CHAPTER 5
A FURTHER EXAMINATION OF THE GROWTH AND GUT RESPONSE OF BROILER CHICKENS TO MANNANOLIGOSACCHARIDE

5.1 INTRODUCTION

Net energy (NE) is that part of the dietary energy which is available to the animal for maintenance and production. In comparison to metabolizable energy (ME), NE is a more precise energy system that can accurately reflect the bird's response to the diet. Feed formulated on the basis of NE, as opposed to that formulated on AME, resulted in savings of over 80 g feed per kg liveweight over a rearing period of 35 d in broiler chickens (Choct, 2005).

Gut microflora can modify energy metabolism by exerting a buffering or a counter-productive action on the energy utilization of the chicken (Muramatsu *et al.*, 1994). It is generally agreed that the presence of gut bacteria increases the maintenance energy requirement of chickens (Lan *et al.*, 2005). For example, by attaching to the gut wall, bacteria, especially pathogens, can stimulate the renewal rate of the epithelial lining and thus increase the percentage of the dietary energy spent on gut maintenance.

The initial examination of the effects of MOS on the development of gut microflora showed that the luminal coliform populations of birds were increased by the addition of MOS in early life (Chapter 4). In the GIT, mucosa-associated bacteria could be more important to intestinal and host health (Gaskins, 1998) because they are in close contact with epithelial lining and can largely affect the integrity and function of gut mucosa. In an *in vitro* model, MOS was shown to inhibit enteropathogenic *E. coli* from attaching to the gut mucosa of chickens; a displacement of attached *E. coli* from the mucus was noticed at the same time with a high dose level (2g/kg) of MOS (Peuranen *et al.*, 2006). However, the *in vivo* effects of MOS on the populations of mucosa-associated bacteria are unknown.

The current experiment was designed to examine the effects of MOS on the NE value of a sorghum-wheat based diet and the composition of selected groups of mucosa-associated bacteria, to further elucidate the role of MOS in the growth, energy utilization and gut development of birds.

5.2 MATERIALS AND METHODS

5.2.1 Experimental design and diets

The dietary treatments comprised two MOS levels (1g MOS/kg feed, low MOS treatment; 2 g MOS/kg feed, high MOS treatment), a negative (NC) and a positive control (ZnB as described in Chapter 3). The basal diet composition is shown in Table 5.1.

As only eight calorimetric chambers were available for one NE run, in order to accommodate at least 7 replicates to each treatment, five consecutive NE runs with two replicates per treatment in each run were conducted over a three-month period.

5.2.2 Birds, housing and management

Altogether, five batches of birds were used for the experiment. The birds were obtained from a local hatchery (Baiada hatchery, Kootingal, NSW) with an interval of two weeks per batch so as to ensure that all the birds assigned to the NE run were at 18 days of age. Only the first batch of birds was used for the assessment of growth performance and gut response.

Three hundred and eighty-four (384) day-old male Cobb broilers (41.4g±4.55) were picked up in the first batch and they were randomly allocated to 12 replicates (cages) per treatment, with 8 birds per replicate. Feed intake and BW were measured at the end of weeks 3 and 5. On d 14, two birds per cage, 8 cages per treatment, were randomly selected for the evaluation of gut development. On d 18, four birds of similar body weight were selected from each treatment and transferred to the closed calorimetric chambers for the determination of NE. Birds were given 3 days to get used to the chambers; therefore, NE determiantio started when birds at 21 days of age.

Thirty-two (32) day-old birds were picked up in the subsequent four batches to continue the NE runs. They were randomly divided into four groups (cages) of 8 birds each and fed with the respective experimental diets until d 18 as the adaptive regime before transferring to the chambers for NE assessment. Nutrient digestibility was determined by total collection of excreta.

All other management was similar to that described in Section 3.2.2. The experiment was approved by the Animal Ethics Committee of the University of New England with approval number of AEC05/049.

Table 5.1 Composition (g/kg) of starter and grower basal diets

Ingredients and composition	Starter diet	Grower diet
Wheat	150.0	150.0
Barley	100.0	150.0
Sorghum	296.4	250.7
Millrun	30.0	50.0
Soy	223.3	213.0
Canola	50.0	50.0
Lupin	50.0	70.0
Meat meal	50.0	0.0
Limestone	9.8	15.7
Dicalcium phosphate	1.6	12.0
Lysine-HCl	1.6	1.0
Methionine	1.8	1.5
Salt	3.5	4.2
Tallow	30.0	30.0
Vitamin and mineral Premix[1]	2.0	2.0
Calculated chemical composition		
ME, MJ/Kg	11.91	11.57
Crude protein	230.00	210.00
Crude fibre	40.80	44.00
Crude fat	57.30	52.10
Lys	12.50	11.00
Met+Cys	9.00	8.30
Ca	10.00	10.00
P available	4.00	3.70
Na	2.00	2.00
Cl	3.30	3.30

[1] The same as described in Chapter 3.

5.2.3 Net energy evaluation

Principles and facilities

The evaluation of feed energy content is usually based on their digestible (DE) or metabolizable (ME) energy value (Noblet *et al.*, 1994). However, metabolic utilization of ME or heat increment (HI) vanes according to diet chemical characteristics and type of production (maintenance, growth, milk secretion, protein, and fat deposition, etc.). Consequently, net energy (NE) prediction equations (i.e., energy systems) have been proposed to precisely measure the energy value of feeds.

The NE value of a food is the energy which is available to the animal for body maintenance and for meat or egg production, as shown in Figure 5.1. The subtraction of the heat increment (HI) of a feedd from its ME gives the NE value of the feed.

Therefore, $NE = ME - HI$,

where NE is net energy, ME is metabolizable energy and HI is heat increment.

The HI is the increase in heat production following consumption of feed when the animal is in a thermoneutral environment. Total heat production (THP) includes two parts: HI and heat production for maintenance (HPm), which will leave the animal as heat.

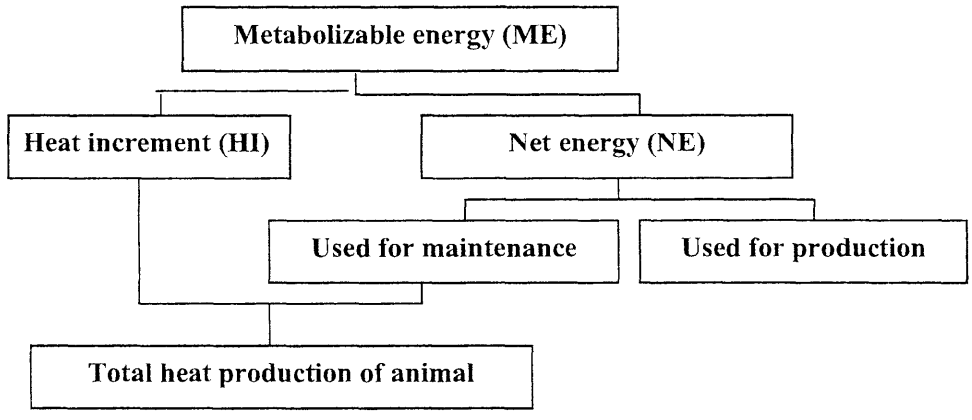

Figure 5.1 The partition of metabolizable energy in the animal.

Closed circuit respiration calorimeters are a way of indirectly measuring the heat production of the subject. By measuring accurately the CO_2 produced and O_2 used by the subject, the heat produced can be calculated. A schematic diagram of the closed-circuit system at UNE is shown in Appendix 2.

Total Heat production

The respiratory quotient (RQ) obtained in each run refers to the ratio between the volume of CO_2 produced by the birds and the volume of O_2 used (RQ = CO_2 produced / O_2 used) and it indicates the degree of oxidation of the diet on trial. By referring to the thermal equivalent of O_2 (kJ/L) for such a mixture, heat production from a known O_2 consumption will be estimated, using the Brouwer equation (Johnson, 1981) incorporated into the Closed Circuit Respiratory Calorimetry (CCRC) computer program (Pesti *et al.*, 1989). Observations of THP was made over a stretch of 4 days with a 2-hour break on each day to allow the replenishment of the feed and water, collections of excreta as well as readjustment of the system for the next run, including increasing the concentration of potassium hydroxide used to trap the carbon dioxide produced. The THP was calculated on an hourly basis and then converted to a 24-hour period.

Heat production for maintenance (Basal metabolism)

The measurement of basal metabolism as estimated by fasting heat, involves the removal of the complicating effect of the heat increment of feeding by starving the birds. The period of fasting required for the complete digestion and metabolism of the previous meals as recommended for poultry is 2 days (Farrell, 1972, 1974; Pym and Farrell, 1977; McDonald *et al.*, 1995). The RQ and HP from the known O_2 consumption and CO_2 produced by each pair of birds, during the starvation period can then be estimated using the Brouwer equation incorporated into the CCRC computer programme. With reference to the above programme, the link between the basal metabolism and the body weight is assumed to be kJ/kg $^{0.75}$/day.

5.2.4 Sampling procedure

The same procedure as described in Section 3.2.4 was followed for the collection of digesta and gut tissue samples. In addition, about 5 cm of the proximal ends of the duodenum and ileum were ligated and prepared according to the method described by Untawale *et al.* (1978), with minor modifications, for the determination of selected groups of mucosa-associated bacteria. Briefly, the section from the small intestine was slit open with a pair of sterile scissors, and the digesta was completely washed off with sterile PBS (pH 7.4). The gut sample was washed three times with new PBS (40 mL sterile PBS in a sterile 50 mL bottle) and then transferred to a wheaton bottle (size 120 mL) containing 65 mL of peptone water. The gut sample was weighed and ground using a tissue grinder (Utra Turrax T25 basic, IKA, Labortedrik, NC, U.S.A) and was then ready for serial dilutions and plating as described below.

5.2.5 Measurements and analyses

Feed intake and mortality were measured as outlined in Section 3.2.3. Digesta samples were processed as described in Section 4.2.3 to examine the counts of lactobacilli (Rogosa, CM 0627), coliforms (MacKonkey, CM 0115); enterococci (Slanetz and Bartly medium, CM 0377) and total anaerobic bacteria (Wilkins-Chalgren Anaerobe agar, CM 0619). Gut tissue samples were serially diluted from 10^{-1} to 10^{-3}. From each dilution, 0.1 mL of the sample was plated onto the appropriate medium for enumeration of lactobacilli, coliforms, and enterococci. All the media were purchased from Oxoid (Sydney, Australia).

The analytical method described by Jensen *et al.* (1995) was adopted with modifications for lactic acid and VFA analysis. Briefly, frozen digesta samples were thawed and homogenized by vigorous shaking. About 2-3 g (wet weight) of the homogenized digesta sample were

weighed and 1 mL internal standard (0.01 M ethylbutyric acid) was added. After the samples were centrifuged (12,000 ×*g*, 15 min), approximately 1 mL of the supernatant was taken out and mixed with 0.5 mL of concentrated HCl and 2 mL of ether. Again, the mixture was centrifuged (8,000 ×*g*, 15 min) and 360 μL of the supernatant were transferred to a GC vial and mixed with 40 μL N-tert-butyldimethylsilyl-N-methyltrifluoro-acetamide. Before running on GC (Varian 3400), the vials were kept in a heating block (80 °C) for 20 minutes and left at room temperature for 48 h.

The analyses for gross energy, starch, protein, fat, histology and pH measurements were performed as described in Sections 3.2.5 and 4.2.3. Procedure for NSP analyses was as described by Englyst and Hundson (1987) and Theander and Westerlund (1993) with minor modifications.

Both groups of free sugars, monosaccharides and oligosaccharides, were analysed by gas-liquid chromatography (GLC) as the alditol acetate derivates of monosaccharides. Diet and digesta samples were ground to a fine powder of 0.5 mm in size. Aliquots of 100-200 mg were placed in screw-capped glass vials and 10 mL of hexane was added. The samples were vortexed, sonicated for 15 min and centrifuged to remove the fat. The residue was extracted with 80% ethanol (5 mL) at 80 °C for 10 min to remove the free sugars and oligosaccharides. After centrifugation, the supernatants were collected and subjected to a series of hydrolysis, reduction and acetylation of the samples, as described by Theander and Westerlund (1993). The supernatants were dried in a vacuum rotary evaporator (6 h at 40 °C), mixed with 3 mL of 1 M H_2SO_4 and hydrolysed at 100 °C for 2 h. The hydrolysates were cooled to room temperature and an aliquot of 0.4 mL was transferred into a clean 30 mL reaction tube and 0.1 mL of 28% NH_3 was added. The samples were thoroughly mixed and 50 μL of precisely measured internal standard (allose 4 mg/mL) was added. The mixture was dried in a vacuum rotary evaporator (4 h at 40 °C). After drying, the residues were recovered in distilled water (0.2 mL) and the monosaccharides were reduced by treatment with sodium borohydrate ($NaBH_4$) (0.3 mL, 50 mg sodium borohydrate per mL 3 M NH_4OH) at 40 °C for 1 h. Any excess amounts of $NaBH_4$ were decomposed with glacial acetic acid. The leftover alditol acetate derivates were acetylated by addition of acetic anhydride (5 mL) in the presence of 1-methylimidazole (0.5 mL) with any excess amount of acetic anhydride being decomposed with 5 mL of distilled water. Finally, the alditols were extracted with 2 mL of dichloromethane and the volatile alditol derivates of monosaccharides were analysed using a Varian 3400 GC equipped with a Varian series 8200 auto-sampler, a capillary column

(BPX70, 25 m, 0.32 mm, SEG International, Australia), and a flame ionisation detector (FID) set at 280 °C. During analysis, the column was held at 195 °C for 1 min and then raised by 5 °C/min until 225 °C was reached and held for 4 min.

The soluble and insoluble NSP portions found in the insoluble residue, after the extraction of samples with 80% ethanol, was dried to a slurry using nitrogen before incubating at 100 °C for 30 min to gelatinise the starch. The gelatinised starch was then digested with a thermostable α-amylase (E.C. 3.2.1.1) set at 95 °C for 30 min and after cooling, with amyloglucosidase (E.C. 3.2.1.3) set at 55 °C for 16 h. After incubation, the samples were centrifuged at 2000 ×*g* for 30 min. Insoluble NSP were determined by drying the insoluble residue under nitrogen and hydrolysing with 12 M H_2SO_4 for 1 h at 30 °C followed by hydrolysis in 1 M H_2SO_4 for 2 h at 100°C. The hydrolysate was cooled to room temperature and an aliquot of 0.8 mL was transferred into a clean 30 mL reaction tube and 0.2 mL of 28 % NH_3 was added. The sample was thoroughly mixed and precisely 50 µL of two internal standards (inositol, 4 mg/mL and allose 4 mg/mL) were added. The mixture was dried in a vacuum rotary evaporator (8 h at 40 °C) followed by reduction and acetylation, carried out using the same procedure as described for the determination of free sugars earlier in this chapter. Soluble NSP were determined by taking an aliquot of the supernatant after starch removal and precipitating soluble NSP in 80 % ethanol by adding 16 mL of absolute ethanol to 4 mL of supernatant. The precipitate was dried under a stream of nitrogen at 40 °C and 1 mL of 2 M triflouroacetic acid was added. The mixture was hydrolysed at 125 °C for 1 h. After cooling, precisely 50 µL of two internal standards (inositol 4 mg/mL and allose 4 mg/mL) were added and the triflouroacetic acid was removed by co-distillation with distilled water under a stream of nitrogen. The dry residue was recovered in distilled water (0.2 mL) and the monosaccharides were reduced and acetylated as described in the preceding section. The levels of polysaccharides were calculated from the levels of the component sugars using polymerisation factors of 0.88 for pentoses (ribose, xylose and arabinose), 0.9 for hexoses (mannose, galactose and glucose), 0.89 for deoxysugars (fucose and ribose) and 0.91 for rhamnose (Theander and Westerlund, 1993).

5.2.6 Calculations and statistical analyses

Excreta digestibility (%) was calculated as: $\frac{\text{nutrient} \times \text{FI} - \text{nutrient} \times \text{excreta}}{\text{nutrient} \times \text{FI}} \times 100$

Net energy was calculated as: NE = AME – Heat increment

where heat increment was estimated as the difference between heat production during feeding and fasting periods. Statistical analysis was conducted as described in Section 3.2.6.

5.3 RESULTS

5.3.1 Growth performance, AME and NE

No significant differences in FI and BWG were observed among the treatments (Table 5.2). Birds given the high MOS diet tended (P=0.10) to have lower FCR than those in the negative control group during d 1 to 21. Diet did not affect the growth performance of birds in the last two weeks. In the entire experimental period, the growth performance as well as the mortality rates of birds were unaffected by MOS or ZnB.

Table 5.2 Feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR) and mortality of broilers fed the experimental diets[1]

	NC	Low MOS	High MOS	ZnB	SEM	P values
1-3 weeks						
FI (g/bird)	1310	1308	1312	1308	34.9	0.98
BWG (g/bird)	984	997	1012	1004	24.8	0.43
FCR (g/g)	1.33	1.32	1.28	1.29	0.032	0.10
4-5 weeks						
FI (g/bird)	2414	2447	2474	2387	37	0.37
BWG (g/bird)	1293	1268	1318	1289	25	0.57
FCR (g/g)	1.87	1.93	1.88	1.86	0.035	0.15
1-5 weeks						
FI (g/bird)	3724	3762	3787	3659	78.3	0.13
BWG (g/bird)	2286	2265	2331	2293	46.7	0.57
FCR (g/g)	1.63	1.66	1.64	1.61	0.036	0.28
Mortality	3/88	5/88	4/88	8/88	-	-

[1] Values are means of 12 replicates.
[a,b] Means within a row not sharing a common superscript letter are significantly different (P<0.05).

The addition of MOS at both low and high level significantly improved (P<0.05) the AME of the diet compared to the negative control but did not affect (P>0.05) the NE value (Table 5.3). Compared to the positive control, the AME of the diet was significantly lower (P<0.05) in birds fed the MOS-supplemented diets and similar results were observed with the NE value. No significant differences were noticed in the AME and NE values between different levels of MOS. The heat production of birds was not affected by the treatments (Table 5.3).

Table 5.3 Effects of MOS and ZnB supplementation on AME, NE, respiration quotient (RQ) and heat production of broilers[1]

	NC	Low MOS	High MOS	ZnB	P values
AME (MJ/ kg DM)	13.36±0.24[c]	13.60±0.21[b]	13.62±0.27[b]	13.96±0.20[a]	0.000
NE (MJ/ kg DM)	9.41±0.32[b]	9.50±0.40[b]	9.57±0.43[b]	9.98±0.38[a]	0.05
RQ	1.025	1.029	1.018	1.019	0.66
Heat production (KJ/ $kg^{0.75}$ BW/d)	968±46	952±40	961±49	950±53	0.82

[1]Each value represents the mean ± SD of 7 replicates for each treatment group.
[a,b]Means within a row with different superscripts are significantly different (P<0.05).

5.3.2 Nutrient digestibility

Dietary MOS did not affect the total tract digestibility of fat and protein (Table 5.4). In contrast, ZnB significantly improved (P<0.05) the protein digestibility compared to the negative control. There was also a tendency (P=0.09) for ZnB to increase the excreta digestibility of starch.

Table 5.4 Effects of MOS and ZnB on excreta nutrient digestibility of birds at week 4[1]

	NC	Low MOS	High MOS	ZnB	SEM	P values
Starch	97.6	97.7	97.2	98.8	0.90	0.09
Protein	60.6[b]	62.1[b]	62.5[b]	65.6[a]	1.88	0.006
Fat	77.2	78.1	78.0	80.0	2.57	0.42
Free sugar	91.1	91.6	90.8	92.4	2.21	0.75
Soluble NSP	-10.3	11.4	4.1	6.3	13.20	0.12
Insoluble NSP	36.0	37.4	38.6	37.7	5.93	0.94

[1]Values are means of 7 replicates.
[a,b] Means within a row with different superscripts are significantly different (P<0.05).

The addition of MOS or ZnB did not affect the digestibility of free sugar and NSP (Table 5.4). However, a significantly lower (P<0.05) concentration of arabinose in the fraction of soluble NSP was noticed in birds given the low MOS and ZnB diets than those given the negative control diet (Table 5.5). In contrast, the concentration of xylose in the free sugar fraction and the concentrations of arabinose and xylose in the insoluble NSP fraction were significantly increased (P<0.05) by ZnB compared to the negative control and/or MOS treatments (Table 5.5).

Table 5.5 Effects of MOS and ZnB supplementation on free sugars, soluble and insoluble NSP in the excreta of birds at week 4[1]

	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose	Total NSP
					Free sugar (g/kg)				
NC	0.45	0.23	0.00	1.90	5.86[b]	1.22	3.80	3.28	11.35
Low MOS	0.44	0.28	0.00	1.74	6.08[b]	1.00	4.35	3.00	11.32
High MOS	0.44	0.22	0.00	1.88	6.43[b]	1.29	4.14	3.89	13.17
ZnB	0.55	0.23	0.00	2.20	8.79[a]	1.12	3.66	2.64	10.91
SEM	0.081	0.113	0.00	0.441	1.332	0.356	1.105	0.681	2.184
P values	0.21	0.41	0.00	0.52	0.01	0.70	0.80	0.32	0.74
					Soluble NSP (g/kg)				
NC	0.40	0.95	0.39	7.88[a]	8.47	1.94	11.15	8.17	35.14
Low MOS	0.53	0.98	0.37	6.27[b]	7.23	1.63	9.24	7.13	28.94
High MOS	0.50	0.83	0.34	6.77[ab]	7.47	2.23	9.97	7.94	32.16
ZnB	0.65	0.79	0.41	6.62[b]	7.42	2.25	9.54	8.08	33.19
SEM	0.163	0.150	0.084	0.827	0.986	0.737	1.339	1.801	4.227
P values	0.18	0.27	0.69	0.05	0.27	0.62	0.20	0.85	0.26
					Insoluble NSP (g/kg)				
NC	2.99	2.73	0.00	60.95[b]	72.80[b]	3.28	46.13	94.2	273
Low MOS	4.13	2.70	0.00	66.48[ab]	78.80[ab]	3.75	39.56	102.0	273
High MOS	3.23	2.60	0.00	67.33[ab]	79.56[ab]	2.76	46.26	94.2	275
ZnB	3.67	3.16	0.00	75.26[a]	91.11[a]	2.75	42.55	95.6	290
SEM	0.679	0.379	0.00	6.921	9.237	0.789	5.717	3.346	20.2
P values	0.12	0.17	0.00	0.05	0.05	0.26	0.43	0.08	0.60

[1]Values are means of 7 replicates.

[a,b] Means within a row with different superscripts are significantly different ($P<0.05$).

5.3.3 Bacterial populations, pH, and concentrations of lactic acid and VFA

Diet had significant effects on four groups of bacteria in the distal part of the small intestine (Table 5.6). In the ileum, birds in the high MOS and ZnB groups had significantly lower ($P<0.05$) counts of lactobacilli and coliforms than those in the negative control group. In the caeca, the number of lactobacilli was significantly reduced ($P<0.05$) on the low MOS and ZnB treatments. The growth of total anaerobic bacteria was inhibited by ZnB in comparison with the negative control and this effect was significant ($P<0.05$) in the ileum.

Table 5.6 Effects of dietary treatments on the counts (log CFU/g digesta) of luminal bacteria of broilers on day 14[1]

Site and microflora	NC	Low MOS	High MOS	ZnB	SEM	P values
Duodenum						
Total anaerobes	7.96	7.92	7.72	7.52	0.342	0.56
Lactobacilli	7.33	6.90	7.32	7.08	0.352	0.57
Coliforms	3.97	4.18	4.01	3.94	0.446	0.86
Enterococci	3.68	3.33	3.50	3.72	0.451	0.58
Ileum						
Total anaerobes	8.90^{a}	8.67^{ab}	8.56^{ab}	8.22^{b}	0.234	0.05
Lactobacilli	8.72^{a}	8.34^{ab}	8.06^{bc}	7.68^{c}	0.305	0.001
Coliforms	6.25^{a}	6.06^{ab}	5.78^{b}	5.83^{b}	0.195	0.01
Enterococci	5.13	5.52	5.30	5.33	0.241	0.14
Caeca						
Total anaerobes	9.67	9.18	9.27	9.06	0.383	0.15
Lactobacilli	9.41^{a}	8.90^{b}	9.17^{ab}	8.82^{b}	0.281	0.03
Coliforms	8.43	8.42	8.65	8.24	0.276	0.53
Enterococci	6.42	6.42	6.18	5.82	0.354	0.30

[1]Values are means of 8 replicates.
[a,b] Means within a row with different superscripts are significantly different ($P<0.05$).

Bacteria attached to the mucosa in the proximal small intestine were altered by diet (Table 5.7). Supplementation with MOS or ZnB tended ($P=0.07$) to reduce the number of lactobacilli attached to the duodenal mucosa while, at the same site, the number of coliforms tended ($P=0.09$) to be lower in birds given the high MOS diet than in those birds fed the control diets. Both MOS and ZnB had no significant effects on the density of mucosa-associated enterococci.

Table 5.7 Effects of dietary treatments on the counts (log CFU/g wet tissue) of mucosa-associated bacteria of broilers on day 14[1]

Site and microflora	NC	Low MOS	High MOS	ZnB	SEM	P values
Duodenum						
Lactobacilli	6.24	5.56	5.88	5.88	0.239	0.07
Coliforms	4.02	3.94	3.81	4.33	0.280	0.09
Enterococci	4.05	3.81	3.85	3.75	0.429	0.78
Ileum						
Lactobacilli	6.31	5.93	6.11	6.15	0.307	0.67
Coliforms	3.67	3.89	3.97	3.92	0.351	0.68
Enterococci	4.05	3.75	3.77	3.90	0.407	0.72

[1]Values are means of 8 replicates.
[a,b] Means within a row with different superscripts are significantly different (P<0.05).

With regards to the intestinal pH values, no effects were seen on the jejunum and caeca. However, ileal pH values of birds fed the MOS or ZnB diet were higher (P<0.05) than that of birds given the negative control diet (Table 5.8).

Table 5.8 Effects of dietary treatments on pH value of the small intestine of birds on day 14[1]

Segment	NC	Low MOS	High MOS	ZnB	SEM	P values
Jejunum	6.23	6.23	6.22	6.23	0.070	0.99
Ileum	7.39[b]	7.66[a]	7.78[a]	7.80[a]	0.184	0.01
Caeca	6.31	5.99	6.08	6.08	0.295	0.46

[1]Values are means of 8 replicates.
[a,b] Means within a row with different superscripts are significantly different (P<0.05).

A significant decrease (P<0.05) in the concentrations of lactic acid and total VFA in the ileum was observed for MOS treatments, as well as the positive control, compared to the negative control (Table 5.9). In the caeca, MOS reduced (P<0.05) the concentrations of propionic acid, while ZnB increased (P<0.05) the acetic acid concentration. The low MOS and ZnB treatments also increased (P<0.05) the concentration of butyric acid in the caeca. No significant differences in the concentration of total VFA were noticed among the treatments (Table 5.9).

The molar ratios of the individual VFAs in the ileum were not affected by diet (Table 5.9). However, in the caeca, a significant decrease (P<0.05) in the molar ratio of propionic acid was noticed in those birds fed the diet supplemented with MOS or ZnB. The concentration of butyric acid was higher (P<0.05) in birds given the low MOS treatment diet than those in the negative control group.

Table 5.9 Concentrations and molar ratios of volatile fatty acids and lactic acid in the ileum and caeca of broiler chickens fed the experimental diets on day 14[1]

	NC	Low MOS	High MOS	ZnB	SEM	P values
Concentration (μmol/g digesta)						
Ileum						
Lactic acid	13.6[a]	7.5[ab]	7.2[b]	3.6[b]	4.38	0.02
Acetic acid	1.5	1.4	1.1	1.1	0.32	0.26
Total VFA	16.3[a]	5.5[b]	9.5[b]	4.8[b]	4.20	0.002
Caeca						
Acetic acid	29.8[b]	32.2[b]	26.3[ab]	47.2[a]	9.08	0.02
Propionic acid	2.2[a]	1.1[b]	0.9[b]	1.9[a]	0.51	0.002
Butyric acid	3.9[b]	7.5[a]	5.0[ab]	6.1[a]	1.77	0.01
Total VFA	44.1	49.0	51.0	57.9	12.7	0.49
Molar ratio (%/total VFA)						
Ileum						
Lactic acid	84.8	70.2	79.6	78.2	10.23	0.26
Acetic acid	11.4	13.8	14.9	17.3	5.88	0.52
Caeca						
Acetic acid	68.0	65.5	74.2	76.5	6.7	0.08
Propionic acid	4.8[a]	2.1[b]	1.9[b]	2.9[b]	1.10	0.001
Butyric acid	5.4[b]	11.6[a]	7.4[ab]	8.3[ab]	2.82	0.02

[1]Values are means of 8 replicates.
[a,b] Means within a row with different superscripts are significantly different (P<0.05).

5.3.4 Gut morphology

No significant effects of the treatments were observed in villus height in the jejunum (Table 5.10). The ileal villi of birds fed the low MOS diet were significantly longer (P<0.05) than those of the birds fed the high MOS and the negative control diets. The crypt depth and the villus height:crypt depth ratio of birds remained unaffected by the diets.

Table 5.10 Effects of dietary treatments on the villus height, crypt depth and villus height:crypt depth ratio (Ratio) of the small intestine of broilers on day 14[1]

	NC	Low MOS	High MOS	ZnB	SEM	P values
Jejunum						
Villus height (μm)	858	935	935	913	78.0	0.46
Crypt depth (μm)	117	126	123	123	4.8	0.31
Ratio	6.9	7.4	7.4	7.3	0.795	0.80
Ileum						
Villus height(μm)	477[bc]	546[a]	473[c]	515[ab]	29.2	0.005
Crypt depth (μm)	121	120	119	119	5.5	0.97
Ratio	4.1[b]	4.6[a]	3.9[b]	4.3[ab]	0.28	0.01

[1]Values are means of 8 replicates.
[a,b] Means within a row with different superscripts are significantly different (P<0.05).

5.4 DISCUSSION

5.4.1 Growth performance, AME, NE and heat production

In contrast to the findings in the preceding experiments of this study (Chapters 3 and 4) and the work of Hughes (2003), the current trial showed that both MOS and ZnB improved the AME of the diets, with greater effects seen on ZnB treatment. Similarly, birds fed the MOS diets had a numerically higher NE value than those in the negative control group but the value was lower than that of birds on ZnB treatment.

The effects of the different in-feed additives on gut microflora may probably explain the differences in energy utilization found in this study. Gut microflora increases energy costs by modifying the rate of energy-consuming reactions such as protein turnover within the chicken GIT (Choct, 1999). By binding pathogenic and opportunistic pathogenic bacteria possessing type-1 fimbriae, MOS can prevent them from attaching to the gut lining, improve the integrity of gut lining (Loddi *et al.*, 2002) and thus energy, which otherwise would be used to combat the growth of pathogenic bacteria, can be saved for body growth. The antibiotic supplement is active mainly against Gram-positive bacteria (Butaye *et al.*, 2003), which means the colonization of some predominant bacteria, such as lactobacilli, is inhibited and more energy is saved for body growth compared to the negative control and MOS treatments. Results of gut microbial population tended to support this postulation. In comparison with the negative control, MOS supplementation reduced the number of coliforms and lactobacilli in the small intestine, whereas a larger effect was noticed with ZnB treatment which also inhibited the growth of total anaerobic bacteria in the small intestine. Thus, the lower bacterial load of birds on MOS or ZnB treatment may be associated with the improved energy utilization.

Heat production did not seem to vary among the dietary treatments, perhaps because the treatments did not greatly affect the processes of digestion or secretion, absorption and metabolism of nutrients, which are considered to be the most important sources of heat increment in the gut, in quite different ways.

5.4.2 Gut microflora, its activity and gut morphology

As reported by Jamroz *et al.* (2003), MOS reduced ileal luminal coliform counts in the current experiment, which contradicts our previous experiment. Differences in basal diets and rearing environments could probably explain the inconsistent results as intestinal

microbiota can be modified when the birds are raised in cages instead of on litter (Willis *et al.*, 2002) and/or when the birds are fed diets containing different ingredients (Apajalahti *et al.*, 2001). The average ileal coliform counts were 5.98 and 7.32 log CFU in identical birds, respectively, for the current and previous experiments, indicating that a different profile of microflora was developed under those specific experimental environments and diets.

Reductions in the number of luminal lactobacilli, as well as lactic acid concentration, in the small intestine were also noticed in birds fed the MOS-supplemented diets. This effect might be associated with the higher content of dietary NSP used in the present trial and also the relative ability of different treatments in controlling the growth of the luminal lactobacilli. To increase the birds' response to the diets so that any effects of MOS supplementation on NE value can be detected, NSP-rich ingredients, such as wheat, barley and lupin, were included in the basal diet. Resistant starch and NSP usually escape digestion in the proximal small intestine (Cummings and Englyst, 1995), to become substrates for certain group(s) of bacteria, such as lactobacilli, which possibly leads to an overgrowth of these bacteria in the distal small intestine. Birds in the negative control group had a higher ileal lactobacilli count than birds in the other three treatment groups. Although lactobacilli are regarded generally as beneficial to bird health and growth, the overgrowth of lactobacilli can negatively affect the growth performance of birds by virtue of their need for dietary energy and nutrients for sustenance. In an earlier research, Eyssen *et al.* (1962) studied the effect of various antibiotics on the microflora of chickens and they found a positive correlation between the sensitivity of lactobacilli to a given antibiotic and the value of this antibiotic as a growth stimulant. These authors concluded that the elimination of the lactobacilli is of importance in the growth-promoting effects of antibiotics. Feighner and Dashkevicz (1987) further pointed out that lactobacilli would compete for nutrient uptake or impair fat absorption due to their ability to deconjugate bile acid. A growth depression, which might be related to a large population of lactobacilli in the small intestine, was also observed by Engberg *et al.* (2000).

O'Carra (1998) suggested that a dual mode of action in exclusion of pathogenic bacteria from the intestine existed with the supplementation of MOS. Firstly, through the direct binding of MOS to specific lectins on the bacteria possessing type 1 fimbriae; and secondly, by stimulating an increased production of IgA (Savage *et al.*, 1996a). The same may hold true for the reduction in the number of lactobacilli in birds fed the MOS-supplemented diets in the current study.

The exact role of mucosa-associated bacteria in the animal is unknown with the exception that by attaching to the gut mucosa-associated pathogens can impair gut integrity, depress growth performance, and cause disease. In the current study, MOS tended to reduce the number of mucosa-associated coliforms, a group of potential pathogenic bacteria, in the proximal small intestine. The reduction in the number of coliforms attached to the gut mucosa of birds in the MOS group signifies a positive health response of birds and supports a role for MOS in the improvement of gut morphology or/and function as observed in the current study and previous research (Santin *et al.*, 2001; Iji *et al.*, 2001).

5.4.3 Nutrient digestibility

In agreement with the report by Kumprecht and Zobac (1997a) and Alves *et al.* (2003), excreta digestibility of fat and protein was not significantly affected by MOS supplementation. No significant differences in total tract digestibility of starch were noticed between MOS treatments and the negative control. However, a tendency for ZnB to improve the starch digestibility and a significant increase in protein digestibility was observed, which may explain why a significant improvement in the NE value of the diet was observed with ZnB treatment but not with MOS treatments, compared to the negative control.

The addition of MOS did not affect the excreta digestibility of free sugars and NSP but the low MOS treatment reduced the concentration of individual sugar in the NSP fraction. Similarly, Ao (2004) noticed that the insoluble xylose content as well as the total insoluble NSP content in ileal digesta was reduced by the addition of the same dosage level of MOS. A large improvement (61.5 to 147.7% increase) in excreta fibre digestibility was noticed in birds given the feed supplemented with 0.5 g/kg MOS up to 3 g/kg MOS by Kumprecht and Zobac (1997a) and the authors suggested that the increased fibre digestibility could be one of the important factors that improves feed conversion of those birds. However, there was only a marked improvement in the FCR of bird given the high MOS diet in the first three weeks in the current trial.

5.5 CONCLUSIONS

The present study clearly demonstrated that MOS could improve the AME of the diet and thus the growth performance of birds, although the effects are not as pronounced as those of ZnB. The NE of the diet was not significantly affected by MOS. The effects of MOS on

excreta digestibility of nutrients were minimal, however, the gut microflora and morphology were altered by MOS.

CHAPTER 6
THE EFFECTS OF MANNANOLIGOSACCHARIDE, OR FRUCTOOLIGOSACCHARIDE ON THE RESPONSE OF BROILER CHICKENS TO PATHOGENIC *ESCHERICHIA COLI* CHALLENGE

6.1 INTRODUCTION

The results obtained in the previous two chapters indicated that dietary MOS could modulate the composition of gut microflora under a relatively clean and hygienic condition and may thus induce beneficial effects on the growth and gut development of young birds. This is important as the gut microflora of young birds is considered to be quite unstable and can easily be disturbed by various external factors, such as subclinical infection posted by pathogenic challenge. Therefore, the ability to maintain a normal or optimal gut microflora becomes one of the key factors in determining the ultimate health status and hence the expression of the genetic growth potential of birds. Although it is believed that MOS has the ability to modulate the gut microflora, their ability to stabilize and restore a disturbed gut microflora, especially in early stage of chickens' life, is unknown. Furthermore, there is no published information regarding the *in vivo* effects of MOS on suppressing the colonization of pathogens associated with the gut wall, one major mode of action by which MOS is thought to improve the gut integrity and growth performance of birds.

Unlike MOS, FOS selectively stimulate the growth of and/or activate the metabolism of a limited number of health-promoting bacteria, such as lactic acid bacteria, in the intestinal tract (Gibson and Roberfroid, 1995), thus inhibiting the growth of pathogens (Chen *et al.*, 1985; Vandenbergh, 1993) and improving the host's microbial balance. Dietary FOS have been shown to improve the growth performance of broiler chickens (Ammerman *et al.*, 1988; Xu *et al.*, 2003) and may be able to replace AGP in broiler chickens diets.

Of all the intestinal and extraintestinal infectious agents, *E. coli* is deemed to be one of the most common causative agents that usually take its course of action by making initial contact with the epithelial surfaces (Smyth *et al.*, 1994). By using an *E. coli* challenge model in the current trial, the protective effects of MOS on the gut ecosystem, in particular on the gut microflora, can be tested and compared with FOS.

6.2 MATERIALS AND METHODS

6.2.1 Birds and diets

Nine hundred and sixty (960) day-old male Cobb broilers, supplied from a local hatchery (Baiada hatchery, Kootingal, NSW), were used in this experiment and placed in brooders located in two separate rooms (groups). Birds in one of the rooms were inoculated with *E. coli* and treated as the challenged group while the unchallenged group of birds in the other room was inoculated with a sterilized brain heart infusion (BHI) broth.

In each of the two groups, four dietary treatments with 8 replicates (cages) of 15 birds each were assigned to a negative control (without any dietary additives), MOS treatment (2g Bio-MOS /kg feed), FOS treatment (10 g oligofructose /kg feed, raftilose P95, Orafti, Tienen, Belgium), or a positive control (ZnB, 50 ppm). The basal diet used in this trial is the same as described in Section 5.2.1. The experiment lasted for 3 weeks.

The experiment was approved by the Animal Ethics Committee of the University of New England (Approval NO.: AEC05/049).

6.2.2 Challenge protocol

A mixture of four specific strains (E3, E30, E956 and E133) of pathogenic *E. coli* was used in this experiment, and they were kindly provided by Prof. G. F. Browning of the University of Melbourne, Parkville Vic, Australia. The agglutination test results showed that the E3 strain strongly agglutinated with MOS, E30 and E956 moderately agglutinated with MOS, and E133 could only weakly agglutinate with MOS. The mixture was inoculated into the BHI broth (Oxoid, CM0225) and grown overnight until the culture reached a concentration of 10^7 cfu/mL, which was the challenge concentration used in the present experiment. One milliliter per bird of this culture was orally gavaged on d 1, 3, 7, and 14 and changed to two mL per bird on d 21. The challenge was also boosted via drinking water on d 2, 5, 12, and 20 at a concentration of approximately 10^5 cfu/mL.

6.2.3 Bird management, sample collection and processing of samples

Samples were taken from two birds per cage (16 birds per treatment) at the end of weeks 1 and 3. Details of chick management, sample collection and processing of samples were as outlined in Sections 3.2.2 and 5.2.3.

6.2.4 Sample analyses

Bacterial enumeration, intestinal histo-morphological studies and analysis of lactic acid and VFA were all as described in Sections 3.2.4 and 5.2.5. Pooled digesta samples from the jejunum, ileum and caeca of two birds per cage were used for bacterial enumeration, lactic acid and VFA analysis. With caecal samples, the VFA analysis was done only with 21-day-old birds. Pooled gut tissue samples from the jejunum and ileum of two birds per cage were used for the determination of mucosa-associated bacteria. The intestinal histo-morphological studies were done with the jejunal and ileal tissue samples from one bird per cage.

6.2.5 Statistical analyses

Data were analysed using the General Linear Model (GLM) procedure of SPSS (SPSS Inc, Version 12) as a 2 (with or without *E. coli* challenge) by 4 (basal diets) factorial array. When a significant ($P<0.05$) *F*-test was detected for the main effects or the interaction, the corresponding means were compared by LSD. Bacterial results obtained from measurements in the jejunum, ileum and caeca were analysed separately.

6.3 RESULTS

6.3.1 Growth performance

At the end of week 1, *E. coli* challenge retarded the growth of chickens with the most obvious effects being a 4% decrease ($P<0.01$) in FI (Table 6.1). With dietary additives, this negative effect was alleviated ($P<0.01$) differently. A significant increase ($P<0.05$) in FI was observed in birds given the FOS or ZnB-supplemented diet compared to the negative control whereas MOS numerically increased ($P>0.05$) the FI of birds. Similar results were noticed for the FI of the unchallenged birds. Diet also affected ($P<0.01$) the BWG of birds. Compared to the negative control, the addition of MOS or ZnB increased ($P<0.05$) the BWG of the challenged birds whereas the same result was observed only for ZnB treatment within the unchallenged group. Both *E. coli* challenge and diet tended ($P=0.09$ and 0.08, respectively) to affect the FCR of birds and the lowest FCR was observed in the challenged birds fed the MOS-supplemented diet among all the treatments. There was no challenge × diet interaction in the growth performance of birds. The mortality level was approximately 10% higher due to *E. coli* challenge; however, diet did not significantly affect the mortality rates of birds and ZnB treatment had the highest mortality within the challenged birds (Table 6.1).

Table 6.1 Feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR) and mortality of birds fed the experimental diets[1]

Challenge[2] (C):	With				Without					P values		
Diet (D):	NC	MOS	FOS	ZnB	NC	MOS	FOS	ZnB	SEM	C	D	C×D
Week 1												
FI (g/bird)	176^{c}	182^{bc}	190^{ab}	187^{ab}	183^{bc}	188^{ab}	193^{a}	195^{a}	4.3	0.01	0.001	0.85
BWG (g/bird)	146^{c}	156^{ab}	151^{abc}	155^{ab}	150^{bc}	152^{ab}	150^{bc}	157^{a}	3.2	0.88	0.003	0.31
FCR (g/g)	1.21	1.17	1.26	1.21	1.23	1.23	1.27	1.24	0.069	0.09	0.08	0.70
Mortality[3]	16/120	13/120	12/120	23/120	0/120	0/120	2/120	2/120	NA[4]	NA	NA	NA
Week 2-3												
FI (g/bird)	1607^{a}	1607^{a}	1684^{a}	1598^{a}	1315^{b}	1314^{b}	1332^{b}	1331^{b}	92.1	0.00	0.41	0.60
BWG (g/bird)	835^{bc}	882^{abc}	899^{a}	889^{ab}	827^{c}	864^{abc}	894^{ab}	863^{abc}	59.2	0.33	0.02	0.95
FCR (g/g)	1.98^{a}	1.83^{bc}	1.95^{ab}	1.80^{c}	1.59^{d}	1.54^{d}	1.51^{d}	1.55^{d}	0.130	0.00	0.07	0.16
Mortality[3]	4/120	1/120	6/120	2/120	1/120	0/120	2/120	0/120	NA	NA	NA	NA

[1]Values are means of 8 replicates.
[2]A mixture of *E. coli* (E3, E30, E956 and E133) was used for the challenge.
[3]Chi-square was used to test the significant differences in mortality among dietary treatments within the challenged or unchallenged group.
[4]NA: not available.
[a,b]Means within one row sharing no common superscripts differ significantly at the levels indicated for the factors ($P<0.05$).

From week 2 to 3, *E. coli* challenge increased (P<0.01) the FI and FCR of birds (Table 6.1). Supplementation of the dietary additives had no ameliorating effects on the FI of birds but improved (P<0.05) BWG. In particular, birds given the FOS-supplemented diet had higher (P<0.05) BWG than those given the negative control diet, regardless of the challenge. Diet tended (P=0.07) to reduce FCR and the challenged birds on MOS and ZnB treatments had lower (P<0.05) FCR compared to the negative control. No challenge × diet interaction was noticed for the growth performance of birds. The mortality level did not differ (P>0.05) among dietary treatments (Table 6.1).

6.3.2 Bacterial populations

Up to 7 days of age, *E. coli* challenge increased (P<0.05) or tended (P=0.07) to increase the number of coliforms in the distal small intestine (Table 6.2). In contrast, diet altered (P<0.05) the coliform number in the proximal small intestine. Within the challenged group, a decrease (P<0.05) in the jejunal coliform number was noticed in birds on FOS treatment compared to the positive control. At the same site, the number of *C. perfringens* tended (P=0.07) to be lower in birds given the diets supplemented with the dietary additives compared to the negative control (Table 6.2).

In the ileum, diet affected (P<0.05) the number of total anaerobic bacteria and the interaction (P<0.05) between the challenge and diet indicated that MOS and FOS could promote the growth of total anaerobic bacteria in the ileum of the unchallenged birds compared to the negative control (Table 6.2). In the caeca, the addition of the dietary additives, particularly ZnB, reduced (P<0.01) the number of *C. perfringens*. A challenge × diet interaction (P<0.05) was noticed for the count of total anaerobic bacteria; birds on ZnB treatment had a lower (P<0.05) number of total anaerobic bacteria than those fed the negative control diet within the unchallenged group (Table 6.2).

On d 21, *E. coli* challenge increased (P<0.01) the number of coliforms in the jejunum and that of lactobacilli in the caeca (Table 6.3). No dietary effects were noticed on the number of the selected groups of bacteria in the small intestine except for the number of *C. perfringens*, which was reduced (P<0.01) by the dietary additives, especially ZnB. There was no challenge × diet interaction in the bacterial populations in the small intestine (Table 6.3).

Table 6.2 Effects of MOS, FOS and ZnB on the counts of four kinds of bacteria (log CFU/g digesta) in the gut lumen of birds on day 7[1]

Challenge[2](C):	With				Without					P values		
Diet (D):	NC	MOS	FOS	ZnB	NC	MOS	FOS	ZnB	SEM	C	D	C×D
Jejunum												
TAB[3]	8.23	8.21	8.13	8.23	8.18	8.29	8.11	8.02	0.349	0.55	0.68	0.70
Lactobacilli	8.30	8.09	8.11	8.20	7.97	8.30	8.06	7.86	0.387	0.51	0.94	0.70
Coliform	3.49^{bc}	3.76^{ab}	3.11^{c}	4.06^{a}	3.30^{bc}	3.47^{bc}	3.56^{abc}	3.62^{abc}	0.508	0.37	0.03	0.11
C. perfringens	3.16	3.07	3.04	3.08	3.43	3.29	3.11	2.99	0.294	0.12	0.07	0.31
Ileum												
TAB[3]	8.93^{ab}	8.95^{ab}	8.61^{bc}	8.62^{bc}	8.53^{c}	9.07^{a}	9.07^{a}	8.63^{bc}	0.377	0.61	0.04	0.02
Lactobacilli	8.88	8.70	8.56	8.65	8.80	8.92	8.85	8.49	0.289	0.64	0.55	0.63
Coliform	4.31^{ab}	4.83^{a}	4.24^{ab}	4.76^{a}	4.35^{a}	4.03^{ab}	3.33^{b}	3.93^{ab}	1.041	0.02	0.28	0.51
C. perfringens	3.13	3.42	3.11	3.42	3.39	3.34	3.11	2.97	0.267	0.63	0.53	0.31
Caeca												
TAB[3]	9.71^{a}	9.62^{ab}	9.62^{ab}	9.81^{a}	9.93^{a}	9.72^{a}	9.90^{a}	9.35^{b}	0.345	0.67	0.22	0.01
Lactobacilli	9.64	9.49	9.45	9.66	10.02	9.65	9.94	9.36	0.230	0.11	0.23	0.09
Coliform	8.26	8.24	8.04	8.17	8.32	7.63	7.93	7.70	0.309	0.07	0.32	0.40
C. perfringens	7.27^{a}	6.61^{ab}	5.96^{bc}	5.18^{c}	7.05^{a}	6.66^{ab}	6.29^{ab}	5.78^{bc}	0.502	0.45	0.00	0.69

[1]Values are means of 8 replicates.
[2]A mixture of *E. coli* (E3, E30, E956 and E133) was used for the challenge.
[3]TAB:Totoal anaerobic bacteria.
[a,b]Means within one row sharing no common superscripts differ significantly at the levels indicated for the factors ($P<0.05$).

Table 6.3 Effects of MOS, FOS and ZnB on the counts of four kinds of bacteria (log CFU/g digesta) in the gut lumen of birds on day 21[1]

Challenge[2] (C):	With				Without					P values		
Diet (D):	NC	MOS	FOS	ZnB	NC	MOS	FOS	ZnB	SEM	C	D	C×D
Jejunum												
TAB[3]	8.30	8.49	8.80	8.53	8.43	8.19	8.30	8.15	0.987	0.30	0.91	0.84
Lactobacilli	8.47	8.21	8.70	8.59	7.72	7.97	8.45	8.06	1.009	0.09	0.49	0.88
Coliform	4.65^{ab}	5.03^{a}	4.82^{a}	4.82^{a}	3.73^{bc}	3.59^{c}	4.10^{abc}	4.71^{ab}	0.521	0.00	0.45	0.35
C. perfringens	3.06	3.09	3.10	3.02	3.13	3.15	3.14	3.08	0.146	0.12	0.44	0.99
Ileum												
TAB[3]	8.70	9.13	9.28	8.43	7.86	8.54	8.74	8.30	1.064	0.06	0.16	0.83
Lactobacilli	8.52	8.65	9.08	8.22	7.92	8.20	8.68	8.26	0.937	0.14	0.18	0.80
Coliform	5.68	6.19	5.92	5.75	5.21	5.35	5.35	5.62	1.208	0.11	0.89	0.86
C. perfringens	3.72	3.44	3.23	2.99	3.97	3.20	3.34	3.90	0.762	0.18	0.14	0.22
Caeca												
TAB[3]	9.30	9.65	9.24	9.20	8.93	9.40	9.25	8.87	0.675	0.17	0.20	0.85
Lactobacilli	8.93^{ab}	9.12^{a}	9.18^{a}	9.11^{a}	8.88^{ab}	8.99^{ab}	8.89^{ab}	8.58^{b}	0.220	0.03	0.47	0.45
Coliform	8.71	8.93	8.06	8.68	8.28	8.48	8.15	8.41	0.801	0.19	0.21	0.77
C. perfringens	5.95^{a}	5.49^{a}	5.62^{a}	4.64^{b}	5.92^{a}	5.84^{a}	5.59^{a}	4.49^{b}	0.772	0.86	0.00	0.80

[1]Values are means of 8 replicates.
[2]A mixture of *E. coli* (E3, E30, E956 and E133) was used for the challenge.
[3]TAB:Totoal anaerobic bacteria.
[a,b]Means within one row sharing no common superscripts differ significantly at the levels indicated for the factors ($P<0.05$).

The effects of *E. coli* challenge and diet on the populations of mucosa-associated bacteria are summarized in Table 6.4. On d 7, *E. coli* challenge increased ($P<0.01$) the number of mucosa-associated coliforms in the jejunum of birds, which was reduced ($P<0.05$) by the addition of MOS as indicated by a challenge × diet interaction ($P<0.05$). There was a tendency ($P=0.08$) for *E. coli* challenge × diet interaction to affect the population of mucosa-associated lactobacilli in the ileum with the highest number noticed in the challenged birds given the ZnB-supplemented diet (Table 6.4).

On d 21, *E. coli* challenge increased ($P<0.05$) the number of lactobacilli attached to the ileal mucosa and this effect tended ($P=0.07$) to interact with the type of the diets (Table 6.4). Within the unchallenged group, the number of mucosa-associated lactobacilli was increased ($P<0.05$) by the dietary additives compared to the negative control. There was also a tendency ($P=0.10$) for diet to increase the number of mucosa-associated lactobacilli in the jejunum with birds given the FOS-supplemented diet having the highest number of mucosa-associated lactobacilli, regardless of the challenge. The number of mucosa-associated coliforms in the jejunum and ileum were increased ($P<0.01$) by *E. coli* challenge. Diet tended ($P=0.07$) to affect the number of mucosa-associated coliforms in the ileum and, interestingly, the unchallenged birds on ZnB treatment had a higher ($P<0.05$) number of mucosa-associated coliforms than those birds on the other three treatments (Table 6.4).

6.3.3 Intestinal pH, lactic acid and VFA concentrations

E. coli challenge reduced ($P<0.01$) the caecal pH value of birds on d 7 but diet did not seem to affect the intestinal pH value of birds at the same age (Table 6.5). On d 21, a challenge × diet interaction ($P<0.05$) was observed in the ileal pH value, whereby FOS reduced ($P<0.05$) the pH value compared to the other three treatments within the unchallenged group (Table 6.5).

Table 6.4 The counts of mucosa associated bacteria (log CFU/g wet tissue) in the gut of birds fed the experimental diets at different ages[1]

Challenge[2] (C):	With				Without					P values		
Diet (D):	NC	MOS	FOS	ZnB	NC	MOS	FOS	ZnB	SEM	C	D	C×D
Day 7												
Lactobacilli												
Jejunum	6.55	6.38	6.92	6.55	6.69	6.61	7.04	6.67	0.289	0.30	0.12	0.99
Ileum	6.62	6.60	6.74	7.08	6.98	6.76	7.03	6.50	0.558	0.68	0.78	0.08
Coliforms												
Jejunum	4.35^{a}	3.73^{bc}	4.41^{a}	4.06^{ab}	3.92^{abc}	3.95^{abc}	3.47^{c}	3.58^{bc}	0.533	0.00	0.33	0.03
Ileum	4.29	3.94	4.38	4.10	4.42	4.52	4.28	4.00	0.728	0.50	0.61	0.53
Day 21												
Lactobacilli												
Jejunum	6.64	6.59	6.79	6.75	5.25	6.63	7.04	6.46	0.549	0.21	0.10	0.17
Ileum	6.96^{a}	6.47^{a}	6.75^{a}	6.70^{a}	5.03^{b}	6.29^{a}	6.64^{a}	6.40^{a}	0.552	0.03	0.33	0.07
Coliforms												
Jejunum	4.43^{abc}	4.37^{abcd}	4.69^{ab}	4.85^{a}	3.67^{d}	3.87^{cd}	4.10^{bcd}	4.24^{abcd}	0.743	0.00	0.21	0.97
Ileum	4.81^{a}	5.03^{a}	5.09^{a}	5.42^{a}	3.92^{b}	3.88^{b}	3.96^{b}	4.76^{a}	0.780	0.00	0.07	0.80

[1]Values are means of 8 replicates.
[2]A mixture of *E. coli* (E3, E30, E956 and E133) was used for the challenge.
[a,b] Means within one row sharing no common superscripts differ significantly at the levels indicated for the factors ($P<0.05$).

Table 6.5 Effects of dietary treatments on pH value in the digestive tract of birds at different ages[1]

Challenge[2] (C):	With				Without					P values		
Diet (D):	NC	MOS	FOS	ZnB	NC	MOS	FOS	ZnB	SEM	C	D	C×D
Day 7												
Jejunum	6.28	6.19	6.29	6.28	6.24	6.33	6.29	6.29	0.145	0.46	0.89	0.38
Ileum	7.10	6.59	6.99	7.10	6.91	7.16	6.79	7.33	0.573	0.49	0.31	0.19
Caeca	6.20^{bc}	5.83^{c}	6.51^{abc}	6.04^{c}	6.55^{abc}	6.88^{ab}	6.95^{a}	6.53^{abc}	0.689	0.00	0.24	0.54
Day 21												
Jejunum	6.25	6.13	6.00	6.10	6.21	6.05	6.12	6.21	0.306	0.77	0.43	0.72
Ileum	7.09^{a}	6.63^{ab}	6.97^{a}	6.71^{ab}	7.38^{a}	7.07^{a}	6.04^{b}	7.24^{a}	0.839	0.69	0.12	0.05
Caeca	6.56	6.59	6.36	6.65	6.54	6.95	6.79	6.60	0.254	0.16	0.61	0.42

[1]Values are means of 8 replicates.
[2]A mixture of *E. coli* (E3, E30, E956 and E133) was used for the challenge.
[a,b] Means within one row sharing no common superscripts differ significantly at the levels as indicated for the factors (P<0.05).

The effects of the treatments on the concentrations of lactic and acetic acids in the ileum are shown in Table 6.6. The concentration of acetic acid was reduced ($P<0.01$) by *E. coli* challenge on d 7 but a reverse pattern was observed on d 21. In contrast, diet affected ($P<0.01$) the concentration of lactic acid on d 7 with a higher value noticed in birds fed the FOS-supplemented diet compared to both controls and/or MOS treatment. On d 21, a challenge × diet interaction ($P<0.05$) was detected for the concentration of lactic acid, which was increased ($P<0.05$) by FOS in the unchallenged birds. Diet tended ($P=0.09$) to affect the concentration of acetic acid on d 7 and birds on FOS and ZnB treatments had a higher ($P<0.05$) concentration of acetic acid than those on MOS treatment within the unchallenged group (Table 6.6).

The concentrations of the individual VFAs and total VFA in the caeca were not affected by either *E. coli* challenge or diet on d 21 (Table 6.7). However, the molar ratio of acetic acid was decreased ($P<0.05$) due to *E. coli* challenge and the opposite trend was observed for the molar ratio of propionic acid. The interaction between the challenge and diet tended ($P=0.10$) to affect the molar ratio of butyric acid with the lowest number noticed in the challenged birds given the ZnB-supplemented diet (Table 6.7).

6.3.4 Gut morphology

E. coli challenge reduced ($P<0.05$) the villus height in the jejunum by approximately 15% on d 7 (Table 6.8). Diet did not alter the jejunal villi; however, MOS and/or ZnB reduced ($P<0.01$) the crypt depth compared to the negative control within the challenged and unchallenged groups. On d 21, there were no significant differences in the mucosal morphology due to either the challenge or diet but a challenge × diet interaction ($P=0.08$) was observed for villus height with the lowest value noticed in the challenged birds fed the MOS-supplemented diet (Table 6.8).

In the ileum, the villus height:crypt depth ratio tended ($P=0.06$) to be reduced by the challenge on d 7 (Table 6.9). The opposite trend was noticed in the villus height as well as the villus height:crypt depth ratio on d 21. There were no dietary or *E. coli* challenge × diet interactive effects on the mucosal morphology of ileum at both ages.

Table 6.6 Effects of dietary treatments on lactic and acetic acids concentrations (μmol/g digesta) in the ileal content of chickens at different ages[1]

Challenge[2] (C):	With				Without					P values		
Diet (D):	NC	MOS	FOS	ZnB	NC	MOS	FOS	ZnB	SEM	C	D	C×D
Day 7												
Lactic acid	11.4^{c}	15.2^{bc}	25.5^{ab}	10.6^{c}	9.4^{c}	18.5^{bc}	37.7^{a}	13.4^{bc}	13.28	0.23	0.00	0.50
Acetic acid	1.0^{bc}	0.9^{bc}	1.2^{bc}	0.8^{c}	1.4^{ab}	1.1^{bc}	1.7^{a}	1.6^{a}	0.469	0.00	0.09	0.33
Day 21												
Lactic acid	25.1^{ab}	38.5^{ab}	28.7^{ab}	45.0^{ab}	24.0^{ab}	14.6^{b}	50.0^{a}	14.4^{b}	30.2	0.26	0.41	0.05
Acetic acid	2.2^{a}	2.0^{ab}	1.8^{ab}	2.0^{ab}	1.3^{b}	1.7^{ab}	1.7^{ab}	1.8^{ab}	0.78	0.05	0.92	0.44

[1]Values are means of 8 replicates.
[2]A mixture of *E. coli* (E3, E30, E956 and E133) was used for the challenge.
[a,b]Means within one row sharing no common superscripts differ significantly at the levels as indicated for the factors ($P<0.05$).

Table 6.7 Effects of dietary treatments on caecal VFA profiles of chickens on day 21[1]

Challenge[2] (C):	With				Without					P values		
Diet (D):	NC	MOS	FOS	ZnB	NC	MOS	FOS	ZnB	SEM	C	D	C×D
Concentrations (μmol/mg digesta)												
Acetic acid	45.6	38.7	45.4	46.0	39.6	45.1	53.0	50.9	25.53	0.62	0.78	0.86
Propionic acid	4.0	2.8	2.0	3.0	3.0	2.5	4.7	3.2	2.63	0.58	0.80	0.21
Butyric acid	8.1	9.7	8.9	5.6	6.6	6.4	8.7	10.3	4.71	0.94	0.85	0.15
Total VFA	64.8	64.9	80.1	67.3	56.4	60.5	80.0	72.4	36.30	0.83	0.43	0.96
Molar ratio (% of total VFA)												
Acetic acid	70^{ab}	68^{b}	70^{ab}	69^{ab}	71^{ab}	74^{a}	72^{ab}	71^{ab}	5.7	0.05	0.94	0.64
Propionic acid	6.5^{a}	5.3^{ab}	4.0^{bc}	4.3^{bc}	4.0^{bc}	4.0^{bc}	4.5^{bc}	3.4^{c}	1.64	0.02	0.12	0.11
Butyric acid	13.0	12.4	12.8	9.0	12.0	13.8	12.8	14.0	3.48	0.14	0.64	0.10

[1]Values are means of 8 replicates.
[2]A mixture of *E. coli* (E3, E30, E956 and E133) was used for the challenge.
[a,b] Means within one row sharing no common superscripts differ significantly at the levels as indicated for the factors ($P<0.05$).

Table 6.8 Effects of dietary treatments on villus height (μm), crypt depth (μm) and the villus height:crypt depth ratio (Ratio) in the jejunum of chickens at different ages[1]

Challenge[2] (C):	With				Without					P values		
Diet (D):	NC	MOS	FOS	ZnB	NC	MOS	FOS	ZnB	SEM	C	D	C×D
Day 7												
Villus height	958^{ab}	860^{ab}	822^{b}	930^{ab}	1027^{a}	1005^{a}	969^{ab}	946^{ab}	166.2	0.03	0.44	0.62
Crypt depth	156^{ab}	137^{c}	158^{ab}	142^{bc}	168^{a}	142^{bc}	152^{abc}	156^{ab}	18.2	0.18	0.01	0.39
Ratio	6.17	6.20	5.27	5.81	6.12	6.55	6.60	6.13	1.248	0.13	0.75	0.46
Day 21												
Villus height	1035	872	1082	1059	1011	1023	916	980	86.8	0.50	0.60	0.08
Crypt depth	154	141	157	168	154	166	149	149	13.1	0.93	0.92	0.13
Ratio	6.72	6.26	7.00	6.41	7.14	6.32	6.15	6.60	1.104	0.88	0.44	0.40

[1]Values are means of 8 replicates.
[2]A mixture of *E. coli* (E3, E30, E956 and E133) was used for the challenge.
[a,b] Means within one row sharing no common superscripts differ significantly at the levels as indicated for the factors ($P<0.05$).

Table 6.9 Effects of dietary treatments on villus height (μm), crypt depth (μm) and the villus height:crypt depth ratio (Ratio) in the ileum of chickens at different ages[1]

Challenge[2] (C):	With				Without					P values		
Diet (D):	NC	MOS	FOS	ZnB	NC	MOS	FOS	ZnB	SEM	C	D	C×D
Day 7												
Villus height	485	504	462	494	504	498	542	458	35.2	0.43	0.71	0.14
Crypt depth	122	132	135	125	124	125	126	115	19.1	0.24	0.44	0.83
Ratio	4.0	3.9	3.4	3.7	4.2	4.0	4.4	4.0	0.73	0.06	0.73	0.36
Day 21												
Villus height	644^{a}	639^{a}	670^{a}	645^{a}	610^{ab}	580^{ab}	533^{b}	593^{ab}	84.3	0.00	0.84	0.33
Crypt depth	116	107	104	107	108	107	105	102	14.3	0.41	0.41	0.81
Ratio	6.0^{ab}	5.7^{ab}	6.4^{a}	6.1^{a}	5.6^{ab}	5.7^{ab}	5.2^{b}	5.9^{ab}	0.86	0.03	0.80	0.22

[1]Values are means of 8 replicates.
[2]A mixture of *E. coli* (E3, E30, E956 and E133) was used for the challenge.
[a,b] Means within one row sharing no common superscripts differ significantly at the levels as indicated for the factors ($P<0.05$).

6.4 DISCUSSION

6.4.1 Growth performance

The improvement in the BWG and FCR of the challenged birds given the MOS-supplemented diet is in agreement with the findings of Fairchild *et al.* (2001), who reported that the addition of MOS improved the growth performance of poults challenged with pathogenic *E. coli*. In line with this, Huan (1999) observed a 5.9% increase in daily growth rate of piglets on MOS treatment when they were reared under an environment contaminated with pathogenic *E. coli*. These observations indicate a growth-promoting effect of MOS in animals challenged with *E. coli*. The growth-promoting effects of MOS were comparable to that of ZnB treatment among the challenged and unchallenged groups, supporting MOS is a promising alternative to AGP in broiler chickens diets (Ao, 2004; Hooge, 2004a).

A large improvement in BWG was observed in birds fed the FOS-supplemented diet. Similar results were noticed by Ammerman *et al.* (1988) and Xu *et al.* (2003). Moreover, this effect of FOS was pronounced in 3-wk-old birds rather than in 1-wk-old birds. This observation may indicate that there is a time lag for the beneficial groups of bacteria to utilize FOS and become the predominant bacteria to help the gut microflora reach a balance that would support the body growth of birds (Xu *et al.*, 2003). However, the effect of FOS on FCR, especially that of the challenged birds, was not evident in the current experiment.

6.4.2 Development of gut microflora and its activity

E. coli challenge increased the coliform number in the small intestine of birds, particularly those associated with the gut wall. The number of mucosa-associated coliforms was suppressed by MOS in the challenged birds, which is in agreement with the report that MOS has a consistent effect against the attachment of *E. coli* in an *in vitro* chicken gut model (Peuranen *et al.*, 2006). In contrast, FOS reduced the coliform number in the gut lumen of the challenged birds; similar results were noticed in the piglets infected with *E. coli* on FOS treatment (Bunce *et al.*, 1995). A mixture of pathogenic *E. coli* was used in the current study. A common feature among pathogenic *E. coli* is to first attach to the gut wall and then cause infection (Smyth *et al.*, 1994). Therefore, it is reasonable to postulate that the balance of mucosa-associated bacteria was more disturbed by *E. coli* challenge compared to the luminal bacteria. By reducing the number of mucosa-associated coliforms, MOS probably stabilized the microbial balance at the gut mucosa and the gut integrity was protected and thus the growth performance of the challenged birds was improved by MOS compared to FOS.

However, MOS had no effects on the mucosa-associated coliforms in the unchallenged birds in the current study. In contrast to this, our previous results (Yang *et al.*, 2006) showed that there was a tendency for MOS to decrease the mucosa-associated coliforms in birds given the same basal diet. The inconsistency in the results might be due to the fact that gut tissue samples were taken from birds at different ages; the gut microflora of young birds is under transition (Lu *et al.*, 2006) and the profile of mucosa-associated coliforms, especially those possessing type-1 fimbriae, may also be transitional.

Dietary FOS did not increase the density of luminal lactobacilli of birds, which is contrary to the report by Xu *et al.* (2003), but a tendency for FOS to increase the mucosa-associated lactobacilli was noticed in the jejunum at the end of week 3. There is no published information regarding the effects of FOS on the mucosa-associated bacteria of birds, but fructans were shown to increase mucosa-associated bifidobacteria in rats (Kleessen *et al.*, 2003). An increase in the number of mucosa-associated lactobacilli was also noticed for MOS and ZnB treatments within the unchallenged group, which may support the growth-improving effects induced by these dietary additives as the adherence of lactic acid bacteria to the mucosa may limit the interactions of pathogens with enterocytes (Bernet *et al.*, 1993) and gut integrity is protected to support body growth.

The growth-promoting effects of the dietary additives, particularly ZnB, can also be related to the suppression of *C. perfringens*, the causative agent of necrotic enteritis in chickens. The inhibitory effects of the in-feed additives were noticed as early as 1 week of age but became less pronounced when the birds got older. The gut microflora and immune system are more mature in older birds and the selection of broiler chicks for early immune response to *E. coli* leads to an overall increase in early immune system maturation (Erf, 1997). Consequentially the natural ability of birds to prohibit and exclude pathogens increases, which might mask the inhibitory effects of dietary additives on the growth of pathogens in the gut.

However, an increase in the coliform population attached to the gut mucosa was observed in 3-week-old birds given the ZnB-supplemented diet. There are no published reports on the effects of ZnB on the mucosa-associated coliform. A reduced coliform number in the gut lumen was noticed by Engberg *et al.* (2000). The inconsistency in the populations of coliform bacteria in the gut of birds on ZnB treatment is difficult to explain but it is known that ZnB does not specifically target *E. coli* as this compound is active mainly against Gram-positive bacteria (Butaye *et al.*, 2003).

The growth of total anaerobic bacteria and lactobacilli was inhibited by ZnB in the present study, which agrees with the findings of Engberg *et al.* (2000). The effect was more obvious in the unchallenged birds than in the challenged birds, indicating that the normal development of gut microflora was disturbed by *E. coli* challenge in the current study, under which condition it might take a longer time for the in-feed additives to stabilize and maintain a normal or healthy gut microflora. This speculation is indirectly supported by the results of the lactic acid contents and the pH values in the small intestine of birds fed the FOS-supplemented diet.

In agreement with the findings of van de Wiele *et al.* (2004), the lactic acid concentration in the gut was increased by FOS and this effect of FOS was noticed in both the challenged and unchallenged birds at the end of week 1 and in the unchallenged birds at the end of week 3. It is well documented that, by lowering the pH, organic acids produced by lactic acid bacteria can inhibit the growth of pathogens (Ouwehand, 1998), such as pathogenic *E. coli* in the coliform group. However, a lower pH value was noticed only in 3-week-old birds given the FOS-supplemented feed within the unchallenged group. Comparatively MOS and ZnB had minimal effects on the profiles of the intestinal lactic acid as well as the pH values of the small intestine.

6.4.3 Development of gut morphology

In the current trial, villus atrophy, in particular in the jejunum, was noticed in the chickens challenged with *E. coli*; a similar result was noticed in piglets infected with enterotoxigenic *E. coli* (Cox *et al.*, 1988). The protective effect of MOS and ZnB on the gut mucosa, mainly in the jejunum, was shown in birds fed diets containing these supplements. For example, compared to the negative control, MOS showed a clear effect on the crypt, decreasing its depth, especially at the end of week 1. A shallow crypt was also noticed by Bradley *et al.* (1994) and Savage *et al.* (1996b) in the birds given a MOS-supplemented diet compared to controls. The depth of the crypt is a function of the rate of cell replacement (Uni *et al.*, 1998). These cells have a life span of 72 hours in chicks from 4 days after hatching and 96 hours in older birds under normal conditions (Sklan, 2001). This high cell turnover is accompanied by an extremely high rate of metabolism, involving 23 to 36% of the whole body energy expenditure (Summers, 1991). A shallow crypt (lower cell turnover rate) of the intestine results in a lower maintenance requirement, which can lead to a higher growth rate or growth efficiency of the animal. Therefore, a reduction in the crypt depth directly indicates the

positive effects of MOS on the growth performance of the birds in the current trial as well as on the energy utilization in a previous trial. The shallow crypt perhaps can be related to the lower counts of mucosa-associated coliforms noticed in birds fed the MOS-supplemented diet, a reduced bacterial load in the gut wall of the challenged birds. By attaching to the gut wall, bacteria, particularly pathogens and/or their toxins, can stimulate cell proliferation and increase depth of crypt (Ichikawa *et al.*, 1999).

6.5 CONCLUSIONS

The present study demonstrated that MOS improved the growth performance of the challenged birds more than FOS did but the opposite trend was noticed with the unchallenged birds. Depending on the challenge and the age of birds, dietary MOS and FOS had different modulatory effects on the populations of the selected groups of bacteria. For example MOS, but not FOS, reduced the number of mucosa-associated coliforms of 7-day-old birds within the challenged group. On the other hand, MOS and FOS shared some similarity in altering the profile of gut bacteria, such as reducing the density of *C. perfringens*. The activity of gut bacteria was altered by FOS rather than MOS whereas the gut morphology was affected only by MOS. In conclusion, the effects of MOS or FOS on the digestive system of birds were related to *E. coli* challenge as well as the age of birds, which may be involved in the observed different growth-improving effects of the tested dietary additives.

A common feature among the different in-feed additives in the modulation of gut microflora seems to be an increase in the populations of mucosa-associated lactobacilli, which might be an interesting subject for future studies regarding mucosal nutrition and integrity in broiler chickens.

CHAPTER 7
USE OF DIETARY MANNANOLIGOSACCHARIDE WITH OR WITHOUT EXOGENOUS ENZYME IN DIFFERENT CEREAL-BASED BROILER CHICKEN DIETS

7.1 INTRODUCTION

Besides being the major source of dietary energy and other essential nutrients for commercial poultry production, cereal grains are also the prime source of anti-nutritive components (Hughes, 2003). The NSP are a group of such components in grains, such as wheat and barely, that have drawn attention over the past two decades (Choct, 2002). Although the resultant increase in intestinal viscosity was shown to be the most important physiological impact on birds, it was suggested that the antinutritive effects of NSP are also mediated through the gut microflora (Choct *et al.*, 1992; Choct *et al.*, 1996), including potential pathogens that adhere to the mucosa of the small intestine (Untawale and Mcginnis, 1979; Danicke *et al.*, 1999).

The beneficial modulatory effects of MOS on the gut microflora were demonstrated in the previous *E. coli* challenge trial (Chapter 6). The hypothesis tested in the present experiment was that supplementation with MOS could improve gut development and growth performance of birds fed different cereal (corn or wheat)-based diets through the modulation of the gut microflora of young birds. An additional aim of this study was to compare the effects of MOS and xylanase, individually or in combination, on the growth performance and gut development of birds fed wheat-based diets.

7.2 MATERIALS AND METHODS

7.2.1 Experimental design and birds

Three basal diets were used, namely: a corn-based diet (corn diet), a wheat-based diet (wheat diet), and a wheat-based diet supplemented with xylanase (wheat plus xylanase diet). The xylanase, Allzyme PT, was obtained from Alltech Biotechnology, Kentucky, USA. The basal diets were formulated to be isocaloric and isonitrogenous, as shown in Table 7.1 (starter diet) and Table 7.2 (grower diet). In addition, the different basal diets were also supplemented either with or without MOS at a dosage of 2g/kg and 1g/kg in the starter and

grower diets, respectively. Acid-insoluble ash (AIA) was included at 0.8% in the diets at the expense of corn or wheat, as a digestibility marker.

Table 7.1 Composition (g/kg) of basal starter diets

Ingredients	Corn diet	Wheat diet	Wheat+xylanase diet
Corn	610.0	-	-
Wheat	-	624.0	653.0
Soy	310.0	265.0	257.0
Meat meal	50.0	50.0	50.0
Limestone	10.0	9.90	12.80
Dicalcium phosphate	3.0	3.0	2.7
Lyscine-HCl	1.9	2.8	3.0
Methionine	1.9	1.9	1.8
Salt	1.0	0.5	0.5
Sodium bicarbonate	3.4	3.3	3.5
Choline chloride	2.2	3.0	3.2
Sun oil	5.0	34.8	10.0
L-threonine	-	0.2	0.3
Xylanase[1]	-	-	0.5
Vitamin and mineral premix[2]	2.0	2.0	2.0
Chemical composition			
ME, MJ/kg	12.33	12.33	12.33
Crude protein	230.00	230.00	230.00
Crude fibre	37.00	29.00	29.00
Crude fat	38.00	55.00	31.00
Lys	13.00	13.00	13.00
Met+Cys	9.00	9.00	9.00
Ca	10.00	10.00	10.00
P available	4.00	4.20	4.20
Na	1.80	1.80	1.80
Cl	2.00	2.00	2.00

[1]1000 XU/kg diet.
[2]The same as described in Chapter 3.

Table 7.2 Composition (g/kg) of basal grower diets

Ingredients	Corn diet	Wheat diet	Wheat+xylanase diet
Corn	662.8	-	-
Wheat	-	672.0	723.0
Soy	257.0	210.0	196.0
Meat meal	50.0	50.0	50.0
Limestone	9.5	13.0	9.2
Dicalcium phosphate	4.2	2.7	2.5
Lyscine-HCl	2.0	3.0	3.3
Methionine	1.4	1.5	1.5
Salt	1.0	1.0	1.1
Sodium bicarbonate	3.1	2.2	1.9
Choline chloride	1.0	2.0	1.3
Sun oil	6.0	39.9	7.3
L-thronine	-	0.5	0.7
Xylanase[1]	-	-	0.5
Vitamin and mineral premix[2]	2.0	2.0	2.0
Chemical composition			
ME, MJ/kg	12.54	12.54	12.54
Crude protein	210.00	210.00	209.50
Crude fibre	36.40	27.00	27.40
Crude fat	40.30	60.40	28.70
Lys	11.90	11.90	11.90
Met+Cys	8.00	8.10	8.20
Ca	10.00	11.00	9.50
P available	4.10	4.10	4.10
Na	1.70	1.70	1.70
Cl	1.80	2.20	2.20

[1] 1000 XU/kg diet.
[2] The same as described in Chapter 3.

Three hundred and eighty-four (384) day-old birds were obtained from a local hatchery (Baiada hatchery, Kootingal, NSW) and randomly allocated to the six treatments, each with 8 replicates (cages) of 8 birds per replicate. The experiment lasted for five weeks and birds were managed as described in Section 3.2.2.

The experiment was approved by the Animal Ethics Committee of the University of New England with approval number of AEC06/050.

7.2.2 Measurements and sample collection

Feed intake and body weight were measured when birds were at 7, 21, and 35 days of age. The individual body weight was measured with birds on d 35 for the determination of flock uniformity, as described in Section 4.2.3.

Intestinal samples were taken from two birds per cage, 16 birds per treatment at the end of weeks 1 and 3. Pooled digesta samples from the ileum and caeca were used for the determination of nutrient contents, bacterial populations and/or VFA concentrations. Pooled gut tissue samples from the duodenum, jejunum, and ileum were used to examine the populations of tissue-associated bacteria. Jejunal and ileal tissue samples from one bird per cage were used for histological analyses. Details of sample collection and processing of samples were outlined in Sections 3.2.2 and 5.2.3. Additionally, on d 21 blood samples were collected with heparinised syringe from the heart of the chickens. Blood samples were collected on ice and centrifuged (3,000 ×*g*, 15 min) to obtain the plasma, which was then stored at -18 °C until further analysis.

7.2.3 Sample analyses

Bacterial enumerations, intestinal histo-morphological studies and analysis of VFA were assessed as described in Sections 3.2.4 and 5.2.5. In addition, the external muscularis thickness was measured as defined in Figure 7.1.

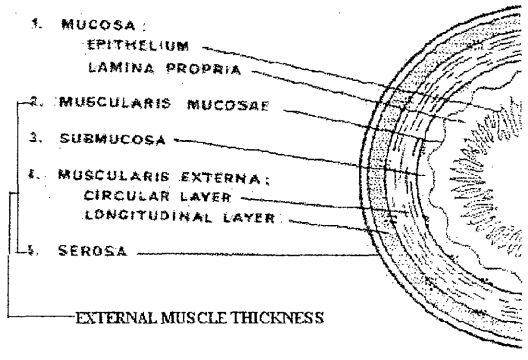

Figure 7.1 Diagrammatic representation of the basic structure of the digestive tract (Jensen, 1976).

The activities of maltase, sucrase and AP were analysed as described in Section 4.2.3 while leucine aminopeptidase (LAP; EC. 3.4.11.2) was assayed according to the method of Miura *et al.* (1983). Twenty-five µL of vesicle samples (diluted 1:60) were incubated with 200 µL of distilled water; 100 µL of 200 mM phosphate buffer, pH 7.0, and 100 µL of 8 mM leucine β-naphthylamide at 39 °C for 30 min. Incubation was terminated with 300 µL of 30 % TCA. The reaction mixture was further incubated at room temperature with 100 µL of 0.3 % sodium nitrite; 100 µL of 1.5 % ammonium sulphamate and 300 µL of 0.1 % (in ethanol) N-

1-naphthylethylene diamine-dihydrochloride, vortexing after the addition of each of these reagents. The absorbance was then read at 560 nm. A standard curve was derived from similarly treating varying concentrations of leucine β-naphthylamide as the samples in the second incubation stage.

At the end of week 1, the specific activities of maltase and AP were enriched by 6 and 3 times, respectively, and at the end of week 3, the specific activities of these two enzymes were enriched by 15 and 7 times, respectively. This indicates that, as in this case, the membrane vesicles had been purified compared to the homogenate samples.

Non-starch polysaccharide contents in the corn and wheat diets were analysed as described in Section 5.2.6. Starch and protein contents in the ileal digesta and feeds were analysed as described in Section 3.2.5. Acid-insoluble ash in the ileal digesta was determined according to the method described by Choct *et al.* (1992). Briefly, diet samples (approximately 3 g) or digesta samples (approximately 1 g) were dried at 105 °C for 16 h. Dried samples were accurately weighed and ashed at 480 °C for 8 h. The ash was then boiled in 4 M HCl for 15 min and rinsed with 4 M HCl followed by distilled water. The residue was dried at 105 °C for 16 h and collected as the AIA. Digestibility of starch and protein were calculated according to the following equation:

$$\text{Digestibility} = (1 - \frac{\text{digesta nutrient (g/kg)/digesta AIA (g/kg)}}{\text{diet nutrient (g/kg)/diet AIA (g/kg)}}) \times 100$$

The salicylate method described by Krom (1980) was adopted to analyse ammonia concentration in blood plasma. In principle, free ammonia reacts with hypochlorite to form monochloramine that reacts with salicylate, in the presence of sodium nitro-ferricyanide, to form 5-aminosalicylate, a greenish complex. The colour intensity was spectrophotometrically measured at 725 nm and results are expressed in μg/mL ammonia-nitrogen, NH_3-N.

7.2.4 Statistical analyses

Data were analysed using the General Linear Model (GLM) procedure of SPSS (SPSS Inc, Version 12) as a 3 (basal diets) × 2 (levels of MOS). When a significant ($P<0.05$) *F*-test was detected for the main effects or the interaction, the corresponding means were compared by LSD. Bacterial results obtained from measurements in the duodenum, jejunum, ileum and caeca were analyzed separately for each gastrointestinal segment. In addition, the effects of

adding MOS or xylanase singly into the wheat-based diet on the growth performance and the parameters of gut physiology were tested with non-orthogonal contrast (P<0.05).

7.3 RESULTS

7.3.1 NSP contents in the corn-based and wheat-based diets

The constituent sugars of NSP in the corn and wheat diets are shown in Table 7.3. The main sugars present were glucose, arabinose and xylose. The contents of arabinose and xylose in the soluble NSP fraction in the wheat diet were about 2 and 3 times higher than those in the corn diet.

Table 7.3 Composition of sugar (g/kg) in the corn and wheat starter diets

	Corn diet			Wheat diet		
	Free sugar	Insoluble NSP fraction	Soluble NSP fraction	Free sugar	Insoluble NSP fraction	Soluble NSP fraction
Rhamnose	0.00	0.00	0.00	0.00	0.00	0.00
Fucose	0.54	0.90	0.00	0.42	0.67	0.00
Ribose	0.00	0.00	0.21	0.00	0.00	0.23
Arabinose	0.53	17.99	1.05	0.40	20.43	2.80
Xylose	0.00	17.13	0.40	0.00	22.21	3.02
Mannose	4.86	1.90	1.42	4.44	2.32	1.30
Galactose	8.76	14.68	1.29	7.94	12.10	1.98
Glucose	26.01	21.75	0.71	22.05	22.67	1.01
Total NSP	40.7	74.35	5.08	35.25	80.4	10.34

7.3.2 Growth performance and flock uniformity

Diet and MOS addition had no significant effect (P>0.05) on the BWG and FI of birds in the first 7 days of life but MOS increased (P<0.05) the FCR of birds (Table 7.4). An interaction (P=0.09) between diet and MOS was noticed in the FI of birds with the highest FI noticed in birds given the corn plus MOS diet. No significant differences in the growth rate of birds were observed between the wheat plus MOS and the wheat plus xylanase treatments.

Between 7 and 21 d, diet affected (P<0.05) the growth performance of birds whereas MOS only tended (P=0.06) to affect the BWG of birds (Table 7.5). Birds given the corn diet had higher BWG but lower FI and FCR than those given the wheat diet. A diet × MOS interaction (P<0.01) indicated that MOS increased the BWG of birds given the corn or wheat diets but the opposite trend was observed with birds on the wheat plus xylanase treatment. A

diet × MOS interaction was also noticed in the FCR of birds, whereby a decrease in FCR, due to MOS, was observed in birds given the wheat diet but not in those given the corn or the wheat plus xylanase diets.

Compared to the xylanase supplement, MOS increased (P<0.05) the BWG and FI of birds fed the wheat-based diet. However, the addition of both xylanase and MOS did not further improve the growth performance of birds.

Table 7.4 Effects of MOS supplementation on body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) of broilers fed the experimental diets in week 1[1]

Treatment		BWG	FI	FCR
Diet	MOS[2]	g/bird	g/bird	g/g
Corn diet	-	157	189	1.22[b]
	+	157	217	1.34[a]
Wheat diet	-	159	199	1.26[ab]
	+	158	200	1.28[ab]
Wheat+xylanase diet	-	162	195	1.21[b]
	+	155	192	1.24[ab]
SEM		9.6	9.9	0.073
Source of variation				
Diet		NS[3]	NS	NS
MOS		NS	0.06	0.05
Diet*MOS		NS	0.09	NS
Contrast wheat+MOS vs wheat+Xylanase		NS	NS	NS

[1]Values are means of 6 replicates. [2] 2g/kg diet.
[3] NS: nonsignificant (P>0.05). [a-b] Means in a column not sharing a common superscript differ (P<0.05).

Table 7.5 Effects of MOS supplementation on body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) of broilers fed the experimental diets during weeks 2 and 3[1]

Treatment		BWG	FI	FCR
Diet	MOS[2]	g/bird	g/bird	g/g
Corn diet	-	846^{ab}	1256^{b}	1.52^{c}
	+	865^{a}	1324^{ab}	1.56^{bc}
Wheat diet	-	740^{c}	1354^{b}	1.83^{a}
	+	864^{a}	1446^{a}	1.63^{bc}
Wheat+xylanase diet	-	814^{ab}	1304^{b}	1.65^{bc}
	+	781^{bc}	1315^{b}	1.70^{ab}
SEM		56.2	88.0	0.123
Source of variation				
Diet		0.03	0.01	0.00
MOS		0.06	NS	NS
Diet*MOS		0.01	NS[3]	0.03
Contrast wheat+MOS vs wheat+xylanase		0.05	0.00	NS

[1]Values are means of 6 replicates. [2] 2g/kg diet.
[3]NS: nonsignificant (P>0.05). [a-c] Means in a column not sharing a common superscript differ (P<0.05).

During weeks 4 and 5, the growth performance was better (P<0.05) among the birds fed the corn diet than those fed the wheat-based diet with or without xyalanase supplementation (Table 7.6). There were no effects of MOS on the growth performance of birds. However, a diet × MOS interaction (P<0.05) was noticed in the FI of birds, which was reduced (P<0.05) when MOS was added to the wheat plus xylanase diet. No differences in the growth rate between the MOS and the xylanase supplements were observed in birds given the wheat-based diet.

The flock uniformity of birds was affected (P<0.01) by diet (Table 7.6). Improved flock uniformity was observed in birds given the corn or the wheat plus xylanase diets compared to the wheat diet. A highly significant diet × MOS interaction (P<0.01) indicated that the addition of MOS to the wheat diet could improve (P<0.05) the flock uniformity of birds. On the wheat-based diet, the xylanase supplement showed a similar improvement in the flock uniformity like MOS.

Table 7.6 Effects of MOS supplementation on body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and flock uniformity of broilers fed the experimental diets during weeks 4 to 5[1]

Treatment		BWG	FI	FCR	Flock uniformity[3]
Diet	MOS[2]	g/bird	g/bird	g/g	%
Corn diet	-	1227^{ab}	1981^{c}	1.55^{c}	90.0^{a}
	+	1281^{a}	2124^{bc}	1.67^{bc}	90.1^{a}
Wheat diet	-	1085^{b}	2250^{ab}	1.97^{a}	86.5^{c}
	+	1185^{ab}	2256^{ab}	1.92^{ab}	89.3^{a}
Wheat+xylanase diet	-	1107^{b}	2436^{a}	2.01^{a}	89.7^{a}
	+	1139^{ab}	2196^{b}	1.94^{a}	88.0^{b}
SEM		76.2	162.3	0.217	0.837
Source of variation					
Diet		0.04	0.00	0.00	0.01
MOS		NS[4]	NS	NS	NS
Diet*MOS		NS	0.02	NS	0.00
Contrast wheat+MOS vs wheat+xylanase		NS	0.07	NS	NS

[1]Values are means of 5 replicates. [2] 1g/kg diet.
[3]Five replicates of 10 birds each on d 35. [4] NS: nonsignificant (P>0.05).
[a-c] Means in a column not sharing a common superscript differ (P<0.05).

7.3.3 Gut microflora and its activity

Mucosa-associated bacteria

At 7 days of age, MOS reduced (P<0.05) the counts of coliform bacteria in all the sections of the gut but did not affect the populations of lactobacilli (Table 7.7). Diet affected (P<0.05) the populations of coliforms in the jejunum and that of lactobacilli in the ileum with the birds given the wheat-based diets having a lower number of coliforms and a higher number of lactobacilli than those given the corn-based diets. The addition of MOS to the wheat-based diet tended (P=0.06) to reduce or reduced (P<0.05) the number of coliform bacteria in the duodenum and ileum compared to the xylanase supplement.

At 21 days of age, the number of mucosa-associated coliforms along the small intestine was still lower (P<0.05) in birds given the MOS-supplemented diets than in those fed the diets without MOS supplementation while the reverse pattern was noticed with the number of mucosa-associated lactobacilli (Table 7.7). There was a strong tendency (P=0.07 or 0.08) for diet to affect the number of mucosa-associated coliforms along the small intestine, with higher numbers noticed in birds given the wheat plus xylanase diet compared to the corn diet (Table 7.7).

Table 7.7 Effects of MOS supplementation on the counts (log CFU/g tissue) of mucosa-associated lactobacilli and coliforms in the small intestine of broilers fed the experimental diets on days 7 and 21[1]

Treatment		Duodenum		Jejunum		Ileum	
Diet	MOS[2]	Coliforms	Lactobacilli	Coliforms	Lactobacilli	Coliforms	Lactobacilli
				Day 7			
Corn diet	-	5.20^{a}	6.75	5.46^{a}	6.67	5.54^{a}	6.13^{b}
	+	4.00^{b}	6.24	4.40^{bc}	6.57	4.49^{b}	6.26^{b}
Wheat diet	-	4.47^{b}	6.57	4.61^{bc}	6.08	4.68^{b}	6.47^{ab}
	+	3.84^{b}	6.48	4.53^{bc}	6.48	4.38^{b}	6.40^{ab}
Wheat+xylanase diet	-	4.52^{ab}	6.48	4.67^{b}	6.82	5.38^{a}	6.61^{ab}
	+	3.93^{b}	6.86	3.90^{c}	6.62	4.33^{b}	6.77^{a}
SEM		0.593	0.506	0.609	0.561	0.533	0.389
Source of variation							
Diet		NS[3]	NS	0.04	NS	NS	0.02
MOS		0.00	NS	0.00	NS	0.00	NS
Diet*MOS		NS	NS	NS	NS	NS	NS
Contrast wheat+MOS vs wheat+xylanase		0.06	NS	NS	NS	0.00	NS
				Day 21			
Corn diet	-	4.61^{a}	4.57^{b}	4.30^{bc}	4.43^{c}	5.11^{ab}	5.32^{b}
	+	3.58^{b}	5.86^{a}	3.94^{c}	5.49^{bc}	4.58^{b}	5.78^{b}
Wheat diet	-	4.66^{a}	5.71^{a}	4.80^{ab}	5.70^{ab}	5.29^{ab}	6.14^{ab}
	+	4.41^{a}	6.56^{a}	4.12^{bc}	6.29^{ab}	4.80^{b}	6.83^{a}
Wheat+xylanase diet	-	4.95^{a}	5.89^{a}	5.13^{a}	5.73^{ab}	5.89^{a}	6.19^{ab}
	+	4.46^{a}	6.82^{a}	4.41^{abc}	6.87^{a}	5.15^{ab}	6.99^{a}
SEM		0.369	0.950	0.386	0.609	0.699	0.443
Source of variation							
Diet		0.07	0.02	0.08	0.01	0.07	0.00
MOS		0.01	0.00	0.01	0.01	0.02	0.02
Diet*MOS		NS	NS	NS	NS	NS	NS
Contrast wheat+MOS vs wheat+xylanase		NS	NS	0.01	NS	0.01	NS

[1] Values are means of 6 replicates. [2] 2g/kg diet.
[3] NS: nonsignificant (P>0.05). [a-c] Means in a column not sharing a common superscript differ (P<0.05).

The number of mucosa-associated lactobacilli was also affected ($P<0.05$) by diet, with higher numbers noticed on the wheat and the wheat plus xylanase treatments compared to the corn diet treatment. The addition of MOS to the wheat-based diet reduced ($P<0.05$) the number of mucosa-associated coliforms in the jejunum and ileum compared to the xylanase supplement.

No interaction between diet and MOS was noticed in the populations of coliforms and lactobacilli attached to the small intestine at both ages.

Luminal bacteria

Diet and MOS supplementation had no effects on the populations of total anaerobic bacteria and lactobacilli in the ileum of 7-day-old birds (Table 7.8). However, both diet and MOS affected ($P<0.05$) the number of coliforms and the interaction ($P<0.05$) between diet and MOS highlights that MOS reduced the number of coliforms in birds given the wheat plus xylanase diet more than in those given the other two basal diets. The count of coliforms was lower ($P<0.05$) in birds given the wheat diet compared to the corn diet but the reverse pattern was observed with the count of *C. perfringens*. The addition of xylanase to the wheat-based diet favored the growth of *C. perfringens*, which was higher ($P<0.05$) compared to MOS treatment.

In the caeca, diet altered the populations of total anaerobic bacteria, lactobacilli as well as coliforms with the wheat plus xylanase diet enhancing the growth of those bacteria compared to the corn diet. The addition of MOS did not affect the populations of the selected groups of bacteria except that of *C. perfringens*, which was higher ($P=0.09$) in birds fed the MOS-supplemented diets than in those without MOS supplementation in the corn and the wheat plus xylanase treatment groups (Table 7.8). A diet × MOS interaction ($P<0.05$) was noticed in the populations of lactobacilli; birds on the wheat plus MOS treatment had the lowest number of lactobacilli among all the treatments. Dietary MOS reduced ($P<0.05$) the number of total anaerobic bacteria and lactobacilli compared to xylanase treatment.

Table 7.8 Effects of MOS supplementation on the counts (log CFU/g digesta) of selected groups of bacteria in the ileum and caeca of birds fed the experimental diets on day 7[1]

Treatment		TAB[3]	Lactobacilli	Coliforms	*C. perfringens*
Diet	MOS[2]				
				Ileum	
Corn diet	-	8.74	8.28	6.70^{a}	4.85^{bc}
	+	9.01	8.51	6.21^{ab}	3.88^{c}
Wheat diet	-	8.45	8.40	6.39^{ab}	5.38^{b}
	+	8.63	8.47	5.99^{bc}	5.40^{b}
Wheat+xylanase diet	-	8.65	8.23	6.62^{a}	7.35^{a}
	+	8.94	8.40	5.06^{c}	7.74^{a}
SEM		0.45	0.33	0.67	1.56
Source of variation					
Diet		NS[4]	NS	0.03	0.00
MOS		NS	NS	0.00	NS
Diet*MOS		NS	NS	0.02	NS
Contrast wheat+MOS vs wheat+xylanase		NS	NS	0.04	0.00
				Caeca	
Corn diet	-	9.44^{b}	9.27^{b}	8.49^{b}	5.69
	+	9.48^{b}	9.34^{b}	8.44^{b}	6.00
Wheat diet	-	9.64^{ab}	9.39^{b}	8.90^{ab}	5.68
	+	9.60^{ab}	8.79^{c}	8.89^{ab}	5.51
Wheat+xylanase diet	-	10.03^{a}	9.40^{b}	9.23^{a}	5.42
	+	9.92^{a}	9.82^{a}	9.22^{a}	7.32
SEM		0.392	0.443	0.483	1.275
Source of variation					
Diet		0.00	0.00	0.00	NS
MOS		NS	NS	NS	0.09
Diet*MOS		NS	0.01	NS	NS
Contrast wheat+MOS vs wheat+xylanase		0.05	0.02	NS	NS

[1]Values are means of 6 replicates. [2]2g/kg diet.
[3]TAB: total anaerobic bacteria. [4]NS: nonsignificant (P>0.05).
[a-c]Means in a column not sharing a common superscript differ (P<0.05).

At 21 days of age, MOS had no effects on the populations of the selected groups of bacteria in the ileum but reduced (P<0.05) the number of *C. perfringens* in the caeca of birds (Table 7.9). The wheat diet displayed higher bacterial growth, with the exception of coliforms in the caeca, compared to the corn diet. The addition of MOS to the wheat-based diet increased (P<0.05) the number of lactobacilli in the ileum but reduced (P<0.05) the number of coliforms in the caeca compared to the xylanase supplement.

Table 7.9 Effects of MOS supplementation on the counts (log CFU/g digesta) of selected groups of bacteria in the ileum and caeca of birds fed the experimental diets on day 21[1]

Treatment		TAB[3]	Lactobacilli	Coliforms	*C. perfringens*
Diet	MOS[2]				
				Ileum	
Corn diet	-	8.32^{ab}	6.94^{c}	6.51^{ab}	7.14
	+	8.01^{b}	7.29^{bc}	5.85^{b}	6.97
Wheat diet	-	8.96^{a}	9.06^{a}	6.23^{b}	7.61
	+	8.77^{a}	8.64^{a}	6.01^{b}	7.37
Wheat+xylanase diet	-	8.37^{ab}	7.22^{bc}	6.65^{ab}	7.85
	+	8.91^{a}	8.05^{ab}	7.09^{a}	7.87
SEM		0.565	0.911	0.710	0.832
Source of variation					
Diet		0.02	0.00	0.03	0.07
MOS		NS[4]	NS	NS	NS
Diet*MOS		NS	NS	NS	NS
Contrast wheat+MOS vs wheat+xylanase		NS	0.01	NS	NS
				Caeca	
Corn diet	-	9.52^{ab}	8.63^{c}	8.55	7.52^{b}
	+	9.20^{b}	8.72^{c}	8.46	6.36^{c}
Wheat diet	-	9.82^{a}	9.69^{ab}	8.56	8.36^{ab}
	+	9.80^{a}	9.70^{ab}	8.19	8.09^{ab}
Wheat+xylanase diet	-	9.77^{a}	9.22^{bc}	8.60	8.65^{a}
	+	9.92^{a}	9.85^{a}	8.61	8.39^{ab}
SEM		0.224	0.488	0.290	0.783
Source of variation					
Diet		0.01	0.00	NS	0.00
MOS		NS	NS	NS	0.04
Diet*MOS		NS	NS	NS	NS
Contrast wheat+MOS vs wheat+xylanase		NS	NS	0.02	NS

[1]Values are means of 6 replicates. [2] 2g/kg diet.
[3]TAB: total anaerobic bacteria. [4] NS: nonsignificant (P>0.05).
[a-c] Means in a column not sharing a common superscript differ (P<0.05).

The corn diet showed higher pH value in the caeca on d 7 and in the ileum on d 21 than the wheat-based diets did (Table 7.10). The addition of MOS reduced (P<0.05) the ileal pH value of birds on d 7 but did not affect the intestinal pH of birds on d 21.

Table 7.10 Effects of MOS supplementation on the intestinal pH of birds fed the experimental diets on days 7 and 21[1]

Treatment		Day 7		Day 21	
Diet	MOS[2]	Ileum	Caeca	Ileum	Caeca
Corn diet	-	7.63^{b}	7.37^{a}	7.77^{a}	6.82
	+	7.62^{b}	6.95^{ab}	7.63^{ab}	7.02
Wheat diet	-	8.13^{a}	6.36^{bc}	6.41^{c}	6.82
	+	7.67^{ab}	6.48^{bc}	7.06^{b}	6.72
Wheat+xylanase diet	-	7.84^{ab}	6.58^{bc}	7.25^{ab}	6.48
	+	7.48^{b}	6.27^{c}	7.13^{ab}	6.47
SEM		0.376	0.566	0.537	0.540
Source of variation					
Diet		NS[3]	0.05	0.00	NS
MOS		0.04	NS	NS	NS
Diet*MOS		NS	NS	NS	NS
Contrast wheat+MOS vs wheat+xylanase		NS	NS	NS	NS

[1]Values are means of 6 replicates. [2] 2g/kg diet.
[3] NS:nonsignificant (P>0.05). [a-c] Means in a column not sharing a common superscript differ (P<0.05).

Diet influenced (P<0.05) the concentrations of propionic acid and total VFA in the caeca of birds at 21 days of age (Table 7.11). Birds fed the corn diet had a higher (P<0.05) concentration of propionic acid but less (P<0.05) total VFA than those given the wheat-based diet with or without xylanase supplementation. The molar ratio of acetic and/or propionic acids was affected by diet and MOS and an interaction (P<0.05) between diet and MOS in these two parameters indicated that the effects of MOS on the molar ratio of the individual VFA are largely dependent on the kind of diet. On the wheat diet, MOS increased (P<0.05) the molar ratio of acetic and propionic acids in the caeca of birds; these values were also higher (P<0.05) than the values of birds fed the diet supplemented with xylanase, but the reverse pattern was the case in terms of the total VFA concentration.

Table 7.11 Effects of MOS supplementation on caecal VFA concentrations and molar ratio of birds fed the experimental diets on day 21[1]

Treatment		Concentration (μmol/g digesta)				Molar ratio (% of total VFA)		
Diet	MOS[2]	Acetic acid	Propionic acid	Butyric acid	Total VFA	Acetic acid	Propionic acid	Butyric acid
Corn diet	-	43.5	1.9^{ab}	5.0	34.2^{c}	73.7^{ab}	3.3^{b}	10.7
	+	44.4	2.1^{a}	4.9	48.4^{abc}	77.4^{a}	3.2^{bc}	9.5
Wheat diet	-	41.6	1.6^{ab}	8.2	60.1^{ab}	50.0^{d}	2.7^{bc}	10.3
	+	32.1	2.0^{ab}	5.6	44.6^{bc}	70.6^{b}	4.4^{a}	13.3
Wheat+xylanase diet	-	43.1	1.2^{ab}	4.7	74.2^{a}	58.5^{c}	3.2^{bc}	11.6
	+	30.8	0.9^{b}	6.1	60.3^{ab}	45.6^{d}	2.0^{c}	10.6
SEM		17.92	0.97	3.33	21.19	5.35	0.98	4.06
Source of variation								
Diet		NS[3]	0.05	NS	0.03	0.00	0.09	NS
MOS		NS	NS	NS	NS	0.04	NS	NS
Diet*MOS		NS	NS	NS	NS	0.00	0.01	NS
Contrast wheat+MOS vs wheat+xylanase		NS	NS	NS	0.03	0.00	0.05	NS

[1]Values are means of 6 replicates. [2] 2g/kg diet.
[3]NS: nonsignificant (P>0.05). [a-d]Means in a column not sharing a common superscript differ (P<0.05).

7.3.4 Digestibility of nutrient and plasma ammonia concentration

The ileal digestibility of starch and protein was higher (P<0.01 and P=0.06, respectively) in birds fed the corn diet than in those fed the wheat diet (Table 7.12). A highly significant interaction (P<0.01) between diet and MOS was noticed in the starch digestibility; the positive effect of MOS was more profound on the wheat diet than on the corn diet. The improvement in starch digestibility of the wheat-based diet by MOS was also higher (P<0.05) than that caused by xylanase. The ammonia concentration in blood plasma was not affected (P>0.05) by diet or MOS.

Table 7.12 Effects of MOS supplementation on the apparent digestibility of ileal nutrient and blood plasma ammonia (μgNH4/mL) of birds fed the experimental diets on day 21[1]

Treatment		Starch	Protein	Ammonia
Diet	MOS[2]			
Corn diet	-	93[a]	74	17.0
	+	95[a]	79	16.4
Wheat diet	-	83[b]	68	18.2
	+	97[a]	65	17.2
Wheat+xylanase diet	-	83[b]	73	17.2
	+	74[c]	74	17.5
SEM		6.9	9.9	3.33
Source of variation				
Diet		0.00	0.06	NS
MOS		NS[3]	NS	NS
Diet*MOS		0.00	NS	NS
Contrast wheat+MOS vs wheat+xylanase		0.00	NS	NS

[1]Values are means of 6 replicates. [2] 2g/kg diet.
[3] NS: nonsignificant (P>0.05). [a-c] Means in a column not sharing a common superscript differ (P<0.05).

7.3.5 Gut morphology

Dietary MOS reduced (P<0.05) the jejunal crypt depth of birds given the wheat diet at 7 days of age (Table 7.13). Diet had no effects on the jejunal villus height, however, birds given the wheat diet had deeper (P>0.05) crypts and thicker (P>0.05) external muscle than those given either the corn or the wheat plus xylanase diets. On d 21, the morphology of the jejunal mucosa was not affected by either diet or MOS but the addition of MOS tended (P=0.08) to reduce the thickness of the external muscle. No significant differences in the gut morphology were noticed between the MOS and the xylanase supplements.

Table 7.13 Effects of MOS supplementation on jejunal villus height (μm), crypt depth (μm), villus height:crypt depth ratio (Ratio) and the external muscle thickness[1] (μm) of birds fed the experimental diets on days 7 and 21[2]

Treatment		Villus height	Crypt depth	Ratio	Thickness[1]
Diet	MOS[3]				
				Day 7	
Corn diet	-	730	125[ab]	5.6	175
	+	764	119[b]	6.0	169
Wheat diet	-	763	137[a]	5.7	210
	+	765	114[b]	6.1	148
Wheat+xylanase diet	-	818	120[b]	6.7	173
	+	710	119[b]	6.3	175
SEM		106.7	11.2	1.15	41.9
Source of variation					
Diet		NS[4]	NS	NS	NS
MOS		NS	0.01	NS	0.12
Diet*MOS		NS	0.04	NS	NS
Contrast wheat+MOS vs wheat+xylanase		NS	NS	NS	NS
				Day 21	
Corn diet	-	1142	172	6.2	283
	+	1036	166	6.5	238
Wheat diet	-	1086	170	6.6	298
	+	1101	163	7.1	248
Wheat+xylanase diet	-	1152	162	7.2	272
	+	1117	174	6.6	283
SEM		97.3	17.8	1.61	26.5
Source of variation					
Diet		NS	NS	NS	NS
MOS		NS	NS	NS	0.08
Diet*MOS		NS	NS	NS	NS
Contrast wheat+MOS vs wheat+xylanase		NS	NS	NS	NS

[1]The external muscle includes muscularis mucosae, submucosa, muscularis externa and serosa.
[2] Values are means of 6 replicates. [3] 2g/kg diet. [4] NS: nonsignificant (P>0.05).
[a-b] Means in a column not sharing a common superscript differ (P<0.05).

7.3.6 Specific activities of BBMV enzymes

The activity of membrane-bound maltase was increased (P<0.05), whereas the activity of LAP was reduced (P<0.01), by MOS in 7-day-old birds (Table 7.14). In contrast, diet had no effects on the total protein contents and the specific activities of the enzymes. A diet × MOS interaction (P<0.05) was noticed in BBMV protein content and the addition of MOS to the wheat plus xylanase diet increased (P<0.05) BBMV protein content compared to those birds on the same diet but without MOS supplementation.

Table 7.14 Effects of MOS supplementation on jejunal protein content (mg/g tissue) and specific activities (µmol product/mg protein/min) of membrane-bound digestive enzymes on days 7 and 21[1]

Treatment		Total				Alkaline	Leucine
Diet	MOS[2]	Protein[3]	BBMV Protein[4]	Maltase	Sucrase	phosphatase	aminopeptidase
				Day 7			
Corn diet	-	40	0.34^{ab}	20.8^{ab}	1.01	12.9	114^{ab}
	+	36	0.36^{ab}	22.4^{a}	1.05	15.8	70^{c}
Wheat diet	-	36	0.34^{ab}	18.1^{b}	0.99	14.2	124^{a}
	+	37	0.30^{b}	20.5^{ab}	1.06	13.1	83^{bc}
Wheat+xylanase diet	-	38	0.26^{b}	20.2^{ab}	1.21	14.2	102^{abc}
	+	34	0.41^{a}	23.1^{a}	1.11	11.0	83^{bc}
SEM		5.6	0.095	3.1	0.242	3.47	30.5
Source of variation							
Diet		NS[5]	NS	NS	NS	NS	NS
MOS		NS	NS	0.04	NS	NS	0.00
Diet*MOS		NS	0.05	NS	NS	0.10	NS
Contrast wheat+MOS vs wheat+xylanase		NS	NS	NS	NS	NS	NS
				Day 21			
Corn diet	-	39	0.99^{a}	7.37	1.04^{ab}	5.84^{c}	78^{b}
	+	47	0.82^{b}	7.55	1.15^{a}	7.01^{bc}	83^{ab}
Wheat diet	-	38	0.64^{cd}	8.28	0.84^{b}	7.53^{ab}	86^{ab}
	+	38	0.51^{d}	7.93	0.90^{ab}	8.80^{a}	100^{a}
Wheat+xylanase diet	-	40	0.80^{ab}	9.00	1.06^{ab}	7.24^{bc}	52^{c}
	+	42	0.73^{bc}	7.34	1.05^{ab}	6.72^{bc}	74^{bc}
SEM		6.53	0.144	1.439	0.212	1.186	20.4
Source of variation							
Diet		NS	0.00	NS	0.04	0.01	0.01
MOS		NS	0.02	NS	NS	NS	0.05
Diet*MOS		NS	NS	NS	NS	NS	NS
Contrast wheat+MOS vs wheat+xylanase		NS	0.00	NS	NS	0.03	0.00

[1] Values are means of 6 replicates. [2] 2g/kg diet.
[3] Protein content in the mucosal homogenate. [4] Protein content in the brush-border membrane vesicle.
[5] NS: nonsignificant ($P>0.05$). [a-d] Means in a column not sharing a common superscript differ ($P<0.05$).

At 21 days of age, MOS reduced (P<0.05) BBMV protein but increased (P<0.05) mucosal LAP activities. Diet also altered (P<0.05) these variables as well as the mucosal sucrase and AP activities. Birds given the corn diet had higher (P<0.05) BBMV protein and mucosal sucrase activities than those birds given the wheat diet, however, the opposite trend was noticed with the mucosal AP and LAP activities. No interaction between diet and MOS was noticed in the mucosal protein contents and enzyme activities at 21 days of age. The xylanase supplement increased (P<0.05) BBMV protein content but reduced (P<0.05) the activities of AP and LAP compared to MOS.

7.4 DISCUSSION

7.4.1 Birds' responses to MOS supplementation of the corn and wheat-based diets

In general, the addition of MOS to the basal diets increased the BWG of birds. A detailed analysis of the growth performance data showed the largest effect of MOS was seen in birds given the wheat diet during weeks 2 to 3, where the FCR was also reduced as a result of the supplement (1.83 vs.1.62). This observation indicates that, on a wheat-based diet, the growth performance of birds was greatly improved by MOS and the birds can utilize the feed more efficiently for body growth.

Soluble NSP, especially arabinoxylans, were higher in the wheat diet compared to the corn diet, as expected. Hubener *et al.* (2002) reported that the most striking bacterial responses to the presence of soluble NSP in the broiler chicken intestine are the higher number of mucosa-associated bacteria. A similar pattern was observed in the present experiment. Compared to the corn diet, the wheat diet enhanced the number of mucosa-associated bacteria and the effects were more pronounced in 3-week-old than in 1-week-old birds. However, as opposed to the findings of Hubener *et al.* (2002) that an increase in the counts of enterobacteria and enterococci was observed in the birds given a wheat/rye diet, the mucosa-associated lactobacilli proliferated more than the mucosa-associated coliforms along the intestinal tract of birds given the wheat diet in the present trial. All these observations indicate that diet can affect the profile of mucosa-associated bacteria, thus affecting the growth rate of birds (Untawale and McGinns, 1979; Danicke *et al.*, 1999).

Regardless of type of basal diets, MOS consistently reduced the number of mucosa-associated coliforms, which is in agreement with the results obtained in Chapter 5 and the *in vitro* results reported by Peuranen *et al.* (2006). The largest reduction was shown in the

jejunum, a major site of digestion and absorption, of birds fed the wheat diet at the end of week 3. Furthermore, at the same age, the low number of mucosa-associated coliforms was counterbalanced by a large number of mucosa-associated lactobacilli in birds fed the MOS-supplemented diets. Similar results were noticed in a previous trial (Chapter 6). It is reported that adherent *Lactobacillus* may stabilize the mucosal barrier by increasing mucin expression, reducing bacterial overgrowth, stimulating mucosal immunity and synthetizing antioxidant substances (Gotteland *et al.*, 2001). Therefore, by decreasing the number of mucosa-associated coliforms and increasing the number of mucosa-associated lactobacilli, the profile of mucosa-associated bacteria might be modulated by MOS towards a balance that is optimal for the development and function of gut mucosa.

When MOS was supplemented in the basal diets, the crypt depth was reduced, especially in the birds given the wheat diet in the first week (a 17% decrease in the crypt depth). A decrease in crypt depth was also observed in birds fed the MOS-supplemented diet in Chapter 6, where a sorghum-wheat basal diet rich in NSP was used. The soluble NSP in wheat-based diets probably increased the bacterial load at the mucosal level (Vahjen *et al.*, 1998); as a result, hyperplasia of crypt occurred, particularly in young birds when the gut system is under development. As discussed in Chapter 6, a shallow crypt possibly indicates a lower turnover rate of the intestinal epithelium, which results in a lower maintenance requirement, favouring a faster growth rate or growth efficiency of the whole animal. The reduction in external muscle thickness, where a large number of immune tissues and cells are located, furthermore, supports the nutrient-and-energy-saving effects of MOS on gut development. The digestive tract and the associated lymphoid tissue of a 25 kg pig, for example, utilize approximately 40% of all ingested nutrients in maintaining integrity and mounting a local immune response (Chesson, 1994). The boosting effects of MOS on the immune system were shown in numerous reports (Savage *et al.*, 1996a; O'Carra, 1997; Spring and Pirvulesu, 1998; Shafey *et al.*, 2001b).

However, it is difficult to interpret the effects of MOS on the development of the specific activities of BBMV enzymes. In 7-day-old chickens, the activity of maltase was increased by MOS. Similar results were noticed by Iji *et al.* (2001), indicating that the brush-border digestive function of the gut was improved by MOS since the final states of nutrient hydrolysis (digestion) are performed by membrane anchored enzymes at the brush border (Sklan, 2001). However, the effects of MOS on the activities of LAP seem to indicate that MOS delayed the maturation of aminopeptidase as decreased activities of LAP were noticed

in birds fed the MOS-supplemented diets on d 7 but the reverse was the case on d 21. Iji *et al.* (2001) also reported a higher specific activity of LAP in the jejunum of 28-d old birds on MOS treatment. Further work needs to be done to confirm the effects of MOS on the development of aminopeptidase in order to give possible explanations.

The wheat diet promoted the growth of bacteria, such as coliforms and *C. perfringens*, in the gut lumen compared to the corn diet. The addition of MOS to the wheat diet generally reduced the proliferation of these bacteria and markedly reduced the number of *C. perfringens* in the caeca by the end of week 3. In accordance with the luminal bacterial results, an increase in the molar ratio of acetic acid and propionic acid was noticed in birds fed the MOS-supplemented diets compared to those given the diets without MOS supplementation, which is in agreement with the findings in Chapters 3, 5 and 6. These observations may indicate that the composition and function of gut microflora were altered by MOS to spare nutrients for body growth. Indeed, MOS supplementation improved the ileal nutrient digestibility. Thus, the starch digestibility in the birds given the wheat diet was increased by MOS by up to 16%. It is unclear how MOS led to such a big improvement in the utilization of starch in the wheat diet. A general inhibition of nutrient digestion, such as starch and protein, is one part of the growth depression induced by wheat NSP (Annison, 1993). The anti-nutritive effect of wheat NSP may be mediated by the gut microflora of birds (Choct *et al.*, 1992) and small intestinal bacteria may use 10 to 20% of carbohydrates and amino acids that could be utilized by the host (Apajalahti *et al.*, 2004). Therefore, the improvement in the digestibility of starch perhaps could partly be related to the beneficial modulatory effects of MOS on the gut microflora, both in the lumen and on the mucosa. Another possibility is that a better functioning mucosa with a thick coat of bacteria attached to it may mean a less physical barrier for nutrient transport across the enterocytes. However, the exact mechanisms need to be elucidated in future research.

The toxicity of ammonia, one of the products of microbial activity, varies under different physiological and pathological conditions. Concurrently with reduction in ammonia concentrations significant increases in growth were observed (Visek, 1964). However no significant differences in ammonia concentrations in blood plasma were noticed as a result of MOS supplementation, although MOS numerically reduced the ammonia concentrations in birds fed both cereal types.

7.4.2 A comparison of birds' responses to MOS and/or xylanase supplementation to the wheat-based diet

The improvements in the performance of birds fed the xylanase-supplemented diet are consistent with published reports (Annison and Choct, 1991; Bedford and Classen, 1992; Wu *et al.*, 2004a, 2004b). However, MOS supported a larger improvement in the growth performance of birds than xylanase during weeks 2 to 3 but no significant differences between the two supplements were noticed in growth performance thereafter. This observation agrees with the findings in a previous experiment (Chapter 3) that the growth-promoting effects of MOS are more pronounced in early life, which can be explained by the modulatory effects of MOS on the gut microflora.

In general, MOS supplementation had more antibacterial effects on the growth of coliforms or lactobacilli compared to the xylanase supplement; for example, the counts of mucosa-associated coliforms in the small intestine of birds fed the MOS-supplemented diet were lower than those of the birds on the xylanase treatment. As discussed earlier, a reduced bacterial load in the gut may be expected in the nutrient-and-energy-saving effects induced by MOS at the mucosa and in the lumen of the gut. The ileal starch digestibility was significantly higher in birds fed the MOS-supplemented diet than in those fed the xylanase-supplemented diet up to the end of week 3, which can correlate with the large improvement in the growth performance induced by MOS.

In accordance with the results of Annison and Choct (1991); Steenfeldt *et al.* (1998); Wu *et al.* (2004a), the present results showed that the antinutritive effects of the wheat diet on the nutrient digestibility and gut morphology were counteracted by the addition of xylanase. Similar results were observed for MOS treatment. However, no additive effects occurred on these variables when the combination of MOS and xylanase was added to the wheat-based diet. No research has been reported on the combined effects of MOS and enzymes in a wheat-based diet for broiler chickens. The microbial results in the present trial seem to indicate that there was an interaction between MOS and xylanase, which is not easy to explain. However, no additive effects between these two supplements observed on the growth performance may be due to the interaction between MOS and xylanase on the gut microflora.

7.5 CONCLUSIONS

Birds given the wheat-based diet showed better response to the MOS supplement than those given the corn-based diet. The improvement in the growth performance and flock uniformity of birds induced by MOS may be associated with the inhibitory effects of MOS on the gut microflora, especially mucosa-associated coliforms, as well as the positive effects of MOS on the gut morphology and nutrient digestibility.

The addition of MOS alone to the wheat-based diet improved the growth rate of birds as effectively as xylanase. The combination of MOS and xylanase caused no further improvements in birds' performance. Different effects between the MOS and the xylanase supplements were noticed on the development of gut microflora and mucosal enzyme activities but not on the development of mucosal morphology.

CHAPTER 8
GENERAL DISCUSSION AND CONCLUSIONS

Five experiments were conducted in the present work to examine the effects of MOS on the growth performance, gut microflora, gut morphology, and nutrient digestibility of birds. The major findings of the work and possible correlations among them are further discussed in the following sections.

8.1 GENERAL PERFORMANCE

Dietary MOS can positively affect the growth performance of birds (Chapters 3, 5, 6, and 7); the effects are more pronounced and comparable to ZnB in early life (Chapters 3 and 6). As birds got older, the growth-promoting effects of MOS become less pronounced than those of ZnB; however, MOS showed better effects on flock uniformity (Chapter 7). These observations generally agree with those in the literature (Ao, 2004; Hooge, 2004a; Rosen, 2007). On the other hand, it should be pointed out that, with a balanced diet, birds can express their genetic growth potential under a hygienic condition without the supplementation of any growth-promoting additives (Chapter 4; Hernández *et al.*, 2004). This observation, combined with large growth-promoting effects noticed in birds given a wheat-based diet added with MOS (Chapter 7), indicates that MOS may be more effective when rearing conditions are poorer. Practically such conditions are common, and will be present, even when hygiene is high, in form of stress caused by high stocking densities.

8.2 ENERGY UTILIZATION AND GUT DEVELOPMENT

The growth-promoting effects of MOS were directly supported by the improved AME value in birds given the MOS-supplemented diets (Chapter 5). The energy-saving effects appear to be due to the use of less energy on gut maintenance induced by MOS, as indicated by the modulatory effects of MOS on the development of GIT. The general morphology of the GIT, except the liver size and the relative length of small intestine, was not affected by MOS; however, the mucosal architecture changed as a result of the addition of MOS. Longer villi were noticed in birds fed MOS-supplemented diets by other authors (Spring, 1996; Iji *et al.*, 2001; Loddi *et al.*, 2002) and in the present work (Chapters 3 and 5). However, a shallow crypt is a more consistent observation, as discussed in the individual chapters, which can, in part, explain the energy-saving effects and the improved AME value noticed in birds given MOS-supplemented diets. A shallow crypt was also noticed in broilers gvien the feed

supplemented with ZnB, xylanase or herbal natural feed additives in the present work (Chapters 6 and 7) and other studies (Demir *et al.*, 2003; Wu *et al.*, 2004a), where improved growth performance was noticed. These observations support the notion that GIT utilizes a large proportion of dietary energy and protein (Ferrell, 1988) and, in rapidly growing young animals, the GIT competes for energy and nutrients with body growth (Reeds *et al.*, 1993). The growth-promoting effects of MOS can partly be explained by its energy-and-nutrient-saving effects at the level of GIT development.

8.3 THE DEVELOPMENT OF GUT MICROFLORA

It was shown that MOS has the ability to stabilize and modulate the gut microflora towards a balance that will be beneficial to the development of gut function and body growth. Firstly, the proliferation of mucosa-associated coliforms was inhibited by MOS although this effect is dependent on the age of the bird, diet and/or rearing environment. Santin *et al.* (2001) proposed that MOS has a protective effect on the intestinal mucosa by reducing the stress condition to which the mucosa is subjected, such as the number of bacteria or other toxins present in the gut, which is supported by the findings in the present work. However, the application of DNA-based techniques to further examine the inhibitory effects of MOS on the development of coliforms at a strain-specific level is preferred as increases in the luminal coliforms were noticed in birds fed the MOS-supplemented diet and it was speculated that the profile of luminal coliforms was changed.

Secondly, the decreasing effects of MOS were also noticed on luminal lactobacilli and *C. perfringens* in the present work. Similar results were reported in turkeys on MOS treatment (Sims *et al.*, 2004; Zdunczyk *et al.*, 2005). However, these groups of bacteria do not possess type-1 fimbriae as is common in coliforms. It is speculated that MOS indirectly inhibits the growth of intestinal bacteria by modulating the immune system and exert their antibacterial effects on these groups of bacteria. The immunomodulatory effects of MOS were reported in many species of animal, such as turkey (Savage *et al.*, 1996a), piglets (Privulescu, 1999), layer chickens (Cotter, 1997) and dogs (O'Carra, 1997; Swanson *et al.*, 2002). However, broiler chicken-specific data are sparse. A 16% increase in secretory Ig A has been noticed in turkey (Savage *et al.*, 1996a), combined with the reduced external mucosal thickness observed in Chapter 7, which probably indicate that MOS has a positive role in the development and function of gut immune system. This area deserves more detailed study because an optimal gut immune function means that birds can effectively "fight" against

various stresses from food and/or environment, a prerequisite for birds to stay in health and to express their genetic growth potential.

8.4 NUTRIENT DIGESTIBILITY AND THE SPECIFIC ACTIVITIES OF BRUSH-BORDER ENZYMES

The effects of MOS on the intestinal nutrient digestibility were noticed with a suboptimal diet (Chapter 7). Similar results were found in the specific activities of brush-border enzymes, which are involved in the final digestion and absorption of nutrients in the gut. MOS appeared to have a positive effect on the nutrient utilization in birds. However, more research is warranted in this area. As reported in Chapter 7, a large improvement in starch digestibility was noticed in birds given a wheat-based plus MOS diet. It is unknown whether it is a consistent observation and if so, what mechanisms are involved.

8.5 CONCLUSIONS

The results reported in this thesis show that, by modulating the development of gut system of young birds, MOS may induce the nutrient-and-energy-saving effects in the gut; to improve the AME value of diet and the growth performance of birds. However, the current series of experiments leave some important questions unanswered, such as the mechanisms of MOS to decrease the populations of the bacteria which do not have type-1 fimbriae.

In conclusion, the growth-promoting effects of MOS are dependent on the age of birds, diet, and rearing environment. The supplement can partly replace antibiotics in terms of the growth performance and/or flock uniformity under the experimental conditions reported in this thesis. The growth-promoting effects may be related to the antibacterial effects of MOS in the gut lumen and the protective effects of MOS on gut mucosa. The ultimate effects of MOS seem to reduce the energy requirement for gut maintenance and to improve nutrient utilization for body growth, which needs to be elucidated in future work at cellular and molecular levels. Investigations into the effects of MOS on the development and function of gut immune system are also needed.