 rpm for 5 minutes and transferred to 2 ml Eppendorf tubes which were stored at -20°C. The estimation of serum biochemical constituents viz.,total protein, albumin and globulin, creatinine, alanine transaminase (ALT), aspartate amino transferase (AST) was done by auto-analyzer using commercial diagnostic kits (ERBA Diagnostics, INC).

Humoral immunity

Humoral immunity in broilers was estimated by measuring antibody titers to Newcastle disease (ND) vaccine (antibody production against ND virus). Broilers were vaccinated against ND by ocular route at 7thand 28thday of age with Lasota strain (ND Lasota Vac-1000; Ventri Biologicals, Pune, India). On 37th day blood was collected and serum was separated. Subsequently antibiotic specific for ND were detected in sera of chicks by haemagglutination inhibition (HI) test and were expressed as $\log_2$ titers (Allan et al., 1978).

Hummoral immunity was done in 'U' bottom micro well plate. It involves 2 processes i.e., HA estimation and HI estimation. For HA estimation, 50µL of normal saline was pipetted into 12 micro wells, 50µL of ND virus was added into 1st well and serial dilution was done upto 11th well. Now, 50µL of 1 % RBC is added to 12 wells and incubated at 37! for 30-40 mins. After incubation a button was formed at U bottom upto 8th well and this considered as 2^8 (HA). For HI estimation 50 of 4HA virus is added instead of ND virus and remaining procedure is same as HA estimation, the buttons are calculated at end and titre value was expressed in $\log_2$.

Cell mediated immunity

Cell mediated immune (CMI) response was assessed by measuring *in vivo* cutaneous basophilic hypersensitivity (CBH) to phyto haemagglutinin phosphate (PHA-P).On 40thday of experiment, one bird was selected randomly from each replicate to assess the CMI response . The toe web was injected with 100 µg of PHA-P suspended in 0.1 ml of phosphate buffer saline (PBS). The web swelling of the feet was measured by micrometer at 24 h after injection. The thickness was calculated by using the formula (Edelman et al.,1986)

$$\text{CMI (\%)} = \frac{\text{post injection skin thickness of toe web}}{\text{Pre injection skin thickness of toe web}} \times 100$$

RESULTS AND DISCUSSION

The present study evaluated the effects of dietary inclusion of raw and fermented rapeseed meal (RSM and FRSM) at levels of 5%, 10%, and 15% on serum biochemical constituents and immune responses in broilers. The results indicated in the Table.2 that the serum albumin, globulin, creatinine, and the AST/ALT ratio were not significantly affected by the inclusion of raw or fermented rapeseed meal. However, a significant difference was observed in serum total protein, with birds fed 10% FRSM showing the highest levels, while the lowest levels were recorded in the 15% RSM diet group. This suggests that, while raw and fermented rapeseed meal had little effect on most of the biochemical parameters, FRSM at 10% inclusion may have a positive impact on total protein levels in broilers.

Table 2. Effect of dietary inclusion of raw and fermented rapeseed meal on serum biochemical constituents in broilers

Diet	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Creatinine (mg/dl)	AST/ALT (IU/L)
Control	4.01^{ab}	1.97	2.04	2.073	1.046
5% RSM	3.60^{ab}	1.98	1.62	2.074	1.119
10% RSM	3.61^{ab}	1.96	1.64	2.041	1.189
15% RSM	3.57^{b}	1.96	1.61	2.003	1.302
5% FRSM	4.35^{ab}	2.14	2.22	2.069	1.155
10% FRSM	4.38^{a}	2.13	2.25	2.004	1.129
15% FRSM	4.34^{ab}	2.12	2.21	2.003	1.145
SEM	0.986	0.374	0.981	0.417	0.302
P-Value	0.04	0.62	0.21	0.99	0.46

^{abc} Means with different superscript in a column differ significantly: P<0.05, P-value: Probability value.

The significant effect on serum total protein observed in this study corroborates findings from previous research. Hu et al. (2012) reported that inclusion of 28.41% RSM and 24.27% FRSM in the diet resulted in higher serum total protein levels in birds fed FRSM compared to control groups. Czech et al. (2020) also found that the inclusion of 4% FRSM increased serum total protein and albumin levels in broilers. These studies suggest that fermented rapeseed meal may improve protein metabolism in broilers, potentially due to the breakdown of anti-nutritional factors during fermentation. However, these findings contrast with those of Shi et al. (2016), who observed no significant changes in serum total protein or albumin when 10% RSM or FRSM was included in the diet. This discrepancy may be attributed to differences in experimental conditions, including diet composition and the specific fermentation processes used.

Regarding other serum biochemical markers, including creatinine and AST/ALT ratios, no significant effects were observed in this study, aligning with the results of Elbaz et al. (2021), who found no changes in these parameters when 20% raw or fermented rapeseed meal was included in broiler diets. This suggests that, at the inclusion levels tested in this study, neither raw nor fermented rapeseed meal had a notable impact on kidney or liver function in broilers.

Table 3. Effect of dietary inclusion of raw and fermented rapeseed meal on humoral immune response against ND vaccine and cell mediated response against PHA-P in broilers

Diet	Humoral immune response (log_2 titer)	Cell mediated response (thickness index)
Control	6.20	40.16
5% RSM	6.10	45.13
10% RSM	6.10	43.92
15% RSM	6.40	35.87
5% FRSM	6.70	47.84
10% FRSM	6.70	36.01
15% FRSM	6.50	33.16
SEM	0.09	1.947
P-Value	0.427	0.339

In terms of immune responses (Table 3), no significant differences were observed in either humoral immunity, assessed through antibody titres to Newcastle disease virus (NDV), or cell-mediated immunity, evaluated by measuring digital skin thickness after PHA-P injection. These results suggest that raw or fermented rapeseed meal, even at 15% inclusion levels, did not significantly affect the immune function of broilers under the conditions of this study. This is in contrast to the findings of Wlazlo et al. (2021), who observed a reduction in immunoglobulin levels in rabbits fed fermented rapeseed meal at 4%, 8%, and 12% inclusion levels, indicating a potential depression of both humoral and cell-mediated immune responses. However, the current study did not observe such an effect in broilers, which could be due to species-specific differences or variations in study methodologies.

On the other hand, studies by Elbaz et al. (2023) and Zhu et al. (2020) reported that fermented meals, such as fermented canola meal, improved immune responses in broilers, including significant increases in antibody titres against NDV. These contrasting findings highlight that the effects of fermented meals on immune function may vary depending on factors such as the type of fermented meal used, the fermentation process, and the species studied. The differences in results could also be attributed to the bioactive compounds released during fermentation, which may influence immune function in different ways depending on the feed ingredient.

In conclusion, the inclusion of fermented rapeseed meal up to 15% did not significantly affect serum biochemical parameters or immune responses in broilers in this study. However, the variations

observed in other studies involving fermented feeds suggest that the effects of such diets can depend on factors like meal type, fermentation process, and species. Future research exploring a broader range of inclusion levels, types of fermented meals, and immune markers is needed to gain a deeper understanding of how fermented rapeseed meal impacts both nutrient metabolism and immune health in poultry.

CONCLUSION

Results suggest that FRSM can be safely included in broiler diets at inclusion levels up to 15% without any negative impact on the birds' health or immune function.

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