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RESEARCH ARTICLE

Protein Source and Diet Structure Influence Growth Performance and Gut Health in Broilers

Abdul Hameed* and M. Ahmad
Poultry Production, Livestock and Dairy Development, Punjab, Pakistan

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***Corresponding Author:**
dr.abdulhameed.106
@gmail.com

ABSTRACT

A study was conducted by using 1-day-old mixed sex (Ross 308) broilers (n=240) to test the hypothesis that a coarsely ground diet increased growth performance of broilers fed on a low digestible protein source. A high digestible soybean meal was gradually replaced by a poor digestible diet based on rapeseed meal (RSM) in three steps (RSM-0%, RSM-50%, and RSM-100%). Two diet structures (fine and coarse), as an extra factor, were investigated in a factorial design with six dietary treatments. The results indicated that an increase in indigestible dietary protein decreased feed intake (FI) (P=0.005), BW gain (P=0.006) and feed conversion ratio (FCR) (P=0.023). This increase in dietary indigestible protein contents by an increase in the indigestible protein level, from RSM-0% to RSM-100%, resulted in decreased (P=0.023) villi heights (1677 vs. 1462 µm); whereas, crypt depths increased (P=0.013; 227vs. 282µm). A coarse diet improved FI (P=0.006), BW gain (P=0.014) and FCR (P=0.009). Birds fed coarsely ground diets had about 12, 21 and 11% lower relative empty weights of the crop, proventriculus and jejunum, respectively. The gizzard weight was approximately 15% improved in coarsely ground diets fed birds compared with those fed the fine diets. Dietary coarseness resulted in approximately16% lower gizzard pH, 21% greater villi heights, 27% lower crypt depths, in comparison with broilers fed fine diets. In conclusion, irrespective of digestibility of the diet, coarse particles feeding improved the performance of broilers.

INTRODUCTION

Depending on the protein source, the percentage of undigested crude protein in different ingredients for poultry diets varies from 8 to 35%. A greater dietary inclusion level of protein sources with a low amino acids digestibility such as rapeseed meal, therefore, may result in a poor performance (Qaisrani et al., 2015). Diet structure plays an important role in controlling the passage rate of the digesta through the gastrointestinal tract (GIT). Dietary ingredients with larger particles are normally ground into smaller particles in the gizzard and then transported to the duodenum (Qaisrani et al., 2015). A coarse diet, remains for a longer time in the gizzard resulting in an enhanced muscular activity and gizzard weight (Jacobs and Parson, 2013). A large muscular gizzard can maximize the grinding capacities of the GIT. A longer retention time in the gizzard leads to more exposure of feed particles to gastric juices that improves the digestion of dietary proteins there by contributing to a better feed efficiency (Liu et al., 2013; Pacheco et al., 2013). Villus height and crypt depth in the small intestine are important indicators of gut development and animal health (Wang and Peng, 2008). Potential beneficial effects of feeding a coarse diet on digestibility of nutrients might be due to its effects on intestinal morphology. There is, however, a scarcity of published data regarding the effects of particle size on intestinal morphology in broilers fed partly ileal indigestible protein. The objective of the current study was to determine the combined effects of diet structure and increased levels of (in) digestible dietary CP on growth performance and intestinal morphology in broilers. It was hypothesized that the effects of a poorly digestible dietary protein source on growth performance and intestinal health may be ameliorated by feeding coarsely ground diet.

MATERIALS AND METHODS

Birds and husbandry

In total, 240 mixed-sex (Ross 308) 1-day-old broilers were individually weighed and randomly assigned to 24 floor pens (10 birds per pen) in a climate-controlled room. Feed and water were available *ad libitum*. Wood shavings were used as a litter material. The lighting schedule was set at 23L:1D for the first three days and thereafter maintained to 16L:8D throughout the experiment. The room temperature was set at 32°C during the first three days and then it was gradually reduced to 22°C in week 4 and maintained until the end of the experiment.

Experimental design and treatments

The study was conducted as a randomized complete block (3×2) orthogonal design. Three dietary treatments were used where a high digestible protein source, soybean meal (SBM) was gradually replaced by a poor digestible protein source, rapeseed meal (RSM); containing diets with a very low amount of indigestible protein (RSM-0%), a medium level of indigestible protein (RSM-50%), and very high level of indigestible protein (RSM-100%). These three diets were each fed in two different diet structures: finely (250 µm) and coarsely (450 µm) ground. All diets were fed to the birds from day one and randomly assigned to the pens in the room. Each treatment had four replicates resulting in 24 experimental units (3×2×4=24 pens). The diets were formulated to meet or exceed the nutrient recommendations for broilers (Ross 308) (Table 1). Diets were formulated to be iso-energetic on an ME basis and to have similar levels of essential amino acids. The coarse diets were obtained by processing of all the maize and wheat using a roller mill. For the fine diets, maize and wheat, as well as SBM and RSM were further processed with a hammer mill. The diets were offered as pellets.

Traits measured

Feed intake (FI) and body weight (BW) gain per pen were recorded at 7, 14, 21, 28, 35 and 42 days of age; whereas, mortality was recorded on daily basis. Feed conversion ratio (FCR) was calculated by dividing total FI by weight gain of live plus dead birds. At the end of the experiment (d 41 and 42), 6 of the 10 birds from each replicate pen were randomly selected and killed by cervical dislocation, thereafter the abdominal cavity was opened. The different parts of the GIT, i.e. the crop, proventriculus, gizzard, duodenum (from pyloric junction to pancreo-billiary duct), jejunum (from pancreo-billiary duct to Meckle's diverticulum), ileum (from Meckle's diverticulum to ileo-cecal junction), cecum (from ostium) and colon were segmented. The digesta contents from each segment were immediately removed by gentle squeezing and the empty segments were weighed. Ceca content of the four birds in a pen were quantitatively pooled and mixed. The pH was recorded using a calibrated pH meter.

Tissue collection and histological measurements

The duodenal samples (approximately 2 cm in length) from the middle of the duodenum were collected and immediately placed in Bouin's fluid after rinsing with cold physiological saline (0.9% saline). The samples, thereafter, were transferred into 70% ethanol within 24 hours, embedded in paraffin and sectioned at 5 µm thickness (Qaisrani et al., 2015). For histological examination, six cross-sections per bird were processed using standard haematoxylin and eosin methods as described by Owusu-Asiedu et al. (2002) for histological examination. Villus height (the distance from the apex of the villus to the junction of the villus and crypt) and crypt depth (the distance from the junction to the basement membrane of the epithelial cells at the bottom of the crypt) were measured on 8 intact, well-oriented villi (from the 2 cm in the middle of the duodenum) per bird using a compound light microscope equipped with a video camera.

Data analysis

The collected data were analyzed with PROC MIXED of SAS (version 9.2; SAS Inst. Inc., Cary, NC). The following statistical model was used:

$Y_{ijkl} = \mu + PS_i + ST_j + PS_i \times ST_j + e_{ijk}$

Differences were considered significant at a probability level of 5%. The means were compared using least squares means comparison, if significances of main effects or their interactions were detected (Qaisrani et al., 2015).

RESULTS

Bird performance

The FI decreased (P=0.005) with increasing amount of RSM in the diet. Coarseness of the diet also influenced (P=0.003) FI with broilers fed the coarse diet having a greater intake than those fed the fine diet (Table 2). Higher RSM inclusions in the diet decreased (P=0.006) BW gain. Body weight gain was also influenced (P=0.010) by diet structure, with broilers fed coarse diets having a greater gain compared to those fed finely ground diets. The increasing level of indigestible protein resulted in a poor (P=0.023) FCR. Coarseness of the diet, however, resulted in an improved (P=0.006) FCR (Table 2).

Digestive tract measurements

The effects of protein source and diet structure on the relative tissue weights of various empty GIT segments are presented in Table 3. Protein source did not affect the relative empty weights of any of the GIT segments. In contrast, diet structure had a significant effect on relative empty weights of the crop, proventriculus, gizzard and jejunum. Coarsely diets ground fed birds had, on average, 11.1, 23.6 and 9.7% lower empty weights of the crop, proventriculus and jejunum, respectively, as compared to the birds fed the fine diets. Empty gizzard weights, however, were 15.0% greater in broilers on the coarse diet compared to those on the fine diet.

Table 1: Dietary ingredients and analyzed and calculated nutrients of the diets1 (g/kg as fed basis)

Item	RSM (%)		
	0	50	100
Maize starch	64.2	53.1	41.9
Maize	300.0	300.0	300.0
Wheat	150.0	150.0	150.0
Fish meal	8.0	26.5	45.0
Soybean meal	350.0	175.0	0.0
Rapeseed meal	0.0	175.0	350.0
Potato protein	5.0	15.0	25.0
Vegetable fat	62.0	66.0	70.0
Diamol (binding material)	30.0	15.0	0.0
Premix[2]	5.0	5.0	5.0
Chalk	10.2	8.1	6.0
Mono-calcium phosphate	8.0	5.5	3.0
Salt	2.5	2.1	1.7
$NaHCO_3$	2.1	1.6	1.2
DL-methionine	1.7	0.8	0.0
L-threonine	0.7	0.4	0.0
L-valine	0.6	0.3	0.0
L-arginine	0.0	0.6	1.2
Calculated composition			
ME (kcal/kg)	2800	2800	2800
CP	206.1	207.8	210.5
NSP[3]	184.9	186.8	188.6
Crude fiber	35.4	42.3	48.2
Digestible Lys	8.6	8.6	8.6
Digestible M+C	6.2	6.2	6.2
Digestible Met	4.6	4.3	3.9
Digestible Thr	6.2	6.2	6.2

Table 2: Effects of protein source (PS) and diet structure (ST) on growth performance of broilers

Main effects	Feed intake (g/bird/d)	Body weight gain (g/bird/d)	FCR[1] (g/g)
RSM[2]-0%	98.4[a]	59.1[a]	1.66[b]
RSM-50%	92.9[b]	53.7[ab]	1.72[ab]
RSM-100%	87.2[c]	49.7[c]	1.75[a]
SEM	1.74	1.33	0.03
Fine	90.4[b]	50.9[b]	1.73[a]
Coarse	95.1[a]	56.4[a]	1.66[b]
SEM	1.11	0.85	0.02
P values			
PS	0.005	0.006	0.023
ST	0.003	0.010	0.006
PS × ST	0.320	0.152	0.235

[1]FCR=feed conversion ratio; [2]RSM=Rapeseed meal

The results of duodenal histology are shown in Table 4. Villus heights, crypt depths and villus height to crypt depth ratio (VCR) were significantly influenced by diet structure and protein source. There was a significant linear decrease in villus heights and increase in crypt depths with the increase in RSM in the diet. This linear decrease in villus height was greater in broilers fed the fine diets relative to those receiving the coarse diets. Similarly, a linear increase in crypt depths was found in broilers fed the fine diets with increasing concentration of dietary RSM. Crypt depths, however, remained unaffected in broilers fed the coarse diets, despite the increasing dietary concentration of RSM, indicating a clear protein source×diet structure interaction (P=0.017; Table 4).Crypt depths were, on average, 27.2% lower in broilers fed the coarse diets compared with those receiving the fine diets. An interaction (P=0.003) was observed for VCR between protein source and diet structure, showing that VCR remained unaffected in broilers fed the coarse diets with an increasing level of RSM. Contrary to this, VCR decreased in broilers fed the fine diets with an increasing level of RSM. Broilers fed the coarse diets had, on average, 17.5 and 33.2% greater villi heights and VCR, respectively, compared to those fed the fine diets.

Gizzard pH

Protein source did not influence (P = 0.702) gizzard pH, whereas it was influenced ($P < 0.001$) by diet structure with broilers fed the coarse diets having a lower pH compared to those fed the fine diets (Table 5).

DISCUSSION

The present study was conducted to investigate the effect of low digestible protein source and particle sizes on growth performance and intestinal morphology in broilers. It was hypothesized that growth performance on a poorly digestible protein source can be improved by feeding a coarsely ground diet instead of a finely ground diet. Indigestible protein increased with increasing levels of RSM. Digestive tract development and intestinal morphology were studied as explanatory variables. Greater villus heights and lower crypt depths were studied as indicators of intestinal health.

The observed reduced FI and BW gain and poor FCR with increasing dietary RSM contents is in accordance with expectations and are in line with the findings of Qaisrani et al. (2014). Poor growth performance of broilers that received high levels of RSM has also been found in some studies (Tripathi and Mishra, 2007; Saleem, 2013). There may be other factors that may have resulted in poor performance of the broilers consuming high levels of RSM. For instance, the presence of anti-nutritional factors such as glucosinolates, tannins, phytic acid and sinapine may also lead to low performance (Khajali and Slominski, 2012). Glucosinolate contents of 8 µmol/g of diet resulted in severe depression of growth in broilers (Tripathi and Mishra, 2007). Presence of glucosinolates and phenolic compounds cause bitter taste and has been reported to reduce FI in broilers (Zeb, 1998). Similarly, tannins (1.9 to 6.2%) and phytic acid may affect protein digestion due to formation of complexes with protein and proteolytic enzymes in the GIT (Khajali and Slominski, 2012). This poor digestion of nutrients, and, as a consequence, poor performance of RSM fed birds may be related to lower villus heights and greater crypt

Table 3: Effects of protein source (PS) and diet structure (ST) on mean relative weights[1] (g/100 g BW) of empty gastrointestinal segments in broilers at 42 d of age

Main effect	Crop	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Ceca	Colon
RSM[2]-0%	0.35	0.62	1.42	1.11	1.46	1.10	0.39	0.17
RSM-50%	0.29	0.59	1.46	1.09	1.51	1.19	0.36	0.16
RSM-100%	0.34	0.60	1.50	1.06	1.53	1.16	0.33	0.20
Pooled SE	0.06	0.04	0.07	0.05	0.08	0.04	0.05	0.04
Structure								
Fine	0.41[a]	0.66[a]	1.34[b]	1.08	1.56[a]	1.21	0.38	0.18
Coarse	0.34[b]	0.53[b]	1.59[a]	1.05	1.42[b]	1.15	0.36	0.17
Pooled SE	0.01	0.02	0.03	0.03	0.03	0.05	0.02	0.01
P-value								
PS	0.205	0.562	0.345	0.757	0.822	0.342	0.435	0.152
ST	0.003	0.004	0.003	0.232	0.004	0.145	0.251	0.667
PS × ST	0.321	0.666	0.088	0.987	0.121	0.845	0.976	0.326

[a-b]Means without a common superscript within a column and main effect significantly ($P< 0.05$) differ; [1]Each value represents the mean of 4 replicates (10 birds per replicate)

Table 4: Effects of protein source (PS) and diet structure (ST) on villus height (µm), crypt depth (µm) and villus height to crypt depth ratio (VCR) in the duodenum of broilers at 42 d of age

Main effects	Villus height	Crypt depth	VCR
RSM[1]-0%	1702[a]	226.7[b]	7.6[a]
RSM-50%	1675[ab]	284.0[a]	6.4[bc]
RSM-100%	1462[b]	277.0[a]	5.9[c]
Pooled SE	43.0	10.7	0.27
Structure			
Fine	1507[b]	283.2[a]	5.36[b]
Coarse	1730[a]	232.7[b]	8.06[a]
Pooled SE	40.0	8.0	0.20
P-value			
PS	0.042	0.021	0.001
ST	< 0.001	< 0.001	< 0.001
PS × ST	0.476	0.017	0.003

[a-c]Means without a common superscript within a column and main effect significantly ($P< 0.05$) differ; [1]RSM = rapeseed meal

Table 5: Effects of protein source (PS) and diet structure (ST) on gizzard and cecal digesta pH in broilers at 42 d of age

Main effects	Gizzard pH	Cecal pH
RSM[1]-0%	3.67	6.28
RSM-50%	3.62	6.27
RSM-100%	3.41	6.14
Pooled SE	0.13	0.10
Structure		
Fine	3.88[a]	6.12
Coarse	3.24[b]	6.27
Pooled SE	0.08	0.06
P-value		
PS	0.602	0.523
ST	0.003	0.205
PS × ST	0.756	0.324

[1]RSM = rapeseed meal

depths. High levels of digestible nutrients in the small intestine were associated with greater villi (Yamauchi, 2007). Performance data also supported the presence of greater villi in broilers consuming low levels of RSM. With high inclusion levels of RSM (> 20%), the extra energy needed for gut wall renewal and for liver metabolic activities can result in reduced performance (Woyengo et al., 2011). Less protein, therefore, will be available for growth.

However, FI, BW gain and FCR were not negatively affected in broilers consuming coarse diets. In contrast, broilers fed coarse diets performed better than those consuming the fine diets as has been reported in other studies for broilers (Jacobs et al., 2010; Pacheco et al., 2013; Liu et al., 2013). The significantly improved FCR in broilers fed the coarse diet may be explained by a better functioning gizzard which optimizes gut motility and especially gastro-duodenal reflux. The lower rate of passage with coarse particles may, furthermore, result in an extended time available for mixing of feed particles with enzymes. As a result optimal digestion and availability of the nutrients to the birds may be reached. Chyme reflux between proventriculus and gizzard may explain the low gizzard pH in broilers fed the coarse diets compared with those on the fine diets. This low pH improved denaturation and hydrolysis of protein by pancreatic enzymes and this may enhance protein digestion (Pacheco et al., 2013).

The significant greater villus height and VCR in broilers fed the coarse diets compared with those fed the fine diets, is in agreement with the findings of Zang et al. (2009). The later authors reported significantly greater villus height (1,451 vs. 1,353 µm) and higher VCR (10.93 vs. 10.53) in coarse and fine diet fed broilers, respectively. The greater villus height, VCR and less deeper crypts in broilers fed the coarse diets may have stimulated nutrient digestion and absorption because of an increased surface area. Deeper crypts, furthermore, are an indication of more cell division at crypts and of increased tissue renewal of the villus resulting in more energy consumption in the gut wall itself.

The higher relative empty weights of the crop and proventriculus in fine diets fed birds may be credited to a greater residence time of the diets with fine particles in these organs. In the coarsely ground diets fed birds, there is a low frequency of over-eating phenomenon (Amerah and Ravindran, 2009) as the gizzard regulates better FI better on a coarse diet than on a fine diet. The improved effects of coarsely ground diets on gut development and relative weight of the gizzard, in the present study, are in line with results of some previous studies (Jacobs et al., 2010; Pacheco et al., 2013). This improved development may be due to the stimulatory properties of bigger particles that lead to the mechanical stimulation of the gizzard and may result in heavier gizzards (Jacobs et al., 2010). The lesser empty weights of the jejunum in the coarsely ground diets fed birds suggest that the chyme in these broilers may require less peristaltic activity from the jejunum (Duke, 1986). The intestines of coarsely ground diets fed birds may, therefore, have a greater effective action of enzymes to the chyme (Gabriel et al., 2003).

The results of the present study show that greater levels of low digestible dietary protein result in a poor growth performance and compromised intestinal morphology. Inclusion of dietary coarse particles improved gut development. Heavy gizzards, enhanced gut morphology, greater BW gain, and improved FCR were found in broilers fed coarse diets. It can be concluded, therefore, that feeding coarse particles improves the growth performance of birds even with a low digestible protein source.

Authors' contributions

AH contributed in conception of design, acquisition of data, analysis and interpretation of data. MA helped in execution of the trial and manuscript writing.

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