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Effect of feeding fermented rapeseed meal on the performance and gut health of commercial broiler chicken

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ABSTRACT

The aim of this research was to study the effect of feeding fermented rapeseed meal on the performance and gut health of commercial broilers. An experiment of 42 days was conducted on day-old chicks to study the effect of raw (RSM) and fermented (FRSM) rapeseed meal on growth performance, and gut health of broilers. A mixed liquid culture of *Enterococcus faecium* and *Lactobacillus plantarum* were used for fermenting rapeseed meal. Birds were randomly allotted to 7 dietary groups namely 5%, 10%, 15% RSM and 5%, 10% and 15% FRSM groups and these were compared to maize-soyabean based control group. Body weights and feed intake were estimated weekly. On 42nd day, 1 bird from each replicate was sacrificed for histomorphological studies and enumeration of total bacteria, *Lactobacillus*, *Escherichia coli* in the gut. Significantly higher ($P<0.05$) body weight was seen in 5% FRSM group when compared to control and raw rapeseed meal fed groups while the feed intake and FCR remained unchanged. Significant differences ($P < 0.05$) were observed in duodenal villus height, crypt depth, the villus height to crypt depth ratio (VH:CD), and gut *Lactobacilli* counts in birds fed diets supplemented with FRSM at all the inclusion levels. Conclusively, fermented rapeseed meal could be included in diets upto 15% without any adverse effects on performance and there was improved *Lactobacillus* count in gut, villus height and crypt depth in duodenum of broilers with fermented RSM at this level.

Keywords: Duodenal villi height, *Enterococcus faecium*, Fermented rapeseed meal, *Lactobacillus plantarum*

The poultry industry in India is grappling with escalating feed costs, primarily due to the rising prices of essential ingredients like corn and soybean meal due to its increased global demand, particularly from rapidly developing economies. Consequently, nutritionists and feed formulators are actively exploring alternative, locally available feed ingredients to mitigate expenses without compromising the nutritional quality of poultry diets. (Yusuf *et al.* 2021)

One such promising alternative is rapeseed meal, a high-protein byproduct of rapeseed oil extraction with crude protein value of 35%. Rapeseed meal offers a favourable amino acid profile, including a higher concentration of sulphur-containing amino acids, which are beneficial for poultry growth (Wongsirichot *et al.* 2022). However, its utilization is hindered by the presence of anti-nutritional factors. These degradation products can adversely affect palatability, reduce feed intake, impair growth, and interfere with thyroid function in poultry. (Hao *et al.* 2020, Yang *et al.* 2022).

To enhance the nutritional value and safety of rapeseed meal, solid-state fermentation (SSF) has emerged as a viable biotechnological approach. SSF involves cultivating specific microorganisms, such as *Bacillus subtilis*, *Aspergillus niger*, and *Lactobacillus* species, on the solid substrate of rapeseed meal. (Zhu *et al.* 2023). This fermentation process facilitates breakdown of glucosinolates and other anti-nutritional factors like phytic acid and fiber (Vig *et al.* 2001). Beyond the reduction of anti-nutritional factors, SSF also enhances the bioavailability of nutrients in rapeseed meal and increases the levels of crude protein, crude fat, and soluble peptides, which are more readily absorbed by poultry (Konkal *et al.* 2019). Additionally, the activity of beneficial microorganisms during fermentation can produce bioactive compounds that may improve the immune status and gut health in poultry. Incorporating fermented rapeseed meal into poultry diets has been associated with enhanced growth performance,

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including increased body weight and feed conversion efficiency. (Elbaz *et al.*2023; Hu *et al.* 2012; Chiang *et al.* 2010). The present study aims to investigate the chemical and microbial characteristics of rapeseed meal during SSF and evaluate its effects on broiler performance and gut health.

MATERIALS AND METHODS

Fermentation technique: The bacterial culture used for fermentation of rapeseed meal (RSM) was obtained from RS-L HEALTH (M/S Loonshot Ventures Private Limited, India),and contained *Enterococcus faecium* (1x10^8 CFU/g) and *Lactobacillus plantarum* (1x10^8 CFU/g). Solid-state fermentation of RSM was conducted following the protocol provided by RS-L HEALTH (75 mL of culture in 75 L of water for 200 kg of meal), with slight modifications to optimize the process. Different levels of water (1x, 1.5x, and 2x) and culture (1x, 1.5x, and 2x) were tested to achieve a fermented RSM (FRSM) with a pH below 4.5 and lactic acid content greater than 25 g/kg. The RSM was mixed by hand with the inoculum and water, and then fermented in airtight vacuum bags at 33–35°C for 84 hours. After fermentation, samples were collected to measure pH and lactic acid. Based on the results (Table1), the most effective combination of water and culture for fermentation was selected for the broiler study.

Experimental design and experimental diets: A total of 350 day-old commercial broiler chicks were wing-banded, weighed individually, and randomly allocated to 7 dietary treatments, with 10 replicates per treatment and 5 chicks per replicate. A corn-soybean-based control (T1) diet was formulated, and the other treatments included 5%, 10%, and 15% raw rapeseed meal (RSM) (T2, T3 and T4 respectively) and 5%, 10%, and 15% fermented rapeseed meal (FRSM) (T5, T6 and T7 respectively), replacing soybean meal in the diet. All the birds were raised under uniform management and fed with respective *isocaloric* and iso *nitrogenous* diets *ad libitum* from day old to 6 weeks of age. Body weights and feed intake were recorded weekly. Clean and fresh drinking water was provided *ad libitum* daily. On day 42, one bird from each replicate in each treatment group was sacrificed for histomorphological analysis and enumeration of total bacteria, *Lactobacillus*, and *Escherichia coli* in the gut. The data collected were subjected to appropriate statistical analysis following the methods described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Among all the combinations of culture and water tested, the fermentation of RSM with 1.5X culture and 2X water (0.5625 ml of culture in 0.75 liters of water per kg of RSM) resulted in a notable improvement in lactic acid content, i.e. from 2.08±0.4 to 5.88±0.5%, while pH decreased from 5.04±0.3 to 4.32±0.5 (Table 1). Adjustments were made to optimize the water and culture levels, achieving a pH of fermented rapeseed meal (RSM) below 4.5 and a lactic acid content exceeding 25 g/kg. The lactic acid increased by 61.86% when compared to raw RSM, this increase can likely be attributed to the higher bacterial concentration and increased water content, which facilitated more efficient carbohydrate digestion and lactic acid production. Lactic acid levels increased from 2.08 ± 0.4 to 5.88 ± 0.5, probably due to carbohydrate fermentation by *Lactobacillus* spp. The decrease in pH from 5.04±0.3 to 4.32±0.5 during fermentation is likely due to the elevated organic acid concentration produced by *Lactobacillus*, which is consistent with previous studies (Van Winsen *et al.* 2001, Canibe *et al.* 2006, 2008).

In the broiler experiment, significant differences (P<0.05) in body weight gain (BWG) were observed during the 1st and 4th weeks (Table 2). The BWG was similar between FRSM and RSM at the same inclusion levels. Birds fed 5% and 10% FRSM diets exhibited BWG comparable to the control group, while those fed 15% RSM had the lowest BWG. The improved BWG at the 5% inclusion level may be attributed to the reduction of anti-nutritional factors in the fermented meal (Feng *et al.* 2007, Mathivanan *et al.* 2006), which might have enhanced nutrient absorption and utilization. Additionally, the increased villus height and villus height-to-crypt depth (VH/CD) ratio in FRSM-fed birds might have also contributed to more efficient nutrient absorption, resulting in better BWG (Shim *et al.* 2010). The overall lower BWG at 15% FRSM might be due to

Table 1. PH and Lactic acid content of raw rapeseed meal and fermented rapeseed meal

Process	pH	Lactic acid (g/kg)
Raw RSM	5.03^{a}	2.04^{g}
SD-SW[1] FRSM	4.88^{b}	4.56^{e}
SD-DW[2] FRSM	4.71^{c}	6.04^{a}
1.5D-SW[3] FRSM	4.56^{d}	4.74^{d}
1.5D-DW[4] FRSM	4.26^{f}	5.35^{b}
DD-SW[5] FRSM	4.36^{e}	4.39^{f}
DD-DW[6] FRSM	4.09^{g}	5.16^{c}
P-value	0.00	0.00
SEM	1.452	1.468

[1]SD-SW=Single dose-single water; [2]SD-DW= Single dose-double water; [3]1.5D-SW=1.5 dose-single water (); [4]1.5D-DW=1.5 dose-double water (??); [5]DD-SW=Single dose-single water (??); [6]DD-DW= Single dose-double water (??); RSM= Rapeseed meal; FRSM= Fermented rapeseed meal; SEM= Standard Error Mean

Indicate the absolute levels of water and culture used in different treatments

Dose	Culture (ml)	Water (ml)
Single dose- single water	0.375	375
Single dose-double water	0.375	750
1.5 dose- single water	0.5625	375
1.5 dose- double water	0.5625	750
Double dose- single water	0.750	375
Double dose- double water	0.750	750

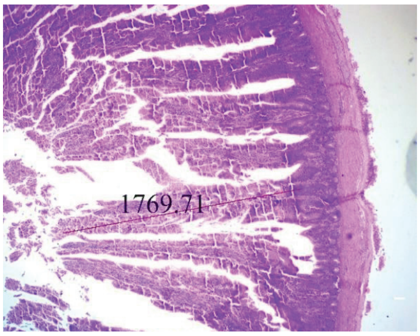

Fig 1. Pictomicrograph showing villus height of duodenum from control group

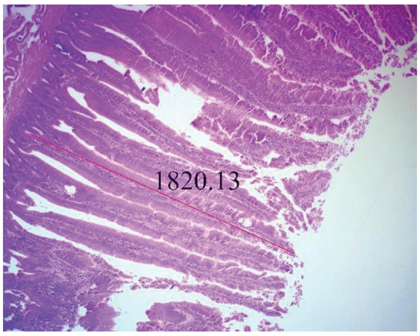

Fig 2. Pictomicrograph showing villus height of duodenum from 5% RSM diet

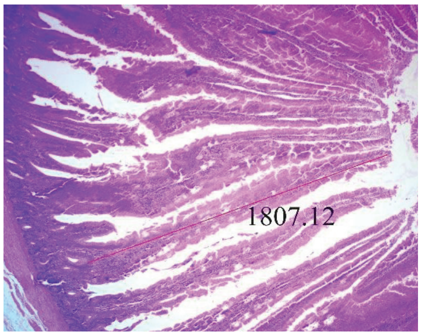

Fig. 3. Pictomicrograph showing villus height of duodenum from 10% RSM diet fed bird

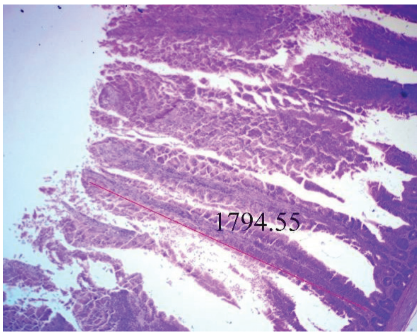

Fig. 4. Pictomicrograph showing villus height of duodenum from 15% RSM diet fed bird

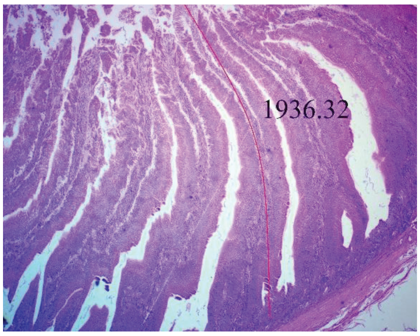

Fig. 5. Pictomicrograph showing villus height of duodenum from 5% FRSM diet fed bird.

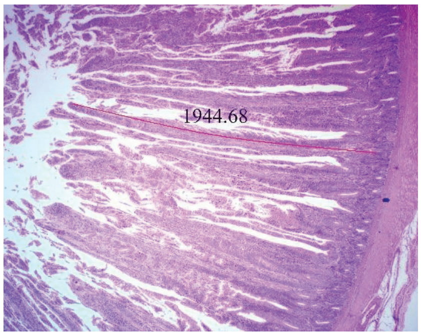

Fig. 6. Pictomicrograph showing villus height of duodenum from 10% FRSM diet fed bird.

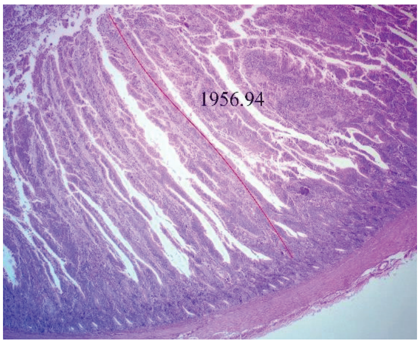

Fig. 7. Pictomicrograph showing villus height of duodenum from 15% FRSM diet fed bird.

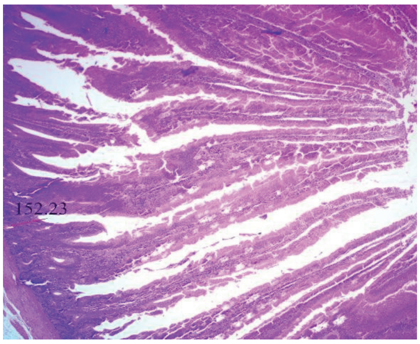

Fig. 8. Pictomicrograph showing Crypt depth of duodenum from control fed bird.

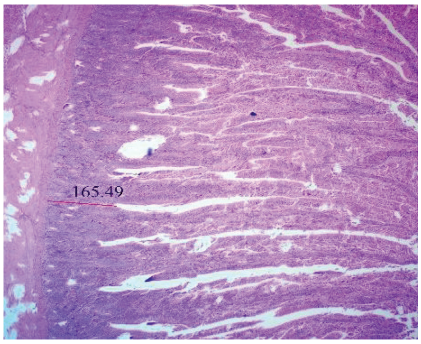

Fig. .9. Pictomicrograph showing crypt depth of duodenum from 5% RSM fed bird.

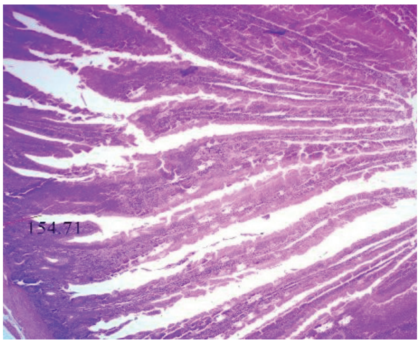

Fig. 10. Pictomicrograph showing crypt depth of duodenum from 10% RSM fed bird.

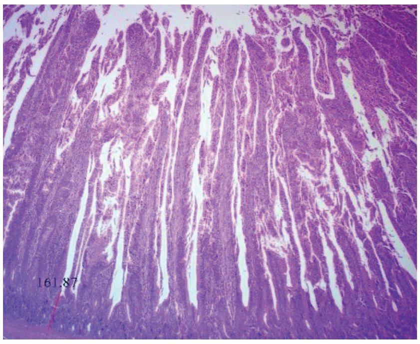

Fig. 11. Pictomicrograph showing crypt depth of duodenum from 5% FRSM fed bird.

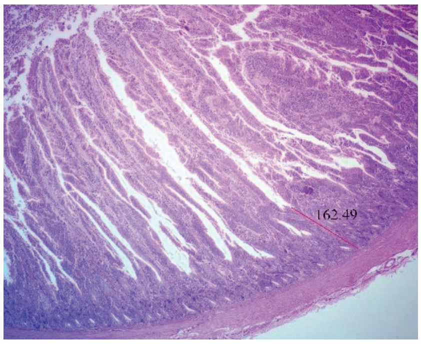

Fig. 12. Pictomicrograph showing crypt depth of duodenum from 15% FRSM fed.

reduced feed intake in this group, which is consistent with findings from Xu *et al.* (2012).

Feed intake differed significantly (P<0.05) during the 1st, 4th, and 6th weeks, with birds fed 15% RSM or FRSM diets showing lower feed intake (Table 3). Overall, birds fed 5% FRSM had the highest feed intake, followed by the control group, with the lowest feed intake observed in birds fed the 15% RSM diet. The higher feed intake in FRSM groups was plausibly to be increased palatability, the enhanced dry matter (DM), crude protein (CP), and crude fiber (CF) content in the fermented feed, and the reduction of anti-nutritional factors in RSM (Chiang *et al.* 2010). However, at the 15% inclusion level of RSM or FRSM, feed intake was reduced. This reduction in feed intake at higher inclusion levels aligns with findings from Wu *et al.* (2022).

The feed conversion ratio (FCR), did not differ significantly (P>0.05) across all weeks (Table 4). Konkol *et al.* (2023) had reported similar findings, observing no significant effect on FCR when soybean meal was replaced with 3% RSM or FRSM. These results suggested that although FRSM might improve gut health and nutrient digestibility, such improvements may not be sufficient to cause measurable changes in FCR under practical broiler production conditions (Konkol *et al.* 2023, 2024) Xu *et al.* (2012) observed that, FCR was poorer (P<0.05) for birds fed 15% FRSM compared to those fed 0%, 5%, and 10% FRSM. Conversely, Shuai *et al.* (2023) found that FRSM diets resulted in better FCR than raw RSM diets.

Lactobacillus spp. plays an important role in regulating the immune system, enhancing gut metabolism, and maintaining gut microbial balance (Valeriano *et al.* 2017). Incorporation of fermented feed in diet affects the

Table 2. Effect of dietary inclusion of raw and fermented rapeseed meal on weekly body weight gain(g) in broilers (0-6 weeks)

Diet	Age (Week)					
	1	2	3	4	5	6
Control	114.22^{ab}	224.26	414.94	522.70^{a}	650.94	615.49
5% RSM	120.38^{a}	223.38	420.36	489.00^{ab}	630.72	602.56
10% RSM	107.08^{bc}	230.26	400.38	466.6^{abc}	617.66	640.90
15% RSM	99.68^{c}	239.67	400.77	407.85^{c}	619.35	659.26
5% FRSM	105.16^{bc}	235.62	433.64	515.53^{a}	710.16	625.29
10% FRSM	106.69^{bc}	232.70	400.78	476.38^{ab}	609.08	640.50
15% FRSM	107.63^{bc}	226.34	393.28	428.48^{bc}	582.00	630.62
N	10	10	10	10	10	10
SEM	1.452	1.942	6.631	8.579	14.642	9.735
P-value	0.003	0.208	0.694	0.001	0.370	0.808

^{abc} Means with different superscript in a column differ significantly: P<0.01, P-value: Probability value.
Each pen is a replicate of 5 chicks, N: number of replicates; SEM: Standard Error Mean
RSM: Rapeseed meal included in diets at 5,10 and 15%; FRSM: Fermented rapeseed meal in diets at 5,10 and15%

Table 3. Effect of dietary inclusion of raw and fermented rapeseed meal on weekly feed intake in broilers (0-6 weeks)

Diet	Age (Weeks)					
	1	2	3	4	5	6
Control	100.62^{abc}	288.1	544.72	803.76^{a}	992.76	1064.78^{b}
5% RSM	109.12^{a}	284.84	542.26	800.02^{a}	992.00	1090.72^{ab}
10% RSM	103.70^{ab}	286.24	533.64	815.86^{a}	978.46	1053.90^{b}
15% RSM	93.24^{c}	282.70	529.60	733.62^{b}	928.64	1070.58^{b}
5% FRSM	98.72^{bc}	284.72	530.32	805.04^{a}	988.52	1085.28^{ab}
10% FRSM	102.06^{abc}	287.38	539.50	764.26^{ab}	973.82	1087.00^{ab}
15% FRSM	98.34^{bc}	287.54	541.94	719.02^{b}	985.66	1114.98^{a}
N	10	10	10	10	10	10
SEM	1.288	0.707	3.044	7.801	8.168	5.066
P-Value	0.041	0.381	0.759	0.001	0.378	0.029

^{abc} Means with different superscript in a column differ significantly: P<0.01, P<0.05 P-value: Probability value
Each pen is a replicate of 5 chicks, N: number of replicates; SEM: Standard Error Mean
RSM: Rapeseed meal included in diets at 5,10 and 15%
FRSM: Fermented rapeseed meal in diets at 5,10,15%

Table 4. Effect of dietary inclusion of raw and fermented rapeseed meal on feed conversion ratio (g intake/g weight gain) in broilers (0-6 weeks)

Diet	Age (Weeks)					
	1	2	3	4	5	6
Control	0.889	1.289	1.359	1.578	1.603	1.780
5% RSM	0.908	1.276	1.291	1.697	1.599	1.851
10% RSM	0.969	1.246	1.337	1.771	1.606	1.671
15% RSM	0.936	1.212	1.366	1.900	1.567	1.684
5% FRSM	0.941	1.241	1.294	1.696	1.522	1.843
10% FRSM	0.961	1.238	1.349	1.617	1.604	1.706
15% FRSM	0.916	1.274	1.379	1.691	1.699	1.823
N	10	10	10	10	10	10
SEM	0.009	0.008	0.013	0.401	0.024	0.029
P-Value	0.285	0.151	0.471	0.443	0.636	0.430

P-value: Probability value.

Each pen is a replicate of 5 chicks, N: number of replicates; SEM: Standard Error Mean

RSM: Rapeseed meal included in diets at 5,10 and 15%

FRSM: Fermented rapeseed meal in diets at 5,10,15%

Table 5. Effect of dietary inclusion of raw and fermented rapeseed meal on microbial counts ($\log_{10}$ CFU/g) in caecum of broilers

Diet	Total aerobic bacteria	*Lactobacillus*	*Escherichia coli*
Control	7.131	6.791[b]	6.184
5%RSM	7.128	6.789[b]	6.191
10% RSM	7.129	6.785[bc]	6.193
15% RSM	7.127	6.779[c]	6.187
5% FRSM	7.130	6.816[a]	6.195
10% FRSM	7.131	6.818[a]	6.198
15% FRSM	7.134	6.883[a]	6.201
N	10	10	10
SEM	0.009	0.002	0.003
P-Value	0.89	0.001	0.825

[abc] Means with different superscript in a column differ significantly: P<0.01, P-value: Probability value

Each pen is a replicate of 5 chicks, N: number of replicates; SEM: Standard Error Mean

RSM: Rapeseed meal included in diets at 5,10 and 15%

FRSM: Fermented rapeseed meal in diet at 5,10,15%

Lactobacillus population in gut (Hoeflinger *et al.* 2015), and such fermented feed could act in similar manner as probiotic to improve the gastrointestinal health, decrease pathogenic bacterial load and enhance growth of beneficial bacteria to protect the gut lining (Paton *et al.* 2006). In the present study, supplementation with FRSM at 5%, 10%, or 15% significantly (P<0.05) increased Lactobacilli counts as compared to control and RSM groups (Table 5). The Lactobacilli count in birds fed 5% and 10% FRSM diets was comparable to the control, while the 15% FRSM group had a reduced Lactobacilli count. These findings are consistent with those of Chiang *et al.* (2010), who reported improved Lactobacilli counts in broilers fed 10% FRSM. Elbaz *et al.* (2021, 2023) also found that fermentation increased Lactobacillus populations in the cecum, contributing to improved gut health.

The total aerobic bacteria and *E. coli* counts did not differ significantly (P>0.05) among the groups (Table 5), which was in line with studies by Chiang *et al.* (2010) and Wlazlo *et al.* (2021), who found no significant effects on total aerobic or Enterobacteria counts when fermented RSM was included in poultry diets.

Gut morphology parameters, including villus height (VH), crypt depth (CD), and VH/CD ratio, are key indicators of intestinal health (Ducatelle *et al.* 2018). Significant differences (P<0.05) were observed in duodenal villus height, crypt depth, and VH/CD ratio (Table 6). Villus height was higher (P<0.01) in all RSM/FRSM groups compared to the control, with the highest villus height in birds fed 15% FRSM. The VH/CD ratio was also higher in FRSM-fed birds, especially those fed 15% FRSM, indicating better intestinal health and nutrient absorption. These changes are likely to be have resulted from beneficial effects of *Lactobacillus* and reduction of anti-nutritional factors during fermentation (Caspary *et al.* 1992). The improvement of intestinal morphology might be mostly due to the degradation of large-size proteins and decrease in anti-nutrients in RRSM during the fermentation process (Feng *et al.* 2007; Hong *et al.* 2004). These findings aligned with previous studies by Hu *et al.* (2012) and Drazbo *et al.* (2018), who observed similar improvements in villus height and VH/CD ratios.

In conclusion, supplementation with FRSM enhanced gut health, and supported improved growth performance in broilers, especially at the 5% and 10% inclusion levels.

Table 6. Effect of dietary inclusion of raw and fermented rapeseed meal on duodenal histomorphometry in broilers

Diet	Villi height (VH) (µm)	Crypth depth (CD) (µm)	Villi height: Crypth Depth ratio (VH/CD)
Control	1769.71^{f}	152.23^{d}	10.09^{e}
5% RSM	1820.13^{c}	165.49^{a}	10.99^{d}
10% RSM	1807.12^{d}	154.71^{c}	11.68^{bc}
15% RSM	1794.55^{e}	152.23^{d}	11.78^{bc}
5% FRSM	1936.32^{b}	161.87^{b}	11.96^{ab}
10% FRSM	1944.68^{b}	162.44^{b}	11.97^{ab}
15% FRSM	1956.94^{a}	162.49^{b}	12.06^{a}
N	10	10	10
SEM	9.101	0.88	0.83
P-Value	0.001	0.001	0.001

^{abc} Means with different superscript in a column differ significantly: P<0.01, P-value: Probability value.
Each pen is a replicate of 5 chicks, N: number of replicates; SEM: Standard Error Mean
RSM: Rapeseed meal included in diets at 5, 10 and 15%
FRSM: Fermented rapeseed meal in diet at 5, 10 and 15%

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