

Influence of pelleted diets and coarse ground mash diets on broilers gut flora

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The gut microflora is of major importance for animal performance and health. Molecular techniques create the possibility to obtain detailed knowledge of the composition and dynamics of this flora. An experiment was performed to assess the effects of pelleted versus coarse mash diets on performance and the composition of the intestinal microflora of broilers. Birds were housed in battery cages and allocated to two dietary treatments. Both treatments were assigned to 6 cages with 20 birds per cage. The experiment was divided into a starter (0-8 d) and grower-finisher phase (9-35 d). All birds were ad libitum fed a pelleted starter diet (2.5 mm) followed by one of the two treatments: a coarse mash or a pelleted (3.0 mm) grower-finisher diet. At 35 days of age, from 5 birds per cage, samples of the content of the crop, proximal ileum, distal ileum and the caeca were taken. The bacterial compositions of the samples were determined using the MCPC method (modification of the T-RFLP method, www.mcpcgenetics.com). FCR was significantly improved in birds fed the mash diet (1.588) compared with the pelleted diet (1.639). However, weight gain was numerically higher for the pelleted diet (2118 g) compared with the mash diet (2063 g). In the crop the most dominantly present peak in the mash diet (28% relative abundance) was decreased ($P<0.05$) with the pelleted diet (12%), whereas in the proximal ileum the opposite effect was found. In the distal ileum and the caeca also shifts ($P<0.05$) between mash and pelleted diets were found in dominant bacterial peaks. Based on available sequence libraries the bacterial shifts in the crop and proximal ileum most probably represent lactobacillus bacteria and in the distal ileum and caeca represent groups of uncultured bacteria. Especially in the proximal ileum, mash diets appear to result in a more balanced bacterial profile (less dominant bacterial groups), which might be one of the factors why birds fed “coarse” mash diets are frequently reported to show less health problems compared to birds fed pelleted diets.

Keywords: Broilers; gut; microflora; mash; pellets; MCPC

Introduction

The gut microflora is of major importance for broiler performance and health. Establishing a microflora that supports both a high level of broiler performance and that causes no health problems is therefore of considerable financial importance for the broiler industry.

Especially feed composition and processing is known to influence the bacterial profile in the gut (Apajalahti et al., 2001; Hume et al., 2003). It has been shown that coarse particles can improve the health status of broilers (Cumming, 1992 and Langhout, 2000). It can be hypothesised that this effect on health status is related to the changes in the bacterial profile that occur due to differences in particle size of the diet. Coarse particles affect crop and gizzard function. This may direct or indirect have an effect on the development of the microflora in the crop and intestines.

The development of molecular techniques has opened new possibilities to study microbial communities because it has overcome the disadvantages of the laborious classical techniques that are not able to grow 99% of the existing bacteria (Apajalahti et al., 2004). Molecular techniques create the possibility to obtain detailed knowledge of the composition and dynamics of the flora. An experiment

was performed to assess the effects of pelleted versus coarse and medium coarse mash diets on performance and the composition of the gut microflora of broilers.

Materials and Methods

Birds were housed in 18 battery cages and had free access to feed and fresh drinking water. Temperature and ventilation were computer controlled. Temperature decreased by 2.5°C a week, from 34°C on the day of arrival (1-d-old chicks) to a final temperature of 21.5°C at 35 d of age. Lights were on 23 h/d. The birds were spray-vaccinated against Newcastle disease directly after hatching and at 21 d of age.

Two-hundred-forty 1-d-old male and 240 1-d-old female chicks of a commercial broiler strain (Ross 308) were purchased and weighed individually. Birds weighing between 39 and 49 g were randomly assigned to a pen and birds outside this weight range were not used in the experiment. The experiment commenced with 20 birds per pen with similar average initial weights of 44 g/chick. The male and female birds were housed in separate rooms. The effects of three different feed forms on broiler performance and gut microflora were compared. The experiment was divided in a starter (0 to 8 d of age), grower (9 to 28 d of age) and a finisher phase (29 to 35 d of age). In the starter phase all birds received the same, pelleted (2.0 mm), starter diet. In the grower and finisher phase the birds were either fed pelleted diets (3.0 mm), coarse mash or medium coarse mash diets. Cages were assigned to the treatments according to a randomised block design (block = sex), with 3 pens with male and 3 pens with female broilers per treatment.

Table 1 Ingredient and nutritional composition of the starter, grower and finisher diets.

Diet	Starter	Grower	Finisher	Diet	Starter	Grower	Finisher
<i>Ingredient composition (g/kg)</i>				<i>Nutritional composition (g/kg)</i>			
Wheat	170.8	337.7	337.6	Crude protein	213	207	205
Maize	398.8	259.0	265.5	Crude fat	77	88	89
Wheat middlings	23.2	-	-	Ash	63	55	54
Soybean meal	261.6	282.7	288.5	AME _{N(Broiler)} kcal/kg	2850	3000	3000
Sunflower meal	23.3	-	-	AFD ² Lys	10.5	10.2	9.9
Fish meal	20.0	10.0	-	AFD Met	5.1	4.9	4.6
Animal fat	-	32.3	33.0	AFD Met + Cys	7.9	7.7	7.4
Soybean oil	48.1	32.3	33.0	AFD Thr	6.6	6.4	6.2
Monocalcium phosphate	12.7	11.4	11.6	AFD Trp	2.1	2.1	2.1
Potato protein	6.0	4.0	-	AFD Ile	7.6	7.5	7.4
Ground limestone	17.0	12.5	12.2	AFD Val	8.8	8.5	8.3
Sodium bicarbonate	2.95	2.60	2.74	AFD Arg	12.3	11.9	11.8
l-Lysine-HCL (80%)	1.74	1.57	1.64	Calcium	10.3	8.0	7.6
dl-Methionine	2.17	2.27	2.05	Phosphorus	7.3	6.6	6.4
l-Threonine	0.14	0.21	0.18	Digestible Phosphorus	3.8	3.4	3.3
Salt, iodised	1.30	1.32	1.55	Potassium	8.9	8.8	8.9
NSP-enzyme	-	0.12	0.12	Sodium	1.7	1.5	1.5
Vitamin-mineral premix ¹	10.0	10.0	10.0	Chloride	1.8	1.6	1.6

¹Contributed per kg diet: riboflavin 5 mg, niacinamide 40 mg, D-pantothenic acid 12 mg, choline chloride 500 mg, cyanocobalamin 15 µg, DL- α -tocopheryl acetate 15 mg, menadione 5 mg, retinyl-acetate 3.44 mg, cholecalciferol 50 µg, biotin 0.1 mg, folic acid 0.75 mg, FeSO₄·H₂O 200 mg, MnO₂ 100 mg, CuSO₄·5H₂O 60 mg, ZnSO₄·H₂O 150 mg, Se (organic) 0.15 mg, KI 1 mg, CoSO₄·7H₂O 1 mg, antioxidant (ethoxyquin), 100 mg.

²AFD = apparent fecal digestible.

The compositions and calculated analyses of the diets are given in Table 1. The wheat and maize used in the diets were coarsely ground, on a roller mill with 1.5 and 2.5 mm spacing between the rollers for wheat and maize, respectively. Roll one and two were operated at 770 and 900 rpm respectively, and the diameter of the rolls was 160mm. For the fine ground diets the roller-milled grains were subsequently ground using a hammer mill with a 5.0 mm screen, operated at 3000 rpm. The pelleted diets were pelleted without steam addition. All diets were analysed for N (Dumas), crude fat (6 h extraction with petroleum-ether), dry matter, ash (incineration at 550°C for 4 h), Ca (ISO

6490.2), and P (ISO 6491) content. Sieve analyses were performed on all mash diets and on the medium and coarse ground wheat and maize (Table 2). The diets were calculated to be adequate in all nutrients.

Table 2 Sieve analyses of the mash diets and of the wheat and maize.

Phase	Grinding size	Sieve size, mm					
		> 2.8 mm	> 2.5 – < 2.8 mm	> 1.4 – < 2.5 mm	> 0.8 – < 1.4 mm	> 0.5 – < 0.8 mm	< 0.5 mm
Grower	Fine	0.0%	2.7%	24.6%	37.3%	10.0%	25.5%
	Coarse	18.0%	8.0%	31.0%	27.0%	9.0%	7.0%
Finisher	Fine	1.3%	3.1%	26.3%	40.6%	12.5%	16.1%
	Coarse	16.3%	8.2%	28.2%	29.9%	10.3%	6.5%
Wheat coarse ground		20.0%	9.7%	34.9%	14.3%	4.6%	16.6%
Wheat fine ground		1.5%	2.6%	41.2%	28.9%	10.8%	14.9%
Maize coarse ground		20.5%	8.2%	32.9%	19.2%	5.5%	13.7%
Maize fine ground		2.2%	2.2%	26.7%	36.6%	11.6%	20.7%

For each cage all birds were weighed together at the start (d 0) and were weighed individually at 8, 28 and 35 d of age and weight gain for both phases was calculated. In addition, feed consumption for each pen was recorded during each phase. FCR was calculated as g feed consumed per g live weight gain. At 35 d of age, 5 birds per pen (ad random chosen) were dissected. The birds were successively weighed, killed by cervical dislocation (method approved by the Ethical Committee of the Erasmus University, Rotterdam, The Netherlands), dissected and swab-samples were taken of the crop, duodenum, proximal and distal ileum and caeca. Immediately after collection the swabs were stored in chilled tubes and subsequently, within 3 h after collection until MCPC-analyses, stored at –20°C.

MCPC analysis (Panneman, 2001) is based on 16S rRNA T-RFLP (Liu et al., 1997). The 16S rRNA genes of the microorganisms present in the sample were amplified by PCR using blue fluorescently labelled primer 8f (5'-AGA GTT TGA TCC TGG CTC AG-3') and yellow fluorescently labelled primer 926r (5'-ACC GCT TGT GCG GGC CC-3') and subsequently digested with restriction endonucleases MspI and HinpII (both from New England Biolabs, Ipswich, MA, USA). The MCPC-database was built using data that were extracted from prokaryote sections of the of the EMBL sequence database, release 82, March 2005, using the Patscan program (Dsouza et al., 1997). For each of the extracted sequences the T-RF size using the aforementioned combination of primers and restriction enzymes was calculated for both primers. To correct for variations in the amount of sample-material the relative peak intensity of each T-RF (referred to as relative abundance) was calculated by dividing the peak height of each specific peak in a sample by the total area of all the peaks in the electropherogram of that sample.

All data were subjected to analysis of variance using the 8th edition of Genstat for Windows (Payne et al., 2005). Cages were treated as experimental units and differences were considered significant for $P < 0.05$.

Results and discussion

FCR was significantly improved in birds fed the coarse and medium mash diet compared with the pelleted diets (Table 3). However, weight gain was numerically higher for the pelleted diet (2118 g) compared with the coarse mash diet (2063 g) and significantly higher compared with the medium mash diet (2017 g).

Bacterial peak 570-572 (Figure 1) was most dominantly present (28% relative abundance) in the crop of birds fed the medium or coarse mash diets. With the pelleted diet the relative abundance of this peak was decreased ($P < 0.05$) to 12%. A similar effect was found for peaks 580-582 and 189, whereas peak 136 showed the opposite effect. However, the relative abundance of those peaks did not exceed the 5% in neither of the treatments.

In contrast with the crop the dietary treatments hardly affected the relative abundance of peak 570-572 in the proximal ileum (Figure 2). However, peak 392-393 was significantly increased in the

pelleted diets (28% relative abundance) compared with the medium and coarse mash diets (13%). For peak 186 and 188 the opposite effects were found.

Table 3. The effect of feed form on technical performance.

Grinding Grower/Finisher feed form	Medium Mash	Coarse Mash	Medium Pellet	<i>LSD</i>
No. of replicates	6	6	6	
Gain, g (d 0-8)	177	182	179	12
Gain, g (d 0-28)	1407 ^b	1428 ^b	1499 ^a	48
Gain, g (d 0-35)	2017 ^b	2063 ^{ab}	2118 ^a	58
Feed intake, g (d 0-8)	202	206	201	12
Feed intake, g (d 0-28)	2060 ^b	2107 ^b	2260 ^a	75
Feed intake, g (d 0-35)	3174 ^b	3268 ^b	3463 ^a	104
FCR (d 0-8)	1.142	1.139	1.122	0.049
FCR (d 0-28)	1.466 ^b	1.478 ^b	1.508 ^a	0.021
FCR (d 0-35)	1.577 ^b	1.588 ^b	1.639 ^a	0.023

(a, b) Means within a row with different superscripts differ significantly.

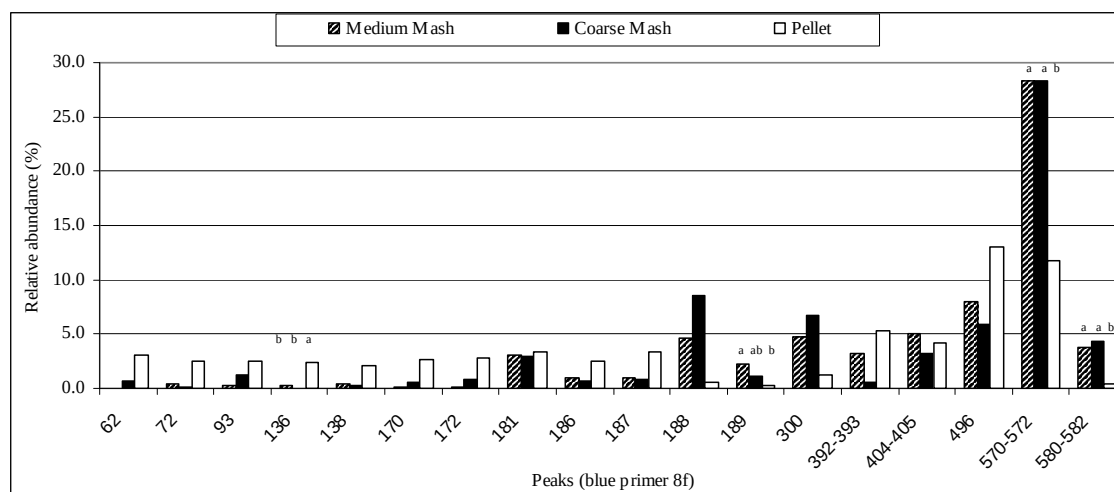


Figure 1 The effect of feed form on bacterial peaks (primer 8f) in the crop at 35 d of age.

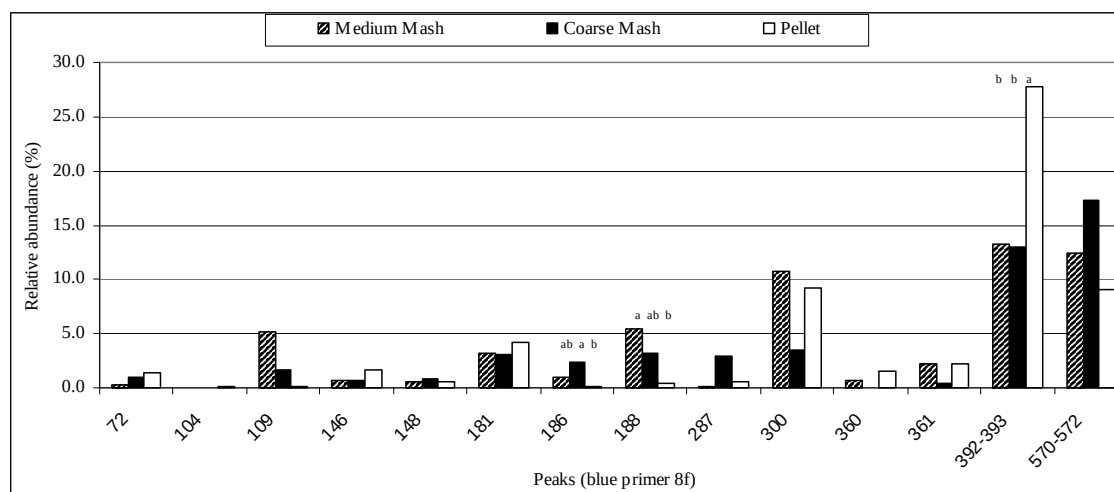


Figure 2 The effect of feed form on bacterial peaks (primer 8f) in the proximal ileum at 35 d of age.

In the caeca (Figure 4) also shifts ($P<0.05$) in bacterial peaks between mash and pelleted diets were found in dominant bacterial peaks. For most peaks similar effects were found with medium and coarse mash diets.

In the distal ileum (Figure 3) some peaks showed similar effects as in the proximal ileum (peak 186) and other peaks (96 and 285) showed similar effects as in the caeca.

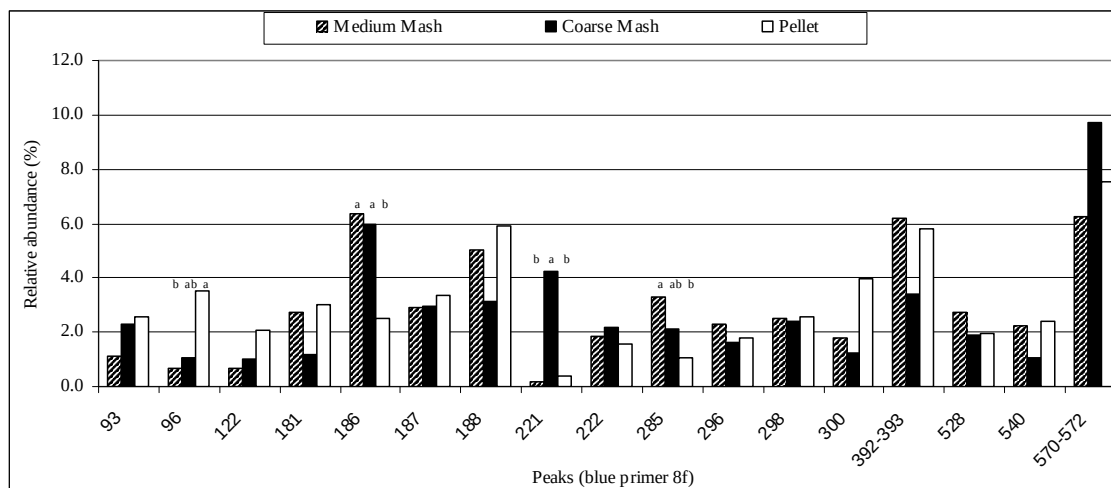


Figure 3 The effect of feed form on bacterial peaks (primer 8f) in the distal ileum at 35 d of age.

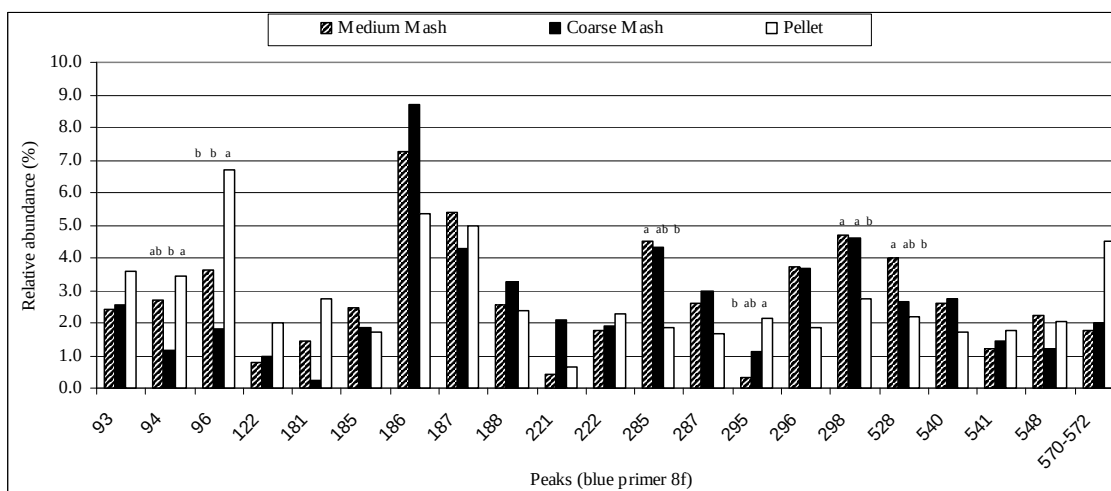


Figure 4 The effect of feed form on bacterial peaks (primer 8f) in the caeca at 35 d of age.

Based on available sequence libraries the bacterial shifts in the crop and proximal ileum most probably represent lactobacillus bacteria and in the caeca represent groups of uncultured bacteria. Especially in the proximal ileum, mash diets appear to result in a more balanced bacterial profile (less dominant bacterial groups), which might be one of the factors why birds fed “coarse” mash diets are frequently reported to show less health problems compared to birds fed pelleted diets.

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