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Research Article

Caecal Bacterial Populations and Growth of Broiler Chickens Fed Diets with Different Particle Sizes and Forms

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Abstract

The main objective of the present study was to evaluate the effects of feed particle size, form and also pellet quality on intestinal pH, population of beneficial bacteria and pathogenic bacteria and performance of broiler chickens during growth period. Chicks were fed the same starter ration, but at day 11 of age, chickens were randomly assigned to eight treatments and four replicates and fed dietary treatments until day 28 of age as grower diet. Dietary treatments were as: pellet diets with (1.8 and 2.2 mm) or without dust, graded diets (0.78 and 1.27 mm) and mash diets (2 and 3 mm diameter), poor quality pellet and high quality pellet with die diameter of 1.8 and 2.2 mm. The lowest pH of duodenal and ileal contents was for pellet 2.2 mm and mash 3 mm, respectively (P<0.05). In mash diet, chicks fed the particle size of 3 mm had 7.3% greater the relative weight of gizzard than those fed particle size of 2 mm. Daily feed intake and daily weight gain of chicks were the highest in pellet diets and the lowest in graded diet 1.27 mm. The worse feed conversion ratio was for chicks fed mash 3 mm and graded diet 1.27 mm (P<0.05). There were no differences (P>0.05) among treatments for bacterial populations, except *Escherichia coli* population. The highest *Escherichia coli* population was found in high quality pellet 1.8 mm and poor quality pellet 1.8 mm and lowest one was found in mash 2 and 3 mm (P<0.05). It was concluded that both feed form and particle size may influence on the gizzard weight and the pH of duodenal and cecal contents and *Escherichia coli* population in the cecal of broiler chicks. High quality pellet 2.2 mm resulted in better feed conversion ratio, although the *Escherichia coli* population was the highest in the cecal content of these chickens.

Keywords: *Broiler chicken, Bacterial population, Feed form, Particle size, Performance*

Değişik Boy ve Formda Diet İle Beslenen Broiler Tavuklarda Büyüme ve Sekum Bakteri Popülasyonu

Özet

Bu çalışmanın amacı broiler tavuklarda büyüme periyodu süresince verilen yemin partikül büyüklüğü, formu ve aynı zamanda pelet kalitesinin bağırsak pH'sı, yararlı ve zararlı bakteri popülasyonları ve performans üzerine etkilerini araştırmaktır. Civcivler aynı başlangıç yemi ile beslendikten sonra 11. günde rastgele olarak dörder tekrar olmak üzere sekiz uygulama grubuna ayrıldı ve 28. güne kadar farklı büyüme yemleri ile beslendi. Yem uygulamaları şu şekilde gerçekleştirildi: tozlu veya tozsuz pelet diyet (1.8 ve 2.2 mm), dereceli diyet (0.78 ve 1.27 mm) ve lapa diyet (2 ve 3 mm çapında), 1.8 ve 2.2 mm çapında düşük kaliteli pelet ve yüksek kaliteli pelet. En düşük duodenum ve ileum içeriklerin pH değerleri sırasıyla 2.2 mm pelet ve lapa 3 mm için bulundu (P<0.05). Lapa diyette 3 mm partikül boyutu ile beslenen civcivler partikül büyüklüğü 2 mm olan ile beslenenlerle karşılaştırıldığında %7.3 daha fazla taşlık ağırlığına sahipti. Civcivlerin günlük yem tüketimi ve günlük ağırlık kazanımı en yüksek olarak pelet diyette gözlemlenirken en düşük 1.27 mm dereceli diyette tespit edildi. En kötü yem dönüşüm oranı lapa 3 mm ve 1.27 mm dereceli diyetle beslenenlerde gözlemlendi (P<0.05). *Escherichia coli* hariç bakteriyel popülasyonlar açısından uygulamalar arasında fark bulunmadı (P>0.05). En yüksek *Escherichia coli* popülasyonu yüksek kaliteli 1.8 mm pelet ve düşük kaliteli 1.8 mm pelet ile tespit edilirken en düşük değer lapa 2 ve 3 mm ile bulundu (P<0.05). Yem formu ve partikül boyutunun taşlık ağırlığı, duodenum ve sekum içerikleri ve sekum *Escherichia coli* popülasyonu üzerine etki yapabileceği sonucuna varıldı. Yüksek kaliteli 2.2 mm pellet daha iyi yem dönüşüm oranına neden olurken sekumdaki *Escherichia coli* popülasyonu bu grupta en yüksek olarak tespit edildi.

Anahtar sözcükler: *Broiler tavuk, Bakteriyel popülasyon, Yem formu, Partikül boyutu, Performans*

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INTRODUCTION

Particle size and form of feed and feed form quality with an important role in broiler nutrition has long remained the basic paradigm of feed manufacturers. From a bird's view, a coarse feed particle without dust is instinctive preference. From a feed manufacturing perspective, smaller feed particle size is preference to improve efficiency and uniformity of mixing, reduce ingredient segregation, and improve performance [1,2]. From a nutritional perspective, smaller feed particle size could increase the relative surface area, improve digestibility, and reduce selective feeding by the bird. From an alternative nutritional perspective, larger particle size could promote gizzard development and activity, longer ingredient retention in the gizzard, more uniformity of particles when pass to the small intestine, and consequently improve digestibility [1,3]. There is a discrepancy like particle size for feed form among different perspectives.

During recent two decades, many papers published concerning the effects of feed particle size and form on performance, nutrient availability, gizzard development, gut morphometry, gizzard or stomach Salmonella population in poultry or pigs, but their effects on population of beneficial and common pathogenic bacteria in broilers has been limited studied. It has been demonstrated that pigs fed finely ground compared with those fed coarsely ground feed had a higher incidence of *Salmonella* [4]. Furthermore, pelleted feed increases the risk of *Salmonella* infection in pigs compared with mash feed [5,6]. In poultry, it was demonstrated that enhance in gizzard activity by large particle size could increase digesta retention time [7] and consequently changed the colon pathogenic bacteria [8].

The main objective of the present study was to evaluate the effects of feed particle size, form and also pellet quality on intestinal pH, population of beneficial bacteria (*Bifidobacterium* and *Lactobacillus)* and pathogenic bacteria *(Clostridium perfringens* and *Escherichia coli)* and performance of broiler chickens during growth period.

MATERIAL and METHODS

Chicks used in this study received human care based on criteria outlined in the Guide for the Care and Use of Laboratory Animals [9] published by the National Institutes of Health in the United State, and the experimental protocol was approved by the Research Committee of Islamic Azad University, Science and Research Branch (19.02.2015; 190215).

A total of 384 chicks 1-day-old male broiler chicks (Ross 308) were obtained from a local hatchery. Chicks were divided in floor pens (100 cm × 90 cm) covered with new wood shavings and raised under environmentally controlled conditions and lighting program based on Ross 308 broiler guides. The chicks had *ad libitum* access to feed and water. Feeders were shaken once per day to prevent variation in feed intake. Chicks were individually weighed, and assigned to pens (12 birds per pen) so that the average weight per pen was similar. Broiler diets were formulated and manufactured according to suggested requirements of Ross 308 broilers.

Chicks were fed the same pre-starter and starter rations, but at day 11 of age, chickens were randomly assigned to eight treatments and four replicates and fed dietary treatments until day 28 of age as grower diet period *(Table 1)*. Dietary treatment were as: mash diet with dust screened at 1.8 and 2.2 mm; mash diet without dust screened to have particles with diameter 0.78 and 1.27 mm; poor quality pellet (75% crumbles and 25% dust) with die diameter of 1.8 and 2.2 mm and high quality pellet with die diameter of 1.8 and 2.2 mm. Corn grain and soybean meal were ground with a hammer mill equipped with two screens and blended in mixer to produce the mash diets. For pelleting, grounded mixed particles were conditioned at 85°C for 45 s and pelleted with a ring die pellet set. In the initial and day 28 of the experiment, feed intake and live body weight of chickens were recorded, and then feed conversion ratio was calculated with considering the weight of dead chickens.

At day 25 of age, two chicks per replicate were randomly chosen, weighed, and euthanized by cervical dislocation. Immediately, the gastrointestinal tract was eviscerated and the pH of different parts of small intestine was quickly recorded by inserting the pH meter directly into the digesta. The gizzards were opened, the contents were removed, weighed and expressed as a percentage of body weight. Also, caecal contents of these chicks (two chicks per each replicate) were collected, pooled and a sample was used to assay beneficiary bacteria (lactic acid bacteria and Bifidobacteria) and pathogen bacteria *(E. coli.* and *Clostridium perfringens)*. The populations of bacteria were estimated as the log 10 of colony forming units (CFU) per gram of content.

One gram of each sample was tenfold serially diluted (10^{-1} to 10^{-9}) in 0.9% sterile bacteriological peptone diluents. *Bifidobacteria* and *Lactobacillus* were cultured on MRS agar in anaerobic and aerobic condition, respectively. Anaerobic cultures jar was used for creating anaerobic conditions. *Clostridium perfringens* was cultured on SPS agar in anaerobic cultures jar. *E. coli* and other C*oliforms* were counted by EMB agar (*Eosin methylene blue)* and *Mac Conkey agar.* All commercial media (Merck, Dusseldorf, Germany) were rehydrated in distilled water. The agar media were sterilized by autoclaving at 121°C for 15 min. All the cultured samples were incubated at 37°C after 48 h, the plates containing 25 to 250 colonies were enumerated and recorded as CFU/g of sample. The number of replicate samples was three, and the experimental program was repeated twice.

Table 1. *Ingredient composition and nutritional values of the experimental diets fed during three phases*

Ingredients (%)	Pre-starter (days 1-7 of age)	Starter (days 8-21 of age)	Grower (days 22-28 of age)
Corn	55.20	59.10	62.00
Soybean meal	38.00	34.44	30.884
Soybean oil	2.200	2.350	3.350
Calcitic limestone	0.950	0.900	0.850
Dicalcium phosphate	1.950	1.800	1.670
Salt	0.266	0.250	0.240
Sodium bicarbonate	0.334	0.360	0.355
DL-methionine	0.255	0.175	0.175
L-lysine	0.345	0.225	0.220
Vit and Min Supp.[1]	0.500	0.400	0.400
Total	100	100	100
Chemical composition			
Metabolizable energy (kcal/kg)	2945	3000	3120
Crude protein (%)	22.05	20.75	19.35
Calcium (%)	0.95	0.90	0.87
Available phosphorus (%)	0.47	0.45	0.42
Methionine (%)	0.58	0.48	0.47
Sulfur amino acids (%)	0.93	0.82	0.80
Lysine (%)	1.47	1.28	1.20
Sodium (%)	0.22	0.21	0.20
Chlorine (%)	0.20	0.20	0.19
Linoleic acid (%)	2.45	2.60	3.15
Potassium (%)	0.85	0.81	0.76

[1] Vitamins and minerals supplement (per kg feed): pantothenic acid 12.8 mg, folic acid 1.25 mg, B.H.T 2.5 mg, biotin 0.125 mg, choline chloride 800.0 mg, niacin 38 mg, Vit-A 13.000 IU, Vit-D_3 2.500 IU, Vit-E 30.0 mg, Vit-K 2.5 mg, Vit-B_1 2.5 mg, Vit-B_{12} 25mg, Vit-B_2 5.5mg, Vit-B_6 5.0 mg, Copper 12.5 mg, Iron 62.65 mg, Iodin 0.025 mg, Manganese 68.0 mg, Selenium 0.23 mg , Zinc 69.0 mg

At first, the normality of data distribution was checked using the Kolmogorov-Smirnov test. The Statistical analysis was done with ANOVA of SAS using GLM procedure for Windows version 14.1 [10]. Means were separated by Tukey test at P≤0.05.

RESULTS

As shown in *Table 2*, significant differences exist among treatments for pH of duodenum and cecum contents, but there was no significant effects of feed forms or feed particle size on pH of jejunum and ileum contents. The lowest pH of duodenum and ileum contents was for high quality pellet 2.2 mm and mash 3 mm, respectively.

The effects of dietary treatments on the relative weight of gizzard and performance of broilers on day 28 of age are presented in *Table 3*. The relative weight of gizzard was influenced by the form of diet. Chicks in mash 3 mm had higher gizzard weight than other treatments. Chickens fed pellet had the lowest gizzard weight. Chicks fed graded

Table 2. *The effect of mash form and particle size on the pH of different section of broilers intestine*

Treatments	Duodenum	Jejunum	Ileum	Cecum
Mash 2 mm	6.12^{a}	6.59	7.11	5.97^{ab}
Mash 3 mm	6.13^{a}	6.52	7.09	5.93^{b}
Good quality pellet 1.8 mm	5.55^{bc}	6.13	6.75	6.18^{a}
Good quality Pellet 2.2 mm	5.41^{c}	6.10	6.72	6.20^{a}
Poor quality Pellet 1.8 mm	6.03^{ab}	6.31	6.93	6.10^{ab}
Poor quality pellet 2.2 mm	6.12^{a}	6.28	6.75	6.17^{ab}
Graded 0.78 mm	6.14^{a}	6.75	7.10	6.08^{ab}
Graded 1.27 mm	6.17^{a}	6.54	7.15	6.15^{ab}
SEM	0.112	0.147	0.124	0.048
P value	0.005	0.0605	0.0876	0.006

Means within a column with different superscripts are significantly different (P<0.05)

diet had higher gizzard weight than poor quality pellet, but lower than those fed mash diet. Regardless of diet form, the gizzard weight of chicks fed different particle size was not significant, but significant difference appeared between mash 1.8 and 2.2 mm. In mash diet, chicks fed the particle size of 3 mm had 12.5% greater relative weight of gizzard than those fed particle size of 2 mm. Daily feed intake and daily weight gain of chicks were the highest in pellet diets and the lowest in graded diet 1.27 mm. The worse feed conversion ratio was for chicks fed mash 3 mm and graded diet 1.27 mm.

The effects of dietary treatments on bacterial populations on day 25 of age are presented in *Table 4*. There were no significant differences among treatments for bacterial populations, except *Escherichia coli* population. The highest *Escherichia coli* population was found in high quality pellet 1.8 mm and poor quality pellet 1.8 mm and lowest one was found in mash diets 2 and 3 mm. Both mash diets have the highest counts of Lactobacillus and Bifidobacterium.

DISCUSSION

In this study, pH of duodenum was the highest in chicks fed mash and graded diets and the lowest in good quality pellet 2.2 mm. As stated by Huang et al.[11] mash diets are retained for longer period in the gizzard of broilers as compared with pelleted diet. Hence, mash and graded diets require further grinding and gizzard muscular activity. The higher activity of gizzard stimulates the proventriculus and increases in the secretion of hydrochloric acid. Thus, the pH of the duodenum in broilers fed mash, graded and poor quality pellet diets is lower compared with those fed pelleted diets as this result is in agreement with the finding of Engberg et al.[12]. Our finding is in contrast to the report of Dahlke et al.[13] who concluded that proventriculus pH and intestinal pH were not affected by different particle sizes.

In the jejunum and ileum of chicks, pancreatic and bile secretion influence on pH of digesta and no difference observed among treatments. The bacterial activity could

Table 3. *The effect of mash form and particle size on the relative weight of gizzard and performance parameters of broilers during grower period*

Treatments	Relative Gizzard Weight (g)	Body Weight Gain (g/day)	Feed Intake (g/day)	Feed Conversion Ratio
Mash 2 mm	1.89^{b}	31.6^{bcd}	41.5^{ab}	1.313^{ab}
Mash 3 mm	2.04^{a}	29.3^{d}	38.7^{bc}	1.320^{a}
Good quality pellet 1.8 mm	1.19^{d}	36.1^{a}	43.1^{a}	1.192^{cd}
Good quality Pellet 2.2 mm	1.22^{d}	36.0^{a}	42.7^{a}	1.187^{d}
Poor quality Pellet 1.8 mm	1.36^{c}	33.0^{b}	41.4^{ab}	1.256^{abcd}
Poor quality pellet 2.2 mm	1.42^{c}	32.7^{bc}	41.5^{ab}	1.269^{abc}
Graded 0.78 mm	1.77^{b}	30.0^{cd}	37.0^{cd}	1.235^{abc}
Graded 1.27 mm	1.82^{b}	26.1^{e}	34.5^{d}	1.323^{a}
SEM	0.028	0.93	0.83	0.024
P value	0.001	0.006	0.005	0.008

Means within a column with different superscripts are significantly different (P<0.05)

Table 4. *The effect of mash form and particle size on the bacteria population (log_{10} cfu/g) in the cecum of broilers during grower period*

Parameter	Lactobacilus	Bifidobacterium	Colostridium	*Escherichia coli*	Coliforms
Mash 2 mm	9.176	8.824	3.853	8.068^{c}	8.927
Mash 3 mm	9.246	8.728	2.606	8.281^{bc}	8.629
Good quality pellet 1.8 mm	8.920	8.042	2.201	10.091^{a}	9.493
Good quality Pellet 2.2 mm	8.795	7.938	2.634	9.704^{a}	9.215
Poor quality Pellet 1.8 mm	9.182	8.188	3.460	10.108^{a}	9.303
Poor quality pellet 2.2 mm	9.188	8.033	2.068	9.553^{ab}	9.508
Graded 0.78 mm	9.477	8.367	2.514	8.904^{abc}	8.609
Graded 1.27 mm	9.437	8.289	1.515	9.283^{abc}	9.545
SEM	0.0655	0.0858	0.3059	0.1701	0.1163
P value	0.081	0.116	0.195	0.008	0.109

Means within a column with different superscripts are significantly different (P<0.05)

change the pH of the cecum content, as in *Table 1* was seen, mash diet with coarse particle size caused a decrease and good quality pellet diet caused an increase in the pH of cecum contents. The availability of nutrient from mash diet in the cecum resulted in a non-significant increase in the lactobacillus population and consequently declined the pH of content. In the present study, the form and particle size of diet influenced on the gizzard weight. Chicks fed good and poor quality pellet diets had lower gizzard weight as compared with mash and graded diets. In line with our finding, several authors [12,14] reported lower gizzard weights in broiler chickens fed the pelleted diet. Lower gizzard weight was also attributed to low coarse particles in pelleted diets [3]. Regardless the diet form, gizzard weight numerically increased as particle size increased in the present study. Mash diet with coarse particle size needs higher muscular activity for grinding, and more activity causes an increase in the weight and size of gizzard. Of course, dietary form had higher impact than dietary particle size on the relative weight of gizzard. Our result is in agreement with finding of Nir et al.[14] who reported that broilers were fed diet with particles in coarse and medium sizes, the gizzard weight increased as compared with the chicks fed fine particles. The gizzard has an important and unique role in the gastrointestinal tract of broilers, as it acts a mechanical grinder for reduction of dietary particle size [15] which effectively resulted in gastrointestinal motility [16]. The gizzard weight has a positive broiler growth response, as Parsons et al.[17] reported an increase in mash feed particle size and coarser diet, chicks had the largest gizzard weight and the highest weight gain. In this study, differences for gizzard weight among particle size in each feed form was not significant, but Amereh et al.[1] reported significant differences. In the study of Parsons et al.[17], the chickens fed the coarse corn mash diets had greater absolute and relative gizzard weights as compared with the chickens fed the fine corn mash diets.

One of strategy for development of gizzard is manipulation of diet as mentioned previously by Nir et al.[14] and Engberg et al.[8] Graded feed in this study was a mash diet with the uniform particle size and compare with pellet diets resulted in greater gizzard weight, but its effect was lower than mash diet. The existence of large uniform particles in graded diet resulted in longer retention of feed in the gizzard [7], the development and activity and consequently the weight of gizzard [1,3]. As seen in *Table 2* and *Table 4*, a higher relative weight of gizzard resulted in a decrease in the population of *E. coli* that support the finding of Bjerrum et al.[18], thus may be reduce the risk of enteric diseases as speculated by Engberg et al.[8] and Bjerrum et al.[18].

Broilers fed pellet diet had the highest feed intake and body weight gain but had the lower feed conversion ratio. The result of this study is inconsistent with finding of Amerah et al.[1] who reported birds fed pellet diets showed lower feed conversion ratio than those fed mash diets. In contrast to feed intake of chicks in this study, Parsons et al.[17] reported no difference in feed intake when birds were fed mash versus pellet diets. In agreement with our study, Lemme et al.[19] indicated that pellet diets of good quality had the highest weight gain than poor-quality pellet feed. In the present study, chickens fed mash diet had lower feed intake and feed conversion ratio. The differences may be attributed to the particle size in mash and pellet diets, the passage rate of digesta and the particles distribution. Amerah et al.[1] demonstrated that large particles in mash diets promoted broiler performance and pelleted diets had controversial results on performance.

An increase in the beneficial bacteria population and a decrease in the pathogenic bacteria population or both is a favorite strategy for broiler industry. In this study, the dietary form had higher impact on the bacterial population than particle size. Diets with larger particle sizes have an indirect effect on reducing enteric disease problems in broilers. These diets may alter the microbial populations of the gastrointestinal tract. The gizzard development stimulated through increase in grinding activity, which leads to an improvement in gut motility [20]. Gizzard activity also increases the secretion of hydrochloric acid, which ultimately reduces the pH of intestinal contents. This event, in combination with the increased retention time of feed in the gizzard, has an antimicrobial effect. When pathogenic bacteria such as *Escherichia coli* entered to the gastrointestinal tract, they inactivated by the highly acidic media of intestine [14,20]. Therefore, the gizzard acts as a barrier in reducing the entrance of pathogenic bacteria to the distal intestinal tract [8].

In this study, lactobacilli populations were numerically higher in mash diets than pellet diets. The finding of this study was inconsistent with the report of Engberg et al.[12]. An increase in lactobacilli populations is usually considered to be beneficial to the host, because they can prevent colonization of diarrhea-causing pathogens such as *E. coli* [8,21,22]. Engberg et al.[8] compared coarse or finely ground mash or pelleted feed and reported that there was an increase in lactobacilli populations in the ceca and rectum when birds were given coarse mash diets, with the lowest counts of lactic acid bacteria in birds given finely ground pellets. There was no additional study concerning the effect of feed form and particle size on bacterial population in the intestine of broiler chickens.

Both feed form and particle size influence on *Escherichia coli* population in the cecal of broiler chicks. *Escherichia coli* population was the highest in the cecal content of chickens fed high quality pellet diets. This finding is in agreement with study of Engberg et al.[8], who observed that broilers fed pelleted diets presented higher coliform and enterococci counts in the ileum and lower lactobacilli and *Clostridium perfringens* numbers in the cecca and rectum compared with mash diet.

The findings of this study suggest that both feed form and particle size may influence on the gizzard weight and the pH of duodenal and cecal contents and *Escherichia coli* population in the cecal of broiler chicks. Based on the results of this study, high quality pellet resulted in better feed conversion ratio, although the *Escherichia coli* population was the highest in the cecal content of chickens. The use of good quality pellet with fine particles could significantly improve the broiler performance and health.

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