Effects of Nutrient Specifications and Xylanase Plus Phytase Supplementation of Wheat-based Diets on Growth Performance and Carcass Traits of Broiler Chicks

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ABSTRACT : The simultaneous addition of xylanase (5,600 EXU/kg) and phytase (500 FTU/kg) feed enzymes to wheat-based broiler diets was investigated. Starter, grower and finisher diets, with three tiers of nutrient specifications, were fed to 1,440 broiler chicks kept on deep litter from 1-42 days post-hatch, without and with xylanase plus phytase, to determine the effects of diet type and enzyme supplementation on growth performance. The nutrient specifications of type A diets were standard; energy density and protein/amino acid levels were reduced on a least-cost basis to formulate type B diets and further reduced to type C diets. Phosphorus (P) and calcium (Ca) levels were adjusted in supplemented diets. From 1-42 days post-hatch, diet type significantly influenced growth performance. Birds on type C diets had lower growth rates (2,429 vs. 2,631 g/bird; $p<0.001$), higher feed intakes (4,753 vs. 4,534 g/bird; $p<0.005$) and less efficient feed conversion (1.96 vs. 1.72; $p<0.001$) than birds offered type A diets. Enzyme supplementation increased growth rates by 3.2% (2,580 vs. 2,501 g/bird; $p<0.005$) and improved feed efficiency by 2.7% (1.80 vs. 1.85; $p<0.05$) over the entire feeding period. There were no interactions between diet type and enzyme supplementation. At 21 days, 5 out of 30 birds per pen were transferred to cages to ascertain treatment effect on apparent metabolisable energy (AME) and nitrogen (N) retention. Xylanase plus phytase enhanced AME (13.48 to 13.91 MJ/kg DM; $p<0.001$) and N retention (56.3 to 59.7%; $p<0.005$). Carcass and breast weights of the caged birds were determined following commercial processing. Diet type significantly influenced breast weight, carcass weight and yield. Birds offered Type A diets, in comparison to Type C diets, supported heavier breast (467 vs. 424 g; $p<0.001$) and carcass weights (1,868 vs. 1,699 g; $p<0.001$) with superior carcass yields (71.8 vs. 70.6%; $p<0.005$). Enzyme addition increased carcass weight by 3.9% (1,752 vs. 1,821 g; $p<0.005$) and breast weight by 5.8% (431 vs. 456 g; $p<0.01$) without influencing yields. Feed ingredient costs per kg live weight gain and per kg carcass weight indicated that enzyme addition was economically feasible, where supplementation of Type A diets generated the most effective results. Importantly, soluble and total non-starch polysaccharide and phytate contents of the wheat used were typical by local standards. This study confirms the potential of supplementing wheat-based broiler diets with xylanase plus phytase but further investigations are required to define the most appropriate inclusion rates and dietary nutrient specifications in this context. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 10 : 1501-1509)***

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INTRODUCTION

It is recognised that wheat-based broiler diets contain variable levels of two poorly digested, anti-nutritive factors: non-starch polysaccharides (NSP), including arabinoxylan, and phytate, which is derived from all plant-sourced feed ingredients in addition to wheat. However, exogenous xylanase and phytase feed enzymes have been developed to degrade these anti-nutritive factors and ameliorate their negative effects. NSP and NSP degrading enzymes have been the subject of very considerable research as evidenced by the reports of Annison (1991), Bedford (1995), Marquardt et al. (1996) and Bedford and Schulze (1998). Similarly, phytate and phytase has been examined by Simons et al. (1990), Ravindran et al. (1995), Lenis and Jongbloed. (1999) and Selle et al. (2000). The routine inclusion of xylanases in wheat-based poultry diets (Silversides and Bedford. 1999) means that, with the increasing acceptance of phytase. the application of these two feed enzymes in tandem will occur increasingly in practice. Nevertheless, information about the simultaneous use of xylanase and phytase and their possible interactions is limited in comparison.

In one of the first assessments. Schwarz et al. (1994) did not report any interactions between phytase and two NSP degrading enzymes. although one phytase plus xylanase combination appeared to support better broiler growth performance. Simbaya et al. (1996) found that very young chicks offered a diet supplemented with phytase. carbohydrase and protease had significantly better growth and conversion than negative control birds; whereas, phytase alone did not significantly influence performance. However. Jacob et al. (2000) concluded that a combination of pentosanase and phytase did not benefit the performance of broilers. Nevertheless. a series of recent reports (Ravindran et al., 1999; Zyla et al., 1999; Peng et al., 2003; Selle et al., 2003b) suggest that the simultaneous use of xylanase and phytase has potential.

Therefore. this experiment was designed to evaluate xylanase plus phytase supplementation of wheat-based

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Table 1. Dietary composition and feed ingredient costs of starter diets

Ingredient (g/kg) \ Diet type	A Minus	A Plus	B Minus	B Plus	C Minus	C Plus
Wheat	575.25	582.13	630.53	638.39	619.66	622.44
Canola meal	75.00	75.00	75.00	75.00	100.00	100.00
Cottonseed meal	25.00	25.00	25.00	25.00	25.00	25.00
Meat & bone meal	50.00	45.22	50.00	45.25	25.86	10.97
Soyabean meal	206.24	209.88	180.88	184.28	196.18	207.52
Tallow	40.00	40.00	13.71	10.84	-	-
Sunflower oil	9.48	7.28	5.11	4.99	5.18	5.15
Dicalcium phosphate	5.30	-	5.30	-	11.20	8.50
Limestone	1.70	3.50	1.40	3.20	6.00	9.40
Salt	1.60	1.60	1.50	1.50	1.90	1.90
Na bicarbonate	2.54	2.62	2.65	2.73	2.55	2.81
K carbonate	0.45	0.33	1.44	1.33	0.53	0.17
Lysine HCl	2.41	2.43	2.51	2.54	1.69	1.85
Methionine	2.44	2.45	2.40	2.40	2.25	2.29
Threonine	0.59	0.56	0.57	0.55	-	-
Premix[1]	2.00	2.00	2.00	2.00	2.00	2.00
Ingredient cost (A$/tonne)[2]	343.69	340.58	328.79	326.04	322.42	321.38

[1] The trace mineral-vitamin premix was added to all diets (Tables 1, 3, 5) at an inclusion rate of 2.0 kg per tonne. This premix supplied, per 1 kg of diet: 12,000 MIU retinyl acetate, 3,500 MIU cholecalciferol, 40.0 mg dl-α-tocopheryl acetate, 4.0 mg menadione, 3.0 mg thiamine, 10.0 mg riboflavin, 15.0 mg calcium pantothenate, 50.0 mg nicotinic acid, 5.0 mg pyridoxine, 0.20 mg biotin, 0.02 mg cyanocobalmin, 70.0 mg Mn as manganous oxide, 60.0 mg Zn as zinc oxide, 50.0 mg Fe as ferrous sulphate, 8.0 mg Cu as copper sulphate, 1.0 mg Mb as sodium molybdate, 0.3 mg Co as cobalt sulphate, 1.0 mg I as calcium iodate and 0.1 mg Se as a 2% selenium premix. [2] A$1.00≅US$0.55.

Table 2. Nutrient specifications and analysed values for starter diets

Item \ Diet type	A Minus	A Plus	B Minus	B Plus	C Minus	C Plus
Specifications (g/kg)						
Calcium	10.14	9.15	10.15	9.16	10.01	10.22
Phosphorus	7.60	6.43	7.62	6.45	7.86	6.72
Nonphytate-P	4.50	3.30	4.50	3.30	4.51	3.31
Phytate-P	3.10	3.13	3.12	3.15	3.36	3.41
ME (MJ/kg)	12.55	12.55	12.05	12.05	11.60	11.61
Protein	235.65	234.67	230.52	230.59	231.72	229.55
Lysine	13.50	13.50	13.10	13.10	12.70	12.70
Methionine	6.00	6.00	5.90	5.90	5.80	5.80
Met.+cystine	10.40	10.42	10.28	10.30	10.36	10.36
Threonine	8.80	8.80	8.50	8.50	8.20	8.20
Tryptophan	2.47	2.49	2.42	2.43	2.52	2.53
Arginine	15.00	15.00	14.54	14.54	14.75	14.63
Analysed values						
Protein (N×6.25)	236.88	234.38	230.63	233.13	231.25	231.88
Calcium	10.90	11.00	10.80	10.10	12.10	9.85
Phosphorus	8.60	8.05	8.40	8.00	9.45	7.25
Gross energy (MJ/kg 'as-is')	17.59	17.56	17.03	16.91	16.70	16.71
Phytase activity (FTU/kg)	<100	600	<100	1130	<100	600
Xylanase activity (EXU/kg)	180	6,790	100	8,320	210	5,870

broiler diets in an applied context. The effects of simultaneous enzyme inclusion in starter, grower, and finisher diets with three tiers of nutrient specifications, on growth performance of broilers kept on deep litter from 1-42 days post-hatch were determined. The diets were formulated on a least-cost basis so the economic feasibility of enzyme addition could be assessed on the basis of feed ingredient costs. To generate additional information, five out of thirty birds were removed from each pen at 21 days post-hatch, identified with wing bands and transferred to wire cages. This permitted total excreta collection to determine the effects of simultaneous enzyme inclusion on AME and N retention and breast and carcass weights and their yields following commercial processing.

MATERIALS AND METHODS

The design was a 3×2 factorial involving starter, grower

Table 3. Dietary composition and feed ingredient costs of grower diets

Ingredient (g/kg) \ Diet type	A Minus	A Plus	B Minus	B Plus	C Minus	C Plus
Wheat	619.07	619.64	674.83	675.66	679.43	678.27
Canola meal	75.00	75.00	75.00	75.00	100.00	100.00
Cottonseed meal	25.00	25.00	25.00	25.00	25.00	25.00
Meat & bone meal	50.00	36.51	50.00	36.51	10.54	-
Soyabean meal	163.28	177.32	138.44	152.41	147.16	158.43
Tallow	45.00	45.00	14.63	13.87	-	-
Sunflower oil	6.21	5.62	4.84	4.80	4.87	4.86
Dicalcium phosphate	2.80	-	2.80	-	12.70	8.90
Limestone	1.30	3.80	1.00	3.50	6.80	9.50
Salt	1.40	1.50	1.30	1.40	1.50	1.50
Na bicarbonate	2.63	2.81	2.75	2.94	3.39	3.71
K carbonate	1.82	1.34	2.79	2.31	2.24	2.89
Lysine HCl	2.41	2.43	2.53	2.55	2.47	2.48
Methionine	1.63	1.65	1.61	1.64	1.65	1.66
Threonine	0.45	0.38	0.48	0.41	0.25	0.20
Premix	2.00	2.00	2.00	2.00	2.00	2.00
Choline chloride	-	-	-	-	-	0.60
Ingredient cost (A$/tonne)	326.75	325.60	312.99	311.95	307.43	308.06

Table 4. Nutrient specifications and analysed values for grower diets

Item \ Diet type	A Minus	A Plus	B Minus	B Plus	C Minus	C Plus
Specifications (g/kg)						
Calcium	9.44	8.43	9.45	8.45	10.21	9.32
Phosphorus	7.02	5.93	7.04	5.96	7.29	6.14
Nonphytate-P	4.00	2.85	4.00	2.85	4.00	2.81
Phytate-P	3.02	3.08	3.04	3.11	3.29	3.33
ME (MJ/kg)	12.68	12.68	12.18	12.18	11.70	11.70
Protein	220.61	219.90	216.85	216.14	210.23	209.59
Lysine	12.50	12.50	12.13	12.13	11.75	11.75
Methionine	5.00	5.00	4.94	4.94	4.88	4.88
Met+cys	9.22	9.25	9.14	9.17	9.17	9.18
Threonine	8.00	8.00	7.75	7.75	7.49	7.49
Tryptophan	2.31	2.34	2.26	2.29	2.30	2.32
Arginine	13.90	13.90	13.46	13.46	13.04	13.04
Analysed values						
Protein (N×6.25)	221.88	220.00	218.13	221.88	206.25	210.00
Calcium	10.90	11.20	11.70	10.70	10.70	11.10
Phosphorus	7.90	7.10	8.25	7.20	7.90	7.20
Gross energy (MJ/kg 'as-is')	17.47	17.47	16.82	16.82	16.28	16.40
Phytase activity (FTU/kg)	<100	600	<100	570	<100	510
Xylanase activity (EXU/kg)	100	4,900	380	5,440	<100	5,680

and finisher diets with three tiers of nutrient specifications (Diet types A, B and C) without and with xylanase (5,600 EXU/kg) plus phytase (500 FTU/kg) supplementation. Samples of relevant feed ingredients were analysed for crude protein, total P and Ca by standard methods and on this basis, starter, grower and finisher diets were formulated and offered to birds from 1-14, 15-28 and 29-42 days post-hatch, respectively. To achieve three tiers of nutrient specifications, the average specified energy density of the type A diets of 12.66 MJ/kg was reduced to 12.16 and 11.70 MJ/kg for type B and C diets, respectively. Similarly, the average crude protein level of 219 g/kg for type A diets was reduced to 215 and 212 g/kg with parallel reductions in critical amino acids. Diets supplemented with the enzyme combination (designated plus) contained an average of 1.17 g non-phytate-P/kg and 1.00 g Ca/kg less than the corresponding, non-supplemented diets (designated minus). The dietary treatments were formulated on a least-cost basis (Agri-Data Systems, Inc. San Diego, CA) and the feed ingredient costs per tonne, inclusive of enzyme addition, are tabulated.

The wheat used was pre-pelleted at approximately 90°C, which reduced its intrinsic phytase activity to only 20

Table 5. Dietary composition and feed ingredient costs of finisher diets

Ingredient (g/kg) \ Diet type	A Minus	A Plus	B Minus	B Plus	C Minus	C Plus
Wheat	684.74	677.69	733.22	726.01	724.82	725.05
Canola meal	85.00	85.00	85.00	85.00	100.00	100.00
Cottonseed meal	25.00	25.00	25.00	25.00	25.00	25.06
Meat & bone meal	50.00	26.19	50.00	26.05	-	-
Soyabean meal	91.37	117.75	73.90	100.45	110.74	114.27
Tallow	42.93	44.52	12.38	14.00	-	-
Sunflower oil	5.14	5.19	4.51	4.56	4.62	4.61
Dicalcium phosphate	0.40	-	0.30	-	12.90	6.50
Limestone	0.70	4.40	0.40	4.10	5.50	6.80
Salt	1.30	1.50	1.30	1.50	1.50	1.20
Na bicarbonate	2.67	2.97	2.71	3.02	3.64	4.57
K carbonate	3.63	2.73	4.31	3.40	4.88	4.91
Lysine	2.38	2.40	2.34	2.36	2.13	2.03
Methionine	1.69	1.73	1.66	1.70	1.69	1.68
Threonine	1.05	0.93	0.97	0.85	0.58	0.52
Premix	2.00	2.00	2.00	2.00	2.00	2.00
Choline chloride	-	-	-	-	-	0.80
Ingredient cost (A$/tonne)	310.85	311.58	298.09	298.86	298.98	297.42

Table 6. Nutrient specifications and analysed values for finisher diets

Item \ Diet type	A Minus	A Plus	B Minus	B Plus	C Minus	C Plus
Specifications (g/kg)						
Calcium	8.75	7.74	8.74	7.73	8.79	7.81
Phosphorus	6.45	5.36	6.48	5.40	6.74	5.58
Nonphytate-P	3.51	2.32	3.50	2.32	3.50	2.32
Phytate-P	2.94	3.04	2.99	3.08	3.24	3.26
ME (MJ/kg)	12.76	12.76	12.26	12.26	11.78	11.83
Protein	201.37	199.78	199.71	198.10	194.23	195.81
Lysine	11.00	11.00	10.68	10.68	10.35	10.37
Methionine	4.80	4.80	4.75	4.75	4.69	4.70
Met + Cystine	8.78	8.81	8.75	8.78	8.78	8.83
Threonine	7.60	7.60	7.36	7.36	7.11	7.13
Tryptophan	2.07	2.13	2.05	2.10	2.14	2.16
Arginine	12.25	12.25	12.00	12.00	11.80	11.92
Analysed values						
Protein (N×6.25)	200.00	198.75	200.00	195.00	193.13	197.50
Calcium	9.80	8.50	9.65	9.75	10.90	9.45
Phosphorus	7.70	6.80	7.80	6.05	7.90	6.45
Gross energy (MJ/kg 'as-is')	17.28	17.14	16.80	16.80	16.46	16.44
Phytase activity (FTU/kg)	<100	510	<100	510	<100	600
Xylanase activity (EXU/kg)	<100	5,170	230	5,310	<100	5,760

FTU/kg. Wheat was analysed for soluble and insoluble NSP fractions by an assay based on the Uppsala method developed by Choct et al. (1999). Wheat and other plant-sourced feed ingredients were analysed for phytate content by a standard ferric chloride precipitation method, which has been described in detail by Miller et al. (1980). In addition all diets were analysed for crude protein, Ca and P and their gross energy was determined on an 'as-is' basis. The dietary compositions, nutrient specifications and analysed values for the starter, grower and finisher diets are detailed in Tables 1 to 6.

Feed enzymes

The liquid enzymes used were Natugrain® Wheat xylanase (28,000 EXU/g) at 200 g and Natuphos® phytase (5,000 FTU/g) at 100 g per tonne of feed, which were supplied by BASF Aktiengesellschaft (Ludwigshafen, Germany). Both enzymes are produced from genetically modified isolates of *Aspergillus niger* and their respective activities are considered to be specific. One phytase unit (FTU) is defined as the amount of enzyme, which liberates 1.00 micromole of inorganic P per minute from 0.0051 mole per litre sodium phytate at 37°C and pH 5.5. One endoxylanase unit (EXU) is defined as the amount of

Table 7. Effects of three tiers of dietary specifications and xylanase (5,600 EXU/kg) plus phytase (500 FTU/kg) inclusion on growth performance from 1-14, 15-28, 29-42 and 1-42 days post-hatch of birds kept on deep litter[1]

Parameter	Treatment						Pooled SEM	Significance (p=)		
	A Minus	A Plus	B Minus	B Plus	C Minus	C Plus		Diet	Enzyme	Interaction
1-14 days post-hatch										
Growth rate (g/bird)	383	397	353	383	328	360	4.935	0.000	0.000	0.145
Feed intake (g/bird)	474	480	466	492	457	481	7.360	0.374	0.003	0.360
Feed:Gain (g/g)	1.24	1.21	1.32	1.29	1.39	1.33	0.0139	0.000	0.001	0.492
15-28 days post-hatch										
Growth rate (g/bird)	998	1034	972	1018	913	934	12.784	0.000	0.002	0.611
Feed intake (g/bird)	1,518	1,500	1,527	1,565	1,595	1,608	32.974	0.026	0.692	0.702
Feed:Gain (g/g)	1.52	1.45	1.57	1.54	1.75	1.72	0.0345	0.000	0.130	0.758
29-42 days post-hatch										
Growth rate (g/bird)	1,224	1,228	1,186	1,209	1,146	1,177	22.193	0.022	0.292	0.825
Feed intake (g/bird)	2,573	2,523	2,565	2,591	2,664	2,701	43.037	0.008	0.898	0.548
Feed:Gain (g/g)	2.11	2.06	2.16	2.15	2.33	2.30	0.0394	0.000	0.287	0.883
1-42 days post-hatch										
Growth rate (g/bird)	2,604	2,659	2,511	2,610	2,388	2,471	29.721	0.000	0.002	0.744
Feed intake (g/bird)	4,565	4,503	4,558	4,648	4,716	4,790	64.468	0.005	0.519	0.440
Feed:Gain (g/g)	1.76	1.69	1.82	1.78	1.98	1.94	0.0230	0.000	0.023	0.813
Ingredient costs per kg live wt gain (A$/kg)	0.560	0.541	0.556	0.547	0.601	0.588				

[1] 8 replicates of 30 birds per treatment from 1-21 days and of 25 birds per treatment from 22–42 days post-hatch.

enzyme, which liberates 4.53 micromole of reducing sugars per minute from xylan, measured as xylose equivalents, at 40°C and pH 3.5. The feed enzymes were weighed out, mixed and diluted immediately prior to application; the combined liquid preparations were either sprayed onto the complete starter and grower diets in a horizontal mixer or onto the wheat component of the finisher diet prior to entry into a vertical mixer. The xylanase activity of the 18 experimental diets was assessed by determining the release rate of reducing sugars from xylan by xylanase under standard conditions. The phytase activity was determined by the method of Engelen et al. (1994), which is based on the liberation of inorganic P from sodium phytate by phytase.

Bird management

A total of 1,500 male, day-old Cobb chicks were obtained from a commercial hatchery and 1,440 were selected on the basis of body weight and allocated into 48 deep litter pens (30 per pen) in an environmentally-controlled facility and given free access to feed and water. The initial temperature of 32°C (week 1) was gradually reduced to 22°C (week 6) with fluorescent lighting being provided for 22 h per day. Each of the 6 dietary treatments was randomly allotted to 8 pens. Birds were weighed on days 1, 14, 21, 28 and 42 post-hatch and corresponding feed intakes recorded. Feed conversion ratios were calculated with allowances made for mortalities, which were recorded on a daily basis.

At 21 days post-hatch, 5 birds were randomly selected from each pen, wing-banded, and transferred to an adjacent facility and similarly distributed to 48 wire-floored battery cages. Total excreta output was collected from 23-26 days post-hatch to determine AME and N retention by standard procedures as outlined by Selle et al., (2003b). At the conclusion of the feeding period feed was withheld overnight and, at 43 days post-hatch, 'empty' live body weights of the caged birds were determined, they were then transported to a commercial processor where carcass weights were determined. Two representative carcasses from each replicate were retained and the breast muscle was dissected to determine the weight and yield.

Statistical analysis

The experimental data was subjected to two-way analyses of variance by a general linear models procedure using a Widows compatible software program (SPSS Inc. Chicago, IL). Main effects of treatments are considered, as are pair-wise comparisons between treatments where appropriate.

RESULTS

The effects of treatment on growth performance of birds are shown in Table 7. Reducing dietary nutrient specifications significantly influenced growth rates in the three feeding phases with an overall reduction ($p<0.001$) of 7.7% from 2,631 g/bird in Type A diets to 2,429 g/bird in Type C diets from 1-42 days post-hatch. Diet types did not influence feed intake in the starter phase but reducing specifications increased intake in the grower ($p<0.05$) and finisher ($p<0.01$) phases. Overall, birds offered Type C diets had a higher feed intake than Type A diets (4,753 vs. 4,534

Table 8. Effects of three tiers of dietary specifications and xylanase (5,600 EXU/kg) plus phytase (500 FTU/kg) inclusion on AME, N retention, breast weight, carcass weight and yields of birds transferred to cages from 22-42 days post-hatch[1]

Parameter	Treatment						Pooled	Significance (p=)		
	A Minus	A Plus	B Minus	B Plus	C Minus	C Plus	SEM	Diet	Enzyme	Interaction
AME (MJ/kg DM)	14.42	14.67	12.90	13.58	13.11	13.49	0.137	0.000	0.000	0.304
N retention (%)	57.2	60.1	51.7	55.9	60.1	63.1	1.45	0.000	0.007	0.902
Breast weight										
Carcass weight (g/bird)	1,840	1,979	1,808	1,844	1,703	1,789	35.48	0.000	0.004	0.357
Breast weight (g/bird)	442	491	442	436	410	437	10.32	0.001	0.009	0.037
Yield (%)	24.0	24.8	24.5	23.7	24.1	24.4	0.365	0.693	0.718	0.071
Carcass weight										
Final live wt. (g/bird)	2,496	27.09	2,514	2,518	2,363	2,448	35.23	0.000	0.001	0.017
Carcass weight (g/bird)	1,793	1,943	1,787	1,797	1,675	1,723	27.93	0.000	0.004	0.045
Yield (%)	71.8	71.7	71.1	71.4	70.9	70.4	0.301	0.002	0.696	0.400
Feed:Carcass wt. (g/g)	2.58	2.45	2.64	2.58	2.77	2.72	0.022	0.000	0.000	0.133
Ingredient costs per kg carcass wt (A$/kg)	0.824	0.795	0.808	0.803	0.842	0.839				

[1] 8 replicates of 5 birds per treatment.

g/bird; $p<0.01$). Feed efficiency declined significantly ($p<0.001$) with reduced dietary specifications in all phases. From 1-42 days post-hatch birds on Type C diets had a feed efficiency of 1.96, which was inferior ($p<0.001$) to the 1.72 feed conversion ratio of birds offered Type A diets.

The effects of xylanase plus phytase supplementation were most evident in the starter phase generating increases of 7.0% in weight gain (380 vs. 355 g/bird; $p<0.001$), 4.1% in feed intake (484 vs. 465 g/bird; $p<0.005$) and 3.0% in feed efficiency (1.28 vs. 1.32; $p<0.005$). In the grower phase, the enzyme combination increased weight gain by 3.5% (995 vs. 961 g/bird; $p<0.005$) but did not influence feed intake nor feed efficiency. In the finisher phase xylanase plus phytase did not alter growth performance. From 1-42 days post-hatch, enzyme supplementation influenced both weight gain (2,580 vs. 2,501 g/bird; $p<0.005$) and feed efficiency (1.80 vs. 1.85; $p<0.05$) with improvements of 3.2% and 2.7%, respectively, without significantly altering feed intake. There were no significant interactions between treatments observed.

The effects of treatment on AME, N retention and carcass traits are shown in Table 8. Xylanase plus phytase supplementation increased AME ($p<0.001$) and N retention ($p<0.005$); the main effect of enzyme addition was to increase AME by 0.43 MJ (13.48 to 13.91 MJ/kg DM) and N retention by 6.0% or 3.4 percentage units (56.3 to 59.7%). Diet type had significant effects ($p<0.001$) on both parameters but the pattern of results was unexpected as relatively low values were recorded for birds receiving the non-supplemented Type B diets.

Both increasing nutrient specifications and enzyme addition enhanced ($p<0.005$) breast weight. Birds offered type A diets had average breast weights of 467 g as opposed to 424 g for birds on type C diets. Enzyme addition increased breast weight by 5.8% from 431 to 456 g/bird, neither treatment had any affect on yield of breast muscle. There was an interaction ($p<0.05$) between treatments as the effect of enzyme addition was more pronounced with Type A and C diets.

Both diet type ($p<0.001$) and enzyme addition ($p<0.005$) significantly influenced carcass weight, with an interaction ($p<0.05$) between treatments. The carcass weight was 1,868 g/bird (71.8% yield) on type A diets as opposed to 1,699 g/bird (70.6% yield) on type C diets and the effect of diet type on yield was significant ($p<0.005$). Enzyme addition increased carcass weight by 3.9% (1,752 to 1,821 g/bird), without influencing yield. The interaction occurred because the effects of enzyme addition on carcass weights were more pronounced with type A and C diets than with type B diets. Both diet type and enzyme addition significantly improved ($p<0.001$) conversion efficiency of feed consumption to carcass weight. Birds on type A diets were 8.4% more efficient than those on type C diets (2.52 vs. 2.75) and enzyme addition resulted in an improvement of 3.0% (2.59 vs. 2.67).

In terms of both feed ingredient costs per kg live weight gain (Table 7) and feed ingredient costs per kg carcass weight (Table 8) the most effective results were obtained with enzyme supplemented Type A diets. Savings of 3.4% and 3.5% respectively were realised in comparison to non-supplemented Type A diets.

The wheat used as the basis of the experimental diets contained 11.51 g soluble NSP and 93.00 g total NSP/kg and 2.40 g phytate-P/kg. The phytate levels in the other relevant feed ingredients were 4.30, 7.80 and 9.70 g phytate-P/kg for soyabean meal, canola meal and cottonseed meal, respectively. Thus from the dietary formulations it can be calculated that the average phytate content was 3.15 g phytate-P/kg with a range of 2.94 to 3.41 g phytate-P/kg. After making adjustments for intrinsic enzyme activities, the supplemented diets contained averages of 5,749 EXU/kg xylanase and 526 FTU/kg

phytase activities of microbial origin, which are in close agreement with the intended inclusion rates of 5,600 EXU and 500 FTU/kg.

DISCUSSION

The wheat used contained 11.51 g soluble and 93.00 g total NSP/kg, which is marginally less than the 12.2 g soluble and 104.8 g total NSP/kg averages recorded in a survey of 81 local wheat samples (Choct et al., 1999). Also wheat contained 2.40 g phytate-P/kg, which is slightly more than the average of 2.20 g phytate-P/kg reported in a survey of 37 Australian wheat samples (Selle et al., 2003c). Based on the phytate content of relevant feed ingredients, the 18 experimental diets had an average level of 3.15 g phytate-P/kg, which is within the normal range of 2.5 to 4 g phytate-P/kg stated for poultry diets (Ravindran, 1995). As apparently subtle differences in substrate levels may influence the magnitude of responses to xylanase plus phytase supplementation of wheat-based broiler diets (Selle et al., 2002), the typical dietary substrate levels in the present study are noteworthy. It follows that the responses to enzyme supplementation observed should be representative of the 'normal' situation.

That three tiers of dietary specifications significantly influenced growth performance from 1-42 days post-hatch and breast and carcass weights at slaughter was anticipated. It is interesting, however, that increasing nutrient specifications significantly enhanced carcass yield.

From 1-42 days post-hatch, xylanase plus phytase significantly improved weight gain and feed efficiency. However, the impact of enzyme supplementation was most pronounced in the starter phase, which was associated with a significant increase in feed consumption. Xylanase plus phytase did not influence bird performance in the finisher phase. The implication is that younger birds are more vulnerable to the anti-nutritive properties of NSP and phytate, which may have posed a constraint on voluntary feed intake during the starter phase as indicated by the feed intake response.

While the modes of action of exogenous enzymes have been considered extensively, the underlying mechanisms of xylanase (Bedford and Schulze, 1998) and, to a greater extent, of the 'extra-phosphoric' effects of phytase (Selle et al., 2000) are still not completely understood. However, it appears that when used simultaneously, the exogenous enzymes may increase apparent ileal digestibility of amino acids to a synergistic extent (Ravindran et al., 1999; Selle et al., 2003b), which may have contributed to the 6.0% improvement in N retention recorded in the present study. Enhanced apparent ileal digestibility of amino acids, as a result of xylanase plus phytase supplementation, could be due to increased substrate access and/or complementary modes of action. NSP exacerbate endogenous amino acid losses in broilers (Angkanaporn et al., 1994) but it has not been established that phytate has a similar effect. Thus the capacity of phytase to increase apparent ileal digestibility of amino acids (Ravindran et al., 2001) may stem more from improved digestibility of dietary amino acids. Therefore, it has been proposed that xylanase plus phytase may have complementary modes of action in this respect (Selle et al., 2003b).

In terms of substrate access, it is relevant that Peng et al. (2003) found that phytase increased the apparent total tract digestibility of phytate from 26.1 to 64.7% of broilers offered wheat-based diets. While xylanase had no effect (29.9%), xylanase plus phytase increased phytate digestibility to 77.8%, which was significantly ($p<0.05$) higher than for phytase alone. These figures suggest that exogenous phytase degraded 38.6% of dietary phytate as opposed to 51.7% for xylanase plus phytase and that xylanase enhanced access of phytase to its substrate. This proposition is supported by *in vitro* data (Parkkonen et al., 1997) that implies xylanase could increase the access of phytase to phytate in the aleurone layer of wheat, where phytate is concentrated (Ravindran et al., 1995). Peng et al. (2003) also reported that xylanase plus phytase improved feed efficiency by 7.3% ($p<0.05$) over a 6 week feeding period. In contrast, the numerical improvements associated with xylanase (3.9%) or phytase (2.4%) individually were not significant and their summed response is less than that of the combination.

In the present study, xylanase plus phytase significantly enhanced dietary AME by 0.43 MJ/kg. This is consistent with earlier findings (Ravindran et al., 1999) where xylanase plus phytase increased energy utilisation of 'low-ME' wheat (Mollah et al., 1983) by 2.30 MJ/kg (12.08 versus 14.38 MJ/kg DM). In contrast, individually, xylanase and phytase increased wheat AME by 1.17 and 0.64 MJ/kg, respectively; thus the summed response is less than that generated by the combination. Soluble NSP levels are inversely related to the AME in wheat (Annison, 1991) and they have the capacity to increase gut viscosity. Reductions in gut viscosities achieved by NSP degrading enzymes are considered to be an important component of their mode of action (Choct, 1998). In contrast, the mechanisms whereby phytase increases AME (Ravindran et al., 20001) and phytate negatively influences energy utilisation by broilers have not been equally clarified (Selle et al., 2003b). However, it follows that if xylanase facilitates the hydrolysis of phytate by phytase the magnitude of AME responses to xylanase plus phytase will be increased.

The relative efficacy of xylanase plus phytase on growth performance following their addition to P-deficient (1.5 g/kg available P) wheat-based diets has been demonstrated by Zyla et al. (1999). These workers found marked

improvements in weight gain (23.2%) and feed efficiency (16.7%) following supplementation with 400 FXU/kg xylanase plus 800 FTU/kg phytase. In contrast, the corresponding responses to 400 FXU/kg xylanase were minimal (-0.3% and 2.0%) and to 1,000 FTU/kg phytase relatively modest (9.9% and 4.9%). Curiously, however, there was no significant difference in bone mineralisation between phytase (107.7 g/kg toe ash) and the combination (95.1 g/kg) at the same inclusion rates.

Reductions in feed ingredient costs per kg of live weight gain (3.4%) and per kg carcass weight (3.6%) following xylanase plus phytase supplementation of type A diets indicate that combined enzyme supplementation of standard wheat-based diets, with typical substrate levels, is viable. It was anticipated that responses to xylanase plus phytase may have been more pronounced in diets with reduced nutrient specifications. For example, Selle et al. (1999) reported significant interactions for feed efficiency and N retention when phytase was used to supplement standard and modified sorghum-based broiler diets, as responses were more pronounced in modified diets with reduced specifications. However, in the present study there were not any similar interactions between diet type and xylanase plus phytase. In fact, it appears that 'over the top' supplementation of Type A diets was the most economically advantageous approach.

Nevertheless, on the basis of pair-wise comparisons, there were no statistical differences in weight gain ($p=0.890$) and feed efficiency ($p=0.354$) between birds offered Type A minus or Type B plus diets. This indicates that enzyme supplementation compensated for the reductions in nutrient specifications in growth performance. However, birds offered Type C plus diets had inferior feed efficiency ($p<0.001$) in comparison to the Type B minus birds.

It is noteworthy that the growth performance of birds transferred to cages was inferior to their counterparts remaining on deep litter from 22-42 days post-hatch. Also enzyme supplementation increased growth rate by 3.1% ($p<0.05$) and feed efficiency by 3.9% ($p<0.001$); whereas enzyme supplementation did not significantly influence growth performance of the better performing birds on deep litter (data not shown). These observations could be attributed to the negative impact of stress associated with the transfer and adjustment to new accommodation and responses to enzyme inclusion may be more pronounced when bird performance is compromised by stress factors. The unexpected pattern of results for diet type on AME and N retention may be associated as excreta were collected shortly after the transfer; nevertheless, enzyme supplementation significantly increased AME and N retention.

Although the enzymes were not assessed individually, the results of this study confirm the simultaneous inclusion of xylanase plus xylanase in wheat-based broiler diets with typical substrate levels has potential. There are indications that lower xylanase inclusion rates could be advantageous when used individually (Selle at al., 2003a) or in combination with 500 FTU/kg phytase activity (Selle at al., 2002). The dose titration study of Huang et al. (2003) illustrates the difficulties in defining the most appropriate inclusion rates for xylanase and phytase in tandem but further research with this objective appears to be entirely justified.

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REFERENCES

Angkanaporn, K., M. Choct, W. L. Bryden and E. F Annison. 1994. Effects of wheat pentosans on endogenous amino acid losses in chickens. J. Sci. Food Agric. 66:399-404.

Annison, G. 1991. Relationship between the levels of soluble nonstarch polysaccharides and the apparent metabolizable energy of wheats assayed in broiler chickens. J. Agric. Food Chem. 39:1252-1256.

Bedford, M. R. 1995. Mechanism of action and potential environmental benefits from the use of feed enzymes. Anim. Feed Sci. Technol. 53:145-155.

Bedford, M. R. and H. Schulze. 1998. Exogenous enzymes of pigs and poultry. Nutr. Res. Rev.11:91-114.

Choct, M. 1998. The effect of different xylanases on carbohydrate digestion and viscosity along the intestinal tract in broilers. In: Proc. Aust. Poult. Sci. Symp. Vol. 10:111-115.

Choct, M., R. J. Hughes and G. Annison. 1999. Apparent metabolisable energy and chemical composition of Australian wheat in relation to environmental factors. Aust. J. Agric. Res. 50:447-451.

Engelen, A. J., F. C. van der Heeft, P. H. G. Randsdorp and E. L. C. Smit. 1994. Simple and rapid determination of phytase activity. J. AOAC Int.

Huang, K., P. H. Selle, X. Li, R. J. Gill, W. I. Muir and W. L. Bryden. 2003. Phytase plus xylanase dose titration and broiler performance on wheat-based diets. In: Proc. Aust. Poult. Sci. Symp. Vol. 15:113.

Jacob, J. P., S. Ibrahim, R. Blair, H. Namkung and I. K. Paik. 2000. Using enzyme supplemented, reduced protein diets to decrease nitrogen and phosphorus excretion of broilers. Asian-Aust. J. Anim. Sci. 11:1561-1567.

Lenis, N. P. and A. W. Jongbloed. 1999. New technologies in low

pollution swine diets: diet manipulation and use of synthetic acids, phytase an phase feeding for reduction of nitrogen and phosphorus excretion and ammonia emission -Review. Asian-Aust. J. Anim. Sci. 12:305-327.

Marquardt, R. R., A. Brenes, Z. Zhang and D. Boros. 1996. The use of enzymes to improve nutrient availability in poultry feedstuffs. Anim. Feed Sci. Technol. 60:321-330.

Miller, G. A., V. L. Youngs and E. L. Oplinger. 1980. Environmental and cultivar effects on oat phytic acid concentration. Cereal Chem. 57:189-191.

Mollah, Y., W. L. Bryden, I. R. Wallis, D. Balnave and E. F. Annison. 1983. Studies of low metabolisable energy wheats for poultry using conventional and rapid assay procedures and the effects of feed processing. Br. Poult. Sci. 24:81-89.

Parkkonen, T., A. Tervila-Wilo, M. Hopeakoski-Nurminen, A. Morgan, K. Poutanen and K. Autio. 1997. Changes in wheat microstructure following *in vitro* digestion. Acta Agric. Scand., Sect. B, Soil and Plant Sci. 47:43-47.

Peng, Y. L., Y. M. Guo and J. M. Yuam. 2003. Effects of phytase replacing partial inorganic phosphorus supplementation and xylanase on the growth performance and nutrient digestibility in broilers fed wheat-based diets. Asian-Aust. J. Anim. Sci. 16:239-247.

Ravindran, V. 1995. Phytases in poultry nutrition. An overview. In: Proc. Aust. Poult. Sci. Symp. Vol. 7:135-139.

Ravindran, V., W. L. Bryden and E. T. Kornegay. 1985. Phytates: occurrence, bioavailability and implications in poultry nutrition. Poult. Avian Biol. Rev. 6:125-143.

Ravindran, V., P. H. Selle and W. L. Bryden. 1999. Effects of phytase supplementation, individually and in combination, with glycanase on the nutritive value of wheat and barley. Poult. Sci. 78:1588-1595.

Ravindran, V., P. H. Selle, G. Ravindran, P. C. H. Morel, A. K. Kies and W. L. Bryden. 2001. Microbial phytase improves performance, apparent metabolizable energy and ileal amino acid digestibility of broilers fed a lysine-deficient diet. Poult. Sci. 80:338-344.

Schwarz, G., F-J. Schoner, H. Weiske and P. P. Hoppe. 1994. Evaluation of a possible interaction between microbial phytase and two carbohydrase preparations in broilers. In: Proc. Soc. Nutr. Physiol. 2:47.

Simbaya, J., B. A. Slominski, W. Guenter, A. Morgan and L. D. Campbell. 1996. The effects of protease and carbohydrase on the nutritive value of canola meal for poultry: In vitro and in vivo studies. Anim. Feed Sci. Technol. 61:219-234.

Selle, P. H., V. Ravindran, R. A. Caldwell and W. L. Bryden. 2000. Phytate and phytase: consequences for protein utilisation. Nut. Res. Rev. 13:255-278.

Selle, P. H., V. Ravindran, P. H. Pittolo and W. L. Bryden. 1999. An evaluation of microbial phytase in sorghum-based broiler diets. In: Proc. Aust. Poult. Sci. Symp. Vol. 11:97-100.

Selle, P. H., V. Ravindran, P. H. Pittolo, R. J. Gill and W. L. Bryden. 2002. Xylanase plus phytase supplementation of broiler diets based on different wheats. In: Proc. Aust. Poult. Sci. Symp. Vol. 14:141-144.

Selle, P. H., P. H. Pittolo, B. Wells, R. J. Gill and W. I. Muir. 2003a. Effects of exogenous xylanases on growth performance of broilers offered steam-pelleted diets from 1-40 days post-hatch. In: Proc. Aust. Poult. Sci. Symp. Vol. 15: 99-102.

Selle, P. H., V. Ravindran, G. Ravindran, P. H. Pittolo and W. L. Bryden. 2003b. Influence of phytase and xylanase supplementation on growth performance and nutrient utilisation of broilers offered wheat-based diets. Asian-Aust. J. Anim. Sci. 16:394-402.

Selle, P. H., A. R. Walker and W. L. Bryden. 2003c. Total and phytate-phosphorus contents and phytase activity of Australian-sourced feed ingredients for pigs and poultry. Aust. J. Exp. Agric. 43:475-479.

Silversides, F. G. and M. R. Bedford. 1999. Enzymes may improve energy, protein digestibility. Feedstuffs 71(9):15-17.

Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, P. Slump, K. D. Bos, M. G. E. Wolters, R. F. Beudeker and G. J. Verschoor. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64:525-540.

Zyla, K., D. Gogol, J. Koreleski, S. Swiatkiewicz and D. R. Ledoux. 1999. Simultaneous application of phytase and xylanase to broiler feeds based on wheat: feeding experiments with growing broilers. J. Sci. Food Agric. 79:1841-1848.