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Functional interactions of manno-oligosaccharides with dietary threonine in chicken gastrointestinal tract. III. Feed passage rate

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Abstract 1. A 3 × 2 factorial experimental design was used to investigate the interaction between threonine concentration (0·70, 1·0 and 1·3 of National Research Council (NRC), 1994, recommendations) and manno-oligosaccharides (0 and 2 g/kg) on feed passage rate in relation to intestinal microbial activities and crude mucin turnover.

2. There was no interaction between the effects of manno-oligosaccharides (MOS) and dietary threonine on total tract transit time. However, an interaction between MOS and threonine was apparent where increasing threonine in the absence of MOS led to a reduction in the mean retention time, but a trend in the opposite direction in the presence of MOS. The ileal mean retention time at deficient and adequate concentrations of threonine was also significantly shorter in the presence of MOS.

3. In the jejunum, dietary MOS interacted with threonine to increase the villus-to-crypt ratio with deficient and adequate concentrations of threonine but not with an excess. In the ileum, MOS had no effect on the villus-to-crypt ratio at the deficient and adequate concentrations of threonine but significantly increased the ileal villus-to-crypt ratio with an excess.

4. There were significant interactions between MOS and dietary threonine in their effects on ileal flow of crude mucin, with MOS supplementation increasing mucin concentration and output when threonine was adequate but not when deficient or in excess.

5. Neither MOS nor threonine affected volatile fatty acids and intestinal musculature. No effects of gut microflora or voluntary feed intake on feed passage rate was attributable to dietary threonine or MOS supplementation.

INTRODUCTION

It was postulated by Ferket *et al.* (2002) that the decrease in thickness of intestinal external muscularis in relation to the feeding of MOS is likely to be associated with an overall improvement in gut viability through a reduced need for gut motility to control gut microbial activities. The role of interdigestive gut motility in nutrient digestion and absorption, as well as intestinal clearance and prevention of bacterial overgrowth, has long been recognised (Vantrappen *et al.*, 1977; Choct *et al.*, 1996). However, the close relationship between feed passage rate and modulation of intestinal commensal microflora and mucin dynamics induced by pro- and prebiotics has not been fully explored. More recently, Lesniewska *et al.* (2006) demonstrated that bifidobacteria and lactobacilli are able to increase both the intestinal motility and the intestinal contractile force in rats. This mechanism is especially important for a proper mixing of the residual food with the enzymatic secretions and to enable a more effective distal propulsion of luminal content, debris and bacterial cell, and to do so over a longer distance.

In addition, results from previous studies also showed that bacterial fermentation products,

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such as short-chain fatty acids (SCFAs), can regulate ileal motility (Cuche *et al.*, 2000). Peristaltic movement plays an integral role in the rapid expulsion of micro-organisms from the small intestine through the formation of antigen–antibody complexes in the mucous coat (Cherbut *et al.*, 1998). This suggests that the binding of pathogens by luminally available MOS could also be beneficial. Furthermore, intestinal mucus acts as a lubricant that aids the passage of digesta along the digestive tract, thus preventing stagnation and bacterial overgrowth.

The objective of this study was to evaluate the interactive effects of MOS and dietary threonine on feed passage rate by focusing on their modulating effects on gut microflora composition, SCFA profiles and intestinal mucin turnover.

MATERIALS AND METHODS

Bird management and experimental diets

A completely randomised 3×2 factorial design was employed, with 6 treatments allocated to each of the starter basal diets, comprising mainly of sorghum, peanut-meal and soybean meal (Table 1). The major essential amino acids of all diets, except for threonine, were formulated to meet or exceed the minimum National Research Council (NRC) (1994) nutrient recommendations for broiler chickens. Three concentrations of dietary threonine were formulated at 0·70 (deficient), 1·0 (adequate) or 1·3 (excess) of the NRC-recommended concentration (Table 2), with L-threonine (Ajinomoto Eurolysine, Paris, France) added to the basal diets at rates of 0, 2·5 and 5·0 g/kg at the expense of L-alanine and L-glutamic acid (1:1 w/w) (Sigma-Aldrich Chemical Co., St Louis, MO, USA) (Atencio *et al.*, 2004).

Diets with MOS supplementation were created by adding Bio-MOS® (Alltech, Nicholasville, KY, USA) at 2·0 g/kg of the experimental diet and at the expense of a Zeolite filler. Titanium dioxide (TiO_2; Sigma-Aldrich) at 0·5% (w/w) inclusion rate was added to the diets for the calculation of feed passage rate and mucin turnover.

A total of 96 d-old male Cobb broiler chicks (Baiada Poultry Pty. Ltd, Kootingal, NSW, Australia), weighing 47·6 ± 0·25 g, were group-brooded according to treatment in a multi-tier brooder for 14 d. At 15 d, 72 birds were reallocated at random to the 6 treatment groups in metabolic cages and 24 birds to individual cages. Birds kept in the metabolic cages, comprising 4 replicates per treatment with 3 birds per replicate, were used for the assessment of feed passage rate using cumulative and non-cumulative titanium dioxide (TiO_2) excretion methods. The individually caged birds with 4 replicates per treatment were used for the evaluation of feed passage rate based on first excreta appearance and mean retention time assessed by intestinal region. All brooding cages were housed in environmentally controlled

Table 1. *Composition (g/kg as-is basis) of experimental basal diet*

Ingredient	Basal starter
Sorghum grain	558·00
Maize flour	90·00
Soybean meal dehulled, 47·5% CP	87·50
Peanut meal, solvent 45% CP	175·00
Vegetable oil	28·05
Filler[a]	2·00
Titanium dioxide[b]	5·00
L-threonine	0·00
L-lysine HCl	9·22
DL-methionine	3·36
Alanine/glutamic acid (1 : 1)	6·00
Calcium carbonate	10·90
Dicalcium phosphate	18·22
Choline chloride	0·30
Sodium bicarbonate	4·00
Sodium chloride	0·45
Vitamin and mineral premix[c]	2·00
Total	1000
Calculated nutrient composition	
CP	196·00
Lysine	13·00
Methionine	5·00
Threonine	5·50
ME poultry (MJ/kg)	12·57

[a]Filler represent inert space (Zeolite), which was replaced by BioMOS®.
[b]Sigma-Aldrich Chemical Co. (St Louis, MO, USA).
[c]Supplied per kg of diet: retinol acetate, 4·12 mg; cholecalciferol, 87·5 μg; DL-alpha-tocopherol acetate, 44·7 mg; menadione, 2 mg; thiamine mononitrate, 2 mg; riboflavin, 6 mg; pyridoxol, 5 mg; cyanocobalamin, 0·2 mg; D-biotin, 0·1 mg; niacin, 50 mg; pantothenic acid, 12 mg; folic acid, 2 mg; Zn ($ZnSO_4 \cdot H_2O$), 90 mg; Mn ($MnSO_4 \cdot H_2O$), 80 mg; Fe ($FeSO_4 \cdot H_2O$), 60 mg; Cu ($CuSO_4 \cdot 5H_2O$), 8 mg; I (KI), 1 mg; Co ($CoSO_4 \cdot H_2O$), 0·3 mg; and Mo ($Na_2Mo_4 \cdot 2H_2O$), 1 mg.

Table 2. *Calculated and analysed nutrient values (g/kg as-is basis) of experimental diets*

	Treatment					
MOS	−			+		
Threonine	Deficient	Adequate	Excess	Deficient	Adequate	Excess
Calculated nutrient composition						
CP	196·00	196·00	196·00	196·00	196·00	196·00
Threonine	5·50	8·00	10·50	5·50	8·00	10·50
Analysed nutrient composition						
CP	192·00	191·00	194·00	193·00	192·00	195·00
Threonine	5·50	8·20	10·60	5·60	8·10	10·90

rooms with an initial room temperature of 34°C for the first 5 d before a gradual decrease to 21°C by the end of the experiment at 21 d. Birds were given feed and water *ad libitum* except for the periods required for the assessment of feed passage rate. Artificial lighting of the room was continuous to minimise development of a diurnal rhythm of feeding. All experimental procedures were approved by the Animal Ethics Committee of the University of New England, Armidale, NSW, Australia.

Feed passage rate and sample collection

Excreta first appearance and regional mean retention time

At 16 d, the individually caged birds were fasted for 12 h before being fed with the marker diets. The time the bird first began to eat and the time of first appearance of solid excreta was recorded. Total tract transit time (h) was calculated as the difference between these two times (Washburn, 1991). At the end of the experiment, excreta samples were collected from each cage and processed immediately for determination of excreta dry matter content. The birds were then allowed to continue to consume the marker diets for 5 d. It was assumed that the birds were already in a steady state at the end of d 21 with respect to the intake and excretion of TiO_2. The quantity of the consumed feed was determined for each individual bird. After live weight was recorded, the birds were killed by CO_2 asphyxiation and quickly dissected to obtain the separate regions of duodenum (from gizzard outlet to the end of the pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum) and ileum (from Meckel's diverticulum to the ileo-caecal junction). Digesta of the respective intestinal regions were squeezed out, frozen, and stored at −20°C for further analysis of gut microflora (without freezing), mucus extraction, SCFA and TiO_2 assays. TiO_2 contents in the digesta samples were measured by the method of Short *et al.* (1996).

Regional mean retention time was calculated from the ratio between TiO_2 present in the respective segments of the small intestine and the daily TiO_2 intake based on the equation described by van der Klis *et al.* (1990). The remaining parts of the respective intestinal segments were collected for enteric morphometric analysis.

Cumulative TiO_2 excretion study

At 21 d, the birds kept in the metabolic cages were fasted for 12 h whilst being allowed water *ad libitum*. At the end of the fasting period, the birds were given their respective TiO_2 marked diets. After 1 h, feed intake was recorded and the marked feed replaced with the normal experimental diets. Excreta were collected hourly for the first 8 h and again 12 and 24 h after the birds had received the marked feed. The excreta collected from each cage were kept in a separate re-sealable plastic bag, and stored at −20°C before being freeze dried, ground to pass through 1 mm sieve and kept in tightly closed plastic containers until used for the analyses. A sample of each of the TiO_2 marked diet was also obtained, processed and analysed through the same procedure as the digesta samples.

The TiO_2 excreted was expressed as cumulative fraction of the total amount of the TiO_2 determined at various sampling times during the 24-h collection period. The cumulative excretion curve of each diet was plotted and fitted to the Hill equation as described by Almirall and Esteve-Garcia (1994) and later transformed into a linear equation as outlined by Ferrando *et al.* (1987).

Times of 1% (T_1) and 50% (T_{50}) excretion, which were the times required to excrete 1% (first appearance) and 50% (total tract mean retention time) of the marker administered, respectively, were determined for each replicate according to the method described by Ferrando *et al.* (1987).

Excreta dry matter content

Excreta dry matter content was determined according to Association of Official Analytical Chemists (AOAC) (1994).

Crude mucin turnover

Crude mucins were isolated by a modification of procedures presented by Allen (1981) and Miller and Hoskins (1981), as described by Lien (1995). The crude mucins recovered expressed as crude mucin concentration (g/100 g DM digesta) and crude mucin output (g/kg DM intake) were computed based on the following equations:

$$\text{Crude mucin concentration} = \frac{\text{Crude mucin}_{\text{digesta}}}{\text{DM}_{\text{digesta}}} \times 100$$

$$\text{Crude mucin output} = \frac{(\text{Crude mucin}_{\text{digesta}} \times \text{TiO}_{2\text{diet}})}{\text{TiO}_{2\text{digesta}}} \times 1000$$

where Crude $\text{mucin}_{\text{diegsta}}$ is the amount of crude mucin (g) extracted out from 1 g of DM digesta ($DM_{digesta}$); and $TiO_{2\ diet}$ or $TiO_{2\ digesta}$ is the TiO_2 concentration expressed as mg/g DM of the diet or digesta as determined for the standardised ileal digestibility calculation.

Microbial and organic acid analysis

Approximately 1 g of fresh samples from the ileal contents and the ileal mucosal samples were mixed in 10 ml pre-reduced salt medium (Holdeman *et al.*, 1997), homogenised, and serially diluted according to the procedure described by Engberg *et al.* (2004) for the enumeration and counting of total anaerobic bacteria (Wilkins–Chalgren anaerobe agar, CM0619, incubated at 37°C for 7 d), lactobacilli (Rogosa agar, CM0627, incubated anaerobically at 39°C for 48 h), lactic acid bacteria (De Man Rogosa and Sharp (MRS) agar, CM0361, incubated anaerobically at 39°C for 48 h) and *C. perfringens* (perfringens agar, CM0587 TSC and SFP, incubated anaerobically for 24 h). Coliforms (red colonies) and lactose-negative enterobacteria (colourless colonies) were grown on MacConkey agar (CM 0007) incubated aerobically at 39°C for 24 h. All the media were obtained from Oxoid (Basingstoke, UK). After incubation, colonies formed on the respective media were carefully counted, converted into logarithmic equivalents, and expressed as number of colony forming units (CFU) per g of wet intestinal content. The analytical method described by Jensen *et al.* (1995) was adopted for lactic acid and SCFA analysis.

Enteric morphometric analysis

Approximately 2 cm of the proximal jejunal and ileal samples were flushed with ice-cold buffered phosphate saline at pH 7·4 and immediately placed in 10% formalin solution. Following 72 h of incubation in 10% buffered formalin, the ileal samples were dehydrated, cleared, and embedded in paraffin. Separate 5 µm sections were placed on glass slides, deparaffinised, rehydrated and stained with a combination of Alcian Blue (AB, pH 2·5, 30 min) and periodic acid-Schiff's reagent (PAS, 20 min each) (Kierrnan, 1990). Histomorphometric analyses were performed with the help of calibrated image analysis software (Video Pro 32, Leading Edge Pty. Ltd, Blackwood, SA, Australia). The parameters measured for villus morphometry on 20 well-oriented villus–crypt units were external muscularis thickness, villus height, crypt depth and villus-to-crypt ratio.

Statistical analysis

Data were analysed using ANOVA of general linear model procedure of SAS Institute (2007) to determine the main effects (MOS and threonine) and their interactions using cage as the experimental unit. Differences were considered significant at $P \leq 0{\cdot}05$, although probability values of $P \leq 0{\cdot}1$ are shown in the text if the data suggested a trend. If significant differences were detected, means were separated with repeated *t*-test using least-squares means option of SAS Institute (2007).

RESULTS

Dietary composition and treatment design

The calculated crude protein and amino acid contents of the diets and their respective analysed values are presented in Table 2. Generally, the analysed values were within 5% of the calculated values.

Enteric morphometric analysis

No significant differences were detected among the treatments for either external muscularis thickness or the crypt depth in any of the intestinal regions (Table 3). In the jejunum, an interaction between MOS and dietary threonine was apparent: MOS increased the villus-to-crypt ratio at deficient and adequate concentrations of threonine but not when in excess. In the ileum, MOS had no effect on the villus-to-crypt ratio when the threonine concentration was deficient or adequate but significantly increased it when threonine was in excess. In addition, birds receiving the threonine-adequate diets had significantly longer villi than those given a threonine-deficient diet.

Characterisation of intestinal microflora by plate counts

Birds deficient in threonine harboured significantly fewer lactobacilli and lactic acid bacteria in the ileal content than those fed on a threonine-adequate diet (Table 4). Interactions between MOS and dietary threonine on coliform counts were observed in both the ileal digesta and mucosa. In the digesta, coliform counts were significantly higher with adequate threonine in the absence of MOS and significantly lower when threonine was in excess. In the mucosa, MOS supplementation significantly reduced the coliform counts only when threonine was adequate; no effect occurred when threonine was deficient or in excess.

No significant differences in lactic and SCFAs attributable to treatment were detected (data not shown).

Crude mucin concentration and output

Flow of crude mucins in the duodenum was not determined due to an insufficient digesta being obtained. Whilst no significant differences were

Table 3. *Effects of dietary threonine concentration and MOS supplementation on jejunal and ileal morphometric parameters of broilers at 21 d of age*[a]

Treatment		Jejunum				Ileum			
Threonine	MOS	Muscularis thickness (μm)	Crypt depth (μm)	Villus height (μm)	Villus/crypt ratio	Muscularis thickness (μm)	Crypt depth (μm)	Villus height (μm)	Villus/crypt ratio
Deficient	−	269	260	1229	4·74^{BC}	261	183	748	4·41^{BC}
Deficient	+	301	230	1398	6·26^{AB}	239	188	722	3·91^{C}
Adequate	−	336	267	1178	4·68^{C}	271	200	938	4·79^{BC}
Adequate	+	327	252	1574	6·32^{A}	261	165	1041	6·66^{AB}
Excess	−	283	245	1497	6·04^{ABC}	264	201	862	4·31^{BC}
Excess	+	337	296	1534	5·21^{ABC}	274	139	984	7·71^{A}
Pooled SEM		21·8	24·0	104·2	0·529	22·2	20·1	38·3	0·742
Main effects									
Deficient		285	245	1314	5·49	250	186	735^{B}	4·16
Adequate		331	260	1376	5·50	266	183	994^{A}	5·72
Excess		310	271	1515	5·63	269	170	923^{A}	6·01
	−	296	258	1301^{B}	5·16	265	195	853^{B}	4·50
	+	322	259	1502^{A}	5·93	258	164	915^{A}	6·09
Source of variation					*P*-values				
Threonine		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0·001	<0·05
MOS		n.s.	n.s.	<0·05	n.s.	n.s.	n.s.	<0·05	<0·05
Thr x MOS		n.s.	n.s.	n.s.	<0·05	n.s.	n.s.	n.s.	<0·05

[a]Each value represents the mean of 4 replicates for each treatment group.
[A–C]Means within a continuous column not sharing a common superscript are significantly different at $P<0·05$. n.s., Not significant.

Table 4. *Effects of dietary threonine concentration and MOS supplementation on the counts (log CFU/g digesta or mucosa) of luminal and mucosa-associated bacteria in the ileum of broiler chickens at 21 d of age*[a]

Treatment		$\log_{10}$CFU/g digesta or mucosa											
Threonine	MOS	Total anaerobes		Lactobacilli		Lactic-acid		Lactose-negative enterobacteria		Coliforms		*C. perfringens*	
		Digesta	mucosa	Digesta	mucosa	Digesta	mucosa	Digesta	mucosa	Digesta	mucosa	Digesta	mucosa
Deficient	−	8·35	6·88	8·29	6·64	8·38	6·64	5·28	4·79	5·30^{B}	4·18^{ABC}	7·58	6·45
Deficient	+	8·12	6·64	8·25	6·68	8·34	6·53	5·65	4·14	5·45^{B}	4·40^{ABC}	6·66	5·93
Adequate	−	7·65	7·35	8·53	6·65	8·58	7·04	5·72	4·21	6·84^{A}	4·97^{A}	6·47	6·10
Adequate	+	8·05	6·87	8·68	6·09	8·69	6·98	5·16	4·07	5·55^{B}	3·94^{BC}	6·66	5·47
Excess	−	8·44	7·18	8·38	6·54	8·85	7·21	6·42	4·12	5·47^{B}	3·55^{C}	6·96	6·20
Excess	+	8·40	7·46	8·59	6·32	8·80	7·20	6·19	4·41	6·69^{A}	4·17^{ABC}	6·72	5·71
Pooled SEM		0·227	0·310	0·118	0·256	0·165	0·311	0·584	0·367	0·288	0·342	0·251	0·335
Main effects													
Deficient		8·23	6·77	8·27^{B}	6·65	8·36^{B}	6·59	5·46	4·47	5·37	4·26	7·10	6·19
Adequate		8·12	7·11	8·61^{A}	6·40	8·73^{A}	7·01	5·44	4·14	6·17	4·46	6·57	5·78
Excess		8·40	7·32	8·48^{AB}	6·43	8·82^{A}	7·20	5·31	4·27	6·12	4·11	6·84	5·95
	−	8·15	7·14	8·39	6·60	8·61	6·96	5·81	4·38	5·90	4·21	7·00	6·25
	+	8·30	6·99	8·51	6·40	8·60	6·91	5·66	4·21	5·88	4·34	6·66	5·71
Source of variation							*P*-values						
Threonine		n.s.	n.s.	<0·05	n.s.	<0·05	n.s.	n.s.	n.s.	<0·05	n.s.	n.s.	n.s.
MOS		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Thr × MOS		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0·01	<0·05	n.s.	n.s.

[a]Each value represents the mean of 4 replicates for each treatment group.
[A–C]Means within the same column not sharing a common superscript are significantly different at $P<0·05$. n.s., Not significant.

detected in jejunal flow of crude mucins (Table 5), there were significant interactions between MOS and dietary threonine, with MOS supplementation increasing mucin concentration and output ($P<0·07$) when threonine was adequate, but not when it was deficient or in excess. Ileal crude mucin concentration and output were highest ($P<0·001$) in birds receiving a threonine-adequate diet, but significantly reduced when threonine was deficient.

Table 5. *Effects of dietary threonine concentration and MOS supplementation on jejunal and ileal crude mucin concentration and output of broilers at 21 d of age*[a]

Treatment	MOS	Crude mucin concentration		Crude mucin output	
Threonine		Jejunum	Ileum	Jejunum	Ileum
		g/100 g DM digesta		g/kg DM intake	
Deficient	−	2·47	1·63^{D}	14·54	4·82
Deficient	+	2·58	1·98^{CD}	15·74	4·88
Adequate	−	2·33	2·70^{B}	16·76	15·00
Adequate	+	2·43	3·94^{A}	17·20	20·57
Excess	−	2·16	2·50^{BC}	16·86	13·30
Excess	+	2·49	2·97^{B}	19·57	12·53
Pooled SEM		0·318	0·208	2·341	1·724
Main effects					
Deficient		2·49	1·81	15·10	4·81^{C}
Adequate		2·33	3·32	17·01	17·78^{A}
Excess		2·32	2·74	18·21	12·92^{B}
	−	2·30	2·28	16·45	11·04
	+	2·32	2·96	17·09	12·63
Source of variation			*P*-values		
Threonine		n.s.	<0·001	n.s.	<0·001
MOS		n.s.	<0·01	n.s.	n.s.
Thr x MOS		n.s.	<0·05	n.s.	0·068

[a]Each value represents the mean of 4 replicates for each treatment group.
[A–D]Means within a continuous column not sharing a common superscript are significantly different at $P<0·05$. n.s., Not significant. Probability values up to $P<0·10$ are also shown if the data suggest a trend.

Table 6. *Parameters to define the cumulative excretion curves and the determined feed intake (FI) and excretion times of broilers fed on diets with different dietary threonine concentrations and with or without MOS supplementation at 21 d of age*[a]

Treatment	MOS	FI[b] (g)	Excretion time[c] (h)	
Threonine		Per cage	T_1	T_{50}
Deficient	−	49·6	2·58	6·53
Deficient	+	43·9	2·08	6·06
Adequate	−	71·7	2·45	6·35
Adequate	+	67·2	2·00	5·80
Excess	−	76·3	2·32	6·13
Excess	+	69·8	2·54	6·40
Pooled SEM		5·19	0·188	0·161
Main effects				
Deficient		46·7^{B}	2·33	6·30
Adequate		69·4^{A}	2·22	6·07
Excess		73·0^{A}	2·43	6·27
	−	65·8	2·45	6·34
	+	60·3	2·20	6·09
Source of variation			*P*-values	
Threonine		<0·001	n.s.	n.s.
MOS		n.s.	n.s.	n.s.
Thr x MOS		n.s.	n.s.	n.s.

[a]Each value represents the mean of 4 replicates for each treatment group.
[b]Feed intake of diets containing 5 g/kg TiO_2.
[c]T_1, excretion time for 1% of TiO_2 in excreta; and T_{50}, excretion time for 50% of TiO_2 in excreta.
[A,B]Means within a continuous column not sharing a common superscript are significantly different at $P<0·05$. n.s., Not significant. Probability values up to $P<0·10$ are also shown if the data suggest a trend.

Feed intake, feed passage rate and excreta characteristics

Total tract transit time and excreta moisture content

Neither the excreta dry matter content nor the total tract transit times, as indicated by first excreta appearance (average 2·2 h), were affected by dietary treatment (data not shown).

Cumulative excretion curves and feed intake

Estimations of the respective first appearance time (T_1, indicated by the excretion time for 1% TiO_2) and total tract transit time (T_{50}, the time taken to excrete 50% of the TiO_2) are shown in Table 6. No significant differences in the first appearance time or total tract mean retention time were attributable to treatment.

Birds fed on diets deficient in threonine consumed significantly less feed ($P<0·01$) than those given diets adequate or excess in threonine. Dietary MOS had no significant effect on the feed intake of the birds.

Mean retention time in various regions of the small intestine and feed intake

The mean retention time in the different regions are shown in Table 7. There was a MOS and

Table 7. *Effects of dietary threonine concentration supplemented with or without MOS on the feed intake (FI) and regional mean retention time of birds at 21 d of age*[a]

Treatment	MOS	FI[b] (g)	Mean retention time (min)		
Threonine		Per cage	Duodenum	Jejunum	Ileum
Deficient	−	103·8	3·2	65·6	121·7^{A}
Deficient	+	96·2	3·1	59·1	102·1^{BC}
Adequate	−	127·6	2·6	62·1	115·8^{AB}
Adequate	+	119·1	1·8	60·5	100·5^{C}
Excess	−	120·8	3·4	56·6	100·2^{C}
Excess	+	107·9	3·0	59·4	112·5^{ABC}
Pooled SEM		6·78	0·72	2·75	4·69
Main effects					
Deficient		100·0^{B}	3·17	62·31	111·89
Adequate		123·4^{A}	3·22	61·28	108·13
Excess		114·4^{AB}	2·89	57·98	106·40
	−	117·0	2·74	61·42	112·59
	+	107·7	2·90	59·64	105·02
Source of variation			*P*-values		
Threonine		<0·01	n.s.	n.s.	n.s.
MOS		n.s.	n.s.	n.s.	n.s.
Thr x MOS		n.s.	n.s.	n.s.	<0·01

[a]Each value represents the mean of 4 replicates for each treatment group.
[b]Feed intake of diets containing 5 g/kg TiO_2.
[A–C]Means within a continuous column not sharing a common superscript are significantly different at $P<0·05$. n.s., Not significant.

threonine interaction in the ileal mean retention time such that increasing threonine in the absence of MOS led to a reduction in the mean retention time whereas a trend in the opposite direction was seen in the presence of MOS. Intriguingly, the ileal mean retention time with deficient and adequate threonine was also significantly shorter ($P < 0{\cdot}01$) in the presence of MOS. Threonine deficiency significantly reduced the feed intake of the birds ($P < 0{\cdot}01$) compared with those receiving adequate or excess threonine. Supplementation with MOS had no significant effect on feed intake.

DISCUSSION

Dietary composition and treatment design

The high degree of consistency in the calculated and analysed CP and threonine values in all the experimental diets validates the treatment effects as the source of variations in this experiment.

Enteric morphometric analysis

The negative effect of threonine deficiency on intestinal villus height is in full agreement with that reported by Hamard *et al.* (2007). Spring *et al.* (2000) and Yang *et al.* (2007*a*) also reported similar findings on the effect of MOS in increasing the intestinal villus height. The measurements of villus height and villus-to-crypt ratio generally give an indication of the likely maturity and functional capacity of the enterocytes (Hampson, 1986). Therefore, it can be deduced that MOS significantly accelerated the maturation of gut function, but this only occurred only in the jejunum when diets were deficient or adequate for threonine and in the ileum only when threonine was adequate or in excess. It is possible that threonine might have a direct effect on the intestinal morphology mediated through its obligatory involvement in the fractional, as well as absolute, synthesis of intestinal mucosa and mucins (Faure *et al.*, 2005; Nichols and Bertolo, 2008). On the other hand, MOS might exert its main influence on villus morphology via its modulating effects on intestinal mucin dynamics and microbial–mucosal interactions (Uni and Smirnov, 2006).

In contrast to the positive finding reported by Ferket *et al.* (2002) in 14-d-old birds, there were no significant effect of MOS or dietary threonine on intestinal muscularis thickness in the current study. This may indicate that older birds, which have a more mature gut microflora, are less dependent on gut motility to control gut microbial activities.

Gut microflora

The coliform counts in the digesta were significantly reduced with adequate threonine in the presence of MOS whilst coliform counts in the digesta of birds given a diet with excess threonine were increased. Supplementation of MOS also significantly reduced the mucosal coliform counts of birds given a threonine-adequate diet, but had no effect in birds receiving either a threonine-deficient or excess diet. The suppressive effect of MOS on intestinal coliform counts at adequate threonine is in accordance with the findings of Stanley *et al.* (1996). Song and Li (2001) and Jamroz *et al.* (2003) also found that MOS significantly decreased the number of *Escherichia coli* in either the small intestine or in the excreta of chickens. It is suggested that MOS, as a receptor analogue, can act as a decoy for those pathogenic or potentially pathogenic bacteria possessing type-1 fimbriae (mannose-sensitive lectins) such as *E. coli*. This, in turn, prevents them from attaching to the gut mucosa to colonise the gut and cause disease (Yang *et al.*, 2007*b*).

The actual mechanism involved in the increase of coliform counts in the digesta of birds supplemented with MOS and given a threonine-excess diet is unknown. One possible reason might be that MOS not only binds but also displaces those type-1 fimbriae bacteria attached to the gut wall (Yang *et al.*, 2007*b*). For example, in an *in vitro* study, attached *E. coli* could be displaced from the epithelial cells within 30 min when exposed to D-mannose (Ofek and Beachey, 1978).

The increase in numbers of ileal lactobacilli and lactic acid bacteria in birds fed on a diet with adequate or excess threonine indicates a close relationship between dietary threonine and intestinal mucin dynamics, gut microflora and mucosal development (Faure *et al.*, 2006). This close relationship has been critically reviewed by Deplancke and Gaskins (2001).

Intestinal mucin dynamics

The effect of dietary threonine deficiency on ileal mucin concentration and output supports the view that dietary threonine is in high demand for the synthesis of intestinal mucin glycoproteins (Faure *et al.*, 2005; Nichols and Bertolo, 2008). The modulating effects of MOS on intestinal mucin dynamics had been reported by Uni and Smirnov (2006), and Solis de los Santos *et al.* (2007). The significant increase in the ileal flow of crude mucins attributable to MOS, when threonine concentration is adequate, can be interpreted as supporting the importance of supplying enough threonine for this modulating

effect of MOS on intestinal mucin synthesis (Stoll *et al.*, 1998). However, the lack of effect with excess threonine is difficult to explain. It is possible that the measurement of crude mucin output only measured the mucin content in the digesta and did not take into account any possible contribution from excess threonine in the presence of MOS on the adherent mucous layer lining the intestinal mucosa.

Feed passage rate

In the present study, the estimations of feed passage rate based on first excreta appearance correlated well with the T_1 values of the cumulative excretion curves. However, these variables, including the total tract mean retention time (T_{50}), were not significantly modified by MOS or dietary threonine.

It is difficult to draw a conclusive explanation for the observed interaction between MOS and threonine effects on the ileal mean retention time when increasing threonine in the absence of MOS led to a reduction in the mean retention time and an increase when MOS was added. This is mainly because the significant interactions between MOS and threonine in their effects on other variables were inconsistent with that observed for the ileal transit time. However, the tendency for a similar numerical trend observed for the ileal flow of crude mucins may partly explain the effects of MOS and threonine on ileal mean retention time, especially on the significantly shorter ileal mean retention time at deficient and adequate concentrations of threonine in the presence of MOS. This apparent relationship between the two variables is likely to be mediated through the lubricating effect of crude mucins (Forstner *et al.*, 1995; Montagne *et al.*, 2000). As explained above, the measurement of crude mucin concentration and output may not be sensitive enough to reflect the total intestinal mucin dynamics. This may warrant future research to investigate the effect of MOS and threonine on intestinal adherent mucous layer conjunctively.

There is no clear indication as to what extent the intestinal musculature, volatile fatty acids and feeding activity may influence the feed passage rate as no significant differences in these parameters were observed.

CONCLUSIONS

This study demonstrated an interaction between MOS and threonine, with increasing threonine concentration in the absence of MOS leading to a significant reduction in the mean retention time, but a trend towards an increase in retention time when MOS was added to the diet. The ileal mean retention time when dietary threonine was either deficient or adequate was also significantly shorter in the presence of MOS. The significant interactions between MOS and threonine in their effects on jejunal and ileal villus-to-crypt ratio, gut microflora and ileal flow of crude mucins were also detected, but were inconsistent with that observed for the ileal mean retention time. However, the tendency for a similar numerical trend observed for the ileal flow of crude mucins may partly explain the effects of MOS and threonine on ileal mean retention time. This apparent relationship between the two variables is likely to be mediated through the lubricating effect of crude mucins.

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