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## Inclusion levels and modes of whole grain incorporation into wheat-based rations differentially influence the performance of broiler chickens

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### ABSTRACT

1. The objective was to compare three whole grain (WG) inclusion levels (7.5, 15 and 30%) offered to broiler chickens by three modes of WG incorporation: (i) pre-pellet WG inclusion, (ii) post-pellet WG inclusion as a blend of WG and pelleted concentrate and (iii) post-pellet WG inclusion where WG and pelleted concentrate were provided in separate feed trays against a ground-grain, wheat-based control diet.  
2. Ten dietary treatments were offered to 6 replicate cages (6 birds per cage) of male Ross 308 chickens from 7 to 28 d post-hatch. The effects of treatment on relative gizzard weights, gizzard contents, pancreatic weights and pH of gizzard digesta were monitored. Parameters of growth performance, nutrient utilisation (apparent metabolisable energy [AME], metabolisable to gross energy [ME:GE] ratios, nitrogen [N] retention and N-corrected AME [AMEn]), apparent starch and protein (N) digestibility coefficients and disappearance rates in for small intestinal segments and concentrations of free amino acids in plasma taken from the anterior mesenteric vein were determined.  
3. Whole grain feeding (WGF) did not influence weight gain, but 30% post-pellet blended and 15 and 30% post-pellet separated treatments significantly depressed ( $P < 0.05$ ) feed intakes while the 30% post-pellet separated treatment improved ( $P < 0.01$ ) feed conversion ratios (FCR). WGF regimes significantly increased relative gizzard weights.  
4. Overall, WGF generated profound responses in AME, ME:GE ratios, N retention and AMEn that were highly correlated with relative gizzard weights. In general, WGF improved starch and protein (N) digestibilities and again there were some correlations with these outcomes and relative gizzard weights.  
5. Post-pellet WG inclusions where WG and pelleted concentrate were offered separately provided chickens with the opportunity to choice feed. Birds showed a preference for the relatively high-protein pelleted concentrate and at 30% WG, this resulted in an improvement in FCR of 7.69% (1.260 versus 1.365;  $P < 0.001$ ) relative to the ground-grain control diet.

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### Introduction

Whole grain feeding (WGF) involves the incorporation of some whole grain, usually wheat, into the broiler diet either prior to, or following, the steam-pelleting process. Under pre-pellet WGF regimes, increases in relative gizzard weights, the hallmark response to WGF, are less robust, which is presumably a consequence of whole grain being 'crushed' in the pellet-mill. This was demonstrated by Wu et al. (2004) in a feeding study in which pre-pellet whole grain did not increase relative gizzard weights but still generated significant improvements in feed efficiency and energy utilisation (AME). This outcome suggests that responses in growth performance and nutrient utilisation generated by WGF should not be simply attributed, in their entirety, to heavier gizzards. Thus, it should be instructive to assess pre-pellet WGF regimes to gain a better comprehension of the mechanisms by which whole grain generates improvements in FCR and energy utilisation which are quite frequently observed (Singh et al. 2014; Liu et al. 2015).

Alternatively, post-pellet WGF regimes may generate profound, but variable, increases in relative gizzard weights and it follows that heavier gizzards will result from increasing proportions of whole grain incorporation. Therefore, whole grain inclusions of 7.5, 15 and 30% were applied to

the three WGF modes in the present study. However, to some extent at least, post-pellet WGF gives birds the opportunity to choice feed or select between the relatively low protein whole grain component and the relatively high protein pelleted concentrate when offered as a blend. Cumming (1992; 1994) championed whole grain feeding in Australia, stressing the advantages that stem from choice feeding and as a possible means to prevent coccidiosis from enhanced gut. According to Gous and Swatson (2000), broiler chickens will choose the best possible combination of protein sources and, if this is the case, the opportunity to choice feed may be contributing to responses generated by whole grain feeding. To investigate this aspect, a second post-pellet WGF regime was assessed where the whole grain and pelleted concentrate were offered to birds in separate feed trays to provide chickens with the opportunity to select either component of the ration and permit individual recordings of feed intakes of the two components with differing protein concentrations.

Thus, the objective of this study was to compare the effects of whole grain inclusions of 7.5, 15 and 30% incorporated into rations via three modes: prior to pelleting or following pelleting, with the two components fed as a blend or separately and on broiler performance against a conventional ground-grain control diet. The assessment of broiler performance

parameters included relative gizzard weights, gizzard contents, pancreatic weights and pH of gizzard digesta. In addition treatment effects on growth performance, nutrient utilisation (AME, ME:GE ratios, N retention, AMEn), apparent starch and protein (N) digestibility coefficients and disappearance rates in for small intestinal segments were determined.

## Materials and methods

### Experimental design

The present study consisted of 10 dietary treatments, with 6 replicates per treatment (6 bird per replicate cage) of ostensibly identical wheat-based diets. A conventional, ground-grain, steam-pelleted diet served as the control. Whole grain was included in the remaining 9 dietary treatments at inclusion levels of 7.5, 15 and 30% via three modes of incorporation. Whole grain was incorporated into the ration either prior to or following steam-pelleting. The 6 post-pelleting whole grain diets were offered to broiler chickens as either a blend of whole grain and the corresponding pelleted concentrate, or these two components were offered separately in two feeding trays. This was to determine the effects of giving birds an opportunity to select either the whole grain or the pelleted concentrate and evaluate the influence of 'choice-feeding'.

### Diet preparation

A basal, wheat-based diet was formulated to standard Ross 308 broiler nutrient specifications as shown in Table 1 from which each of the dietary treatments was derived. The characterised wheat (120 g/kg protein, 683 g/kg starch) was either ground through a 3.2 mm hammer-mill screen prior to incorporation into intact steam-pelleted rations or fed whole as 7.5, 15 and 30% of the diet following either pre- or post-pelleting incorporation. Following post-pelleting incorporation, the whole grain component was offered with the corresponding balancing pelleted concentrate as a blend or separately. The steam-pelleted components of the diets were processed through a Palmer PP330 pellet press (Palmer Milling Engineering, Griffith, NSW, Australia) with an approximate capacity of 3 tonnes per h. The die dimensions were 4 mm in diameter and 45 mm in length. The residence time in the conditioner was 14 s with a computer-controlled conditioning temperature of 75°C. A non-starch polysaccharide degrading enzyme (Danisco Xylanase) was added across all diets at 16 000 BXU/kg. Celite (Celite™ World Minerals, Lompoc, CA, USA) was included in diets

as an inert acid insoluble ash marker in order to determine nutrient digestibility coefficients in 4 small intestinal sites.

### Bird management

At 7 d post-hatch, a total of 360 male Ross 308 chickens were individually identified (wing-tags), weighed and allocated into bioassay cages (6 birds per cage) on the basis of body weights. Bird allocation was such that cage means and variations were almost identical. Each dietary treatment was offered to 6 replicate cages from 7 to 28 d post-hatch. Birds had unlimited access to feed and water under a 18L:6D lighting regime in an environmentally controlled facility. An initial room temperature of  $32 \pm 1^\circ\text{C}$  was maintained for the first week, which was gradually decreased to  $22 \pm 1^\circ\text{C}$  by the end of the third week and maintained at this temperature for the final week. This study fully complied with specific guidelines approved by the Animal Ethics Committee of The University of Sydney.

### Sample collection and chemical analysis

Initial and final body weights were determined and feed intakes recorded, from which FCR were calculated. Any dead or culled birds were removed on a daily basis and their body weights recorded and used to correct FCR calculations. Feed intakes and excreta outputs were monitored from 25 to 27 d post-hatch in order to calculate AME, ME:GE ratio, N retention and AMEn on a dry matter basis. Over this total excreta collection period water intakes were monitored to determine water-to-feed-intake ratios. Excreta were air-forced oven dried for 24 h at 80°C. The GE of diets and excreta were determined via bomb calorimetry using an adiabatic calorimeter (Parr 1281 bomb calorimeter, Parr Instruments Co., Moline, IL). AME was calculated by the following equation:

$$\text{AME}_{\text{diet}} = \frac{(\text{Feed intake} \times \text{GE}_{\text{diet}}) - (\text{Excreta output} \times \text{GE}_{\text{excreta}})}{(\text{Feed intake})}$$

N-corrected AME values were calculated by correcting to zero N retention, using the factor of 36.54 kJ/g (Hill and Anderson 1958).

N retention was calculated by the following equation:

$$\text{N retention}(\%) = \frac{(\text{Feed intake} \times \text{N}_{\text{diet}}) - (\text{Excreta output} \times \text{N}_{\text{excreta}})}{(\text{Feed intake} \times \text{N}_{\text{diet}})} \times 100$$

At d 28, birds were killed by an intravenous injection of sodium pentobarbitone. The abdominal cavity was opened and blood samples were immediately taken from the anterior mesenteric vein of three randomly selected birds per cage for the control, 30% pre-pellet and 30% post-pellet blended whole grain treatments. Concentrations of free amino acids in plasma taken from the portal circulation were determined by methodology outlined in detail in Selle et al. (2016). The pH of digesta within the gizzard *in situ* was determined. Gizzard, gizzard contents and pancreas were removed and weighed to determine their absolute and relative weights. The incidence of any dilated proventriculi was recorded. The small intestine was removed and divided into the 4 segments [proximal jejunum (PJ), distal jejunum (DJ), proximal ileum (PI) and distal ileum (DI)]. The 4 segments were demarcated by the end of the duodenal loop, Meckel's diverticulum and the ileo-caecal junction and their mid-points. Digesta was collected

**Table 1.** Composition and nutrient specifications of basal experimental diet.

Feed ingredient	g/kg	Nutrient specification	g/kg
Wheat	532.8	Metabolisable energy (MJ/kg)	12.96
Soybean meal	310.0	Protein	218.6
Canola meal (expeller)	40.0	Calcium	7.64
Soy oil	70.0	Total phosphorus	7.31
Dicalcium phosphate	17.0	Available phosphorus	3.65
Limestone	5.9	Lysine	12.74
Lysine HCl	1.9	Methionine	5.87
Methionine	2.8	Threonine	8.87
Threonine	1.0	Tryptophan	2.86
Sodium chloride	2.2	Isoleucine	8.94
Sodium bicarbonate	4.3	Sodium	2.01
Vitamin-trace mineral premix	2.0	Potassium	9.40
Celite	10.0	Chloride	2.23

in its entirety from each segment. Digesta samples were gently expressed from each segment, pooled by cage, homogenised, freeze dried and weighed to determine the apparent digestibility of starch and N. Concentrations of starch in diets and digesta was determined by methods described in Mahasukhonthachat et al. (2010). Nitrogen and acid insoluble ash (AIA) concentrations were determined as outlined in Siriwan et al. (1993). Apparent digestibility coefficients of starch and nitrogen were calculated by the following equation:

$$\text{Digestibility coefficient} = \frac{(\text{Nutrient/AIA})_{\text{diet}} - (\text{Nutrient/AIA})_{\text{digesta}}}{(\text{Nutrient/AIA})_{\text{diet}}}$$

Starch: protein disappearance rate ratios were deduced from starch and protein disappearance rates in 4 small intestinal segments which were calculated from the following equation:

$$\text{Disappearance rate (g/bird/d)} = \text{FI} \times \text{nutrient content}_{\text{diet}} \times \text{ADC}.$$

Feed intake (FI) is the total 21-d feed intake expressed on a daily basis, nutrient content<sub>diet</sub> is the dietary starch or protein concentrations (g/kg) and ADC is the apparent digestibility coefficients of the relevant nutrient.

### Statistical analysis

Experimental data were analysed as a one way ANOVA of dietary treatments. Additionally, pre-pellet and post-pellet separate whole grain treatments were analysed as a 2 × 3 factorial design to illustrate the effect of choice-feeding on selected performance parameters. Univariate, general linear models procedures and Pearson correlations were followed using the SPSS®IBM Statistics 20 software program (IBM Corporation, Somers, NY, USA). The experimental units were cage means and differences were considered significant at the 5% level of probability.

**Table 2.** Effects of dietary treatments on growth performance [weight gain, feed intake, feed conversion ratio (FCR)] and mortality/cull rates from 7 to 28 d post-hatch.

Treatment		Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Mortality/cull rates (%)
Description	Whole grain inclusion (%)				
Control	0.0	1651	2252de	1.365bc	2.77
Pre-pellet	7.5	1621	2266e	1.364bc	2.77
	15.0	1644	2240de	1.362bc	0
	30.0	1590	2209bcde	1.355bc	2.77
Post-pellet blended	7.5	1640	2226cde	1.358bc	0
	15.0	1540	2120bcd	1.371c	2.77
	30.0	1532	2086ab	1.359bc	0
Post-pellet separated	7.5	1608	2147bcde	1.334bc	0
	15.0	1532	1959a	1.296ab	2.77
	30.0	1681	2098bc	1.260a	2.77
SEM		48.705	47.276	0.0258	2.143
Significance ( <i>P</i> = )		0.308	<0.001	0.036	0.904
LSD ( <i>P</i> < 0.05)			134.3	0.0733	
Linear effects					
Pre-pellet		<i>r</i> = -0.175 <i>P</i> = 0.414	<i>r</i> = -0.182 <i>P</i> = 0.394	<i>r</i> = -0.095 <i>P</i> = 0.659	
Post-pellet blended		<i>r</i> = -0.513 <i>P</i> = 0.010	<i>r</i> = -0.517 <i>P</i> = 0.010	<i>r</i> = -0.021 <i>P</i> = 0.923	
Post-pellet separated		<i>r</i> = 0.092 <i>P</i> = 0.670	<i>r</i> = -0.375 <i>P</i> = 0.071	<i>r</i> = -0.465 <i>P</i> = 0.022	

<sup>abcde</sup>Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

## Results

The effects of dietary treatments on growth performance and mortality rates from 7 to 28 d post-hatch are shown in Table 2. Overall, birds outperformed 2014 Ross 308 objectives (values given in parentheses) with a mean weight gain of 1604 g/bird (1387 g/bird), feed intake of 2160 g/bird (2052 g/bird) and an FCR of 1.342 (1.479). Dietary treatments did not influence weight gain, which ranged from 1532 to 1681 g/bird. However, increasing whole grain inclusions in post-pellet blended rations linearly depressed (*r* = -0.513; *P* < 0.05) weight gain. Dietary treatments significantly (*P* < 0.001) influenced feed intake; with one exception WGF either numerically or statistically reduced feed intakes. For example, the 15% post-pellet separated ration significantly depressed feed intake by 13% (1959 versus 2252 g/bird; *P* < 0.001) relative to the control, ground-grain treatment on the basis of a pair-wise comparison and increasing whole grain inclusions in post-pellet blended rations linearly depressed (*r* = -0.517; *P* < 0.05) feed intake. Dietary treatments significantly (*P* < 0.05) influenced FCR; the 30% post-pellet separated ration enhanced FCR by 7.69% (1.260 versus 1.365; *P* < 0.001) relative to the control group which was highly significant on the basis of a pair-wise comparison. In addition, increasing whole grain inclusions in post-pellet separated rations linearly improved (*r* = -0.467; *P* < 0.05) FCR. The acceptable overall mortality rate of 1.66% was unrelated to dietary treatment.

Table 3 shows treatment effects on relative gizzard weights, contents and digesta pH, relative pancreas weights and the incidence of dilated proventriculi at 28 d post-hatch. Parameters of gizzard and pancreas functionality were significantly (*P* < 0.001) influenced by dietary treatment. Overall, WGF generated an increase of 21% (17.73 versus 14.01 g/kg) in relative gizzard weights, an increase of 28.3% (8.31 versus 6.48 g/kg) in relative gizzard contents, a reduction in gizzard pH of 0.60 (2.36 versus 2.96) and an increase of 5.80% (2.41 versus 2.28 g/kg) in relative pancreas weights compared to the respective controls. The maximum increase in relative gizzard weight was 53% (21.39 versus 14.01 g/kg) in birds offered the 30% whole grain post-pellet blend and all three modes of whole grain incorporation linearly increased gizzard weights. The maximum increase in relative gizzard contents was 73% (11.21 versus 6.48 g/kg) in birds offered the same treatment and again all three modes of whole grain incorporation linearly increased gizzard contents. Both post-pellet modes of whole grain incorporation linearly reduced gizzard pH where the 15% whole grain post-pellet separate treatment generated the largest reduction in pH from 2.96 to 2.15. The same treatment generated the largest increase in relative pancreas weights of 15.4% (2.63 versus 2.28 g/kg) and whole grain post-pellet blends prompted a linear increase (*r* = 0.558; *P* < 0.01) in pancreas weights. The modest 1.95% incidence of dilated proventriculi was not related to dietary treatment.

The effects of dietary treatments on parameters of nutrient utilisation are shown in Table 4, where all parameters (AME, ME:GE ratios, N retention, AMEn) assessed were significantly influenced (*P* < 0.001) by treatment. Pre-pellet WGF did not influence AME but all post-pellet blended and separated whole grain rations significantly increased AME by an average of 1.04 MJ (12.98 versus 11.94 MJ/kg) and 1.22 MJ (13.16 versus 11.94 MJ/kg), respectively, relative to the control. Both blended



**Table 3.** Effects of dietary treatments on relative gizzard weights, contents and digesta pH, relative pancreas weights and incidence of dilated proventriculi at 28 d post-hatch.

Treatment		Relative gizzard weight (g/kg)	Relative gizzard content (g/kg)	Gizzard digesta pH	Relative pancreas weight (g/kg)	Incidence of dilated proventriculi (%)
Description	Whole grain inclusion (%)					
Control	0.0	14.01a	6.48b	2.96c	2.28ab	2.78
Pre-pellet	7.5	15.17b	4.53a	2.43b	2.05a	2.78
	15.0	15.17b	6.50b	2.43b	2.26ab	5.57
	30.0	15.58b	9.79de	2.72c	2.35bc	0
Post-pellet blended	7.5	16.88c	8.52cd	2.23ab	2.26ab	2.78
	15.0	19.01e	8.80d	2.24ab	2.54cd	0
	30.0	21.39f	11.21e	2.38ab	2.63d	0
Post-pellet separated	7.5	17.94d	6.99bc	2.36ab	2.45bcd	2.78
	15.0	19.22e	8.86d	2.15a	2.69d	0
	30.0	19.23e	9.61de	2.31ab	2.48bcd	2.78
SEM		0.3640	0.5657	0.0885	0.0856	2.2612
Significance ( $P =$ )		< 0.001	< 0.001	< 0.001	< 0.001	0.714
LSD ( $P < 0.05$ )		1.034	1.607	0.251	0.243	
Linear effects						
Pre-pellet		$r = 0.536$ $P = 0.007$	$r = 0.683$ $P < 0.001$	$r = -0.144$ $P = 0.502$	$r = 0.274$ $P = 0.194$	
Post-pellet blended		$r = 0.942$ $P < 0.001$	$r = 0.778$ $P < 0.001$	$r = -0.471$ $P = 0.020$	$r = 0.558$ $P = 0.005$	
Post-pellet separated		$r = 0.743$ $P < 0.001$	$r = 0.688$ $P < 0.001$	$r = -0.531$ $P = 0.008$	$r = 0.309$ $P = 0.142$	

<sup>abcdef</sup>Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

**Table 4.** Effects of dietary treatments on nutrient utilisation (AME, ME:GE ratios, N retention, AMEn) at 25 to 27 d post-hatch.

Treatment		AME (MJ/kg DM)	ME:GE ratio	N retention (%)	AMEn (MJ/kg DM)
Description	Whole grain inclusion (%)				
Control	0.0	11.94a	0.688a	53.68a	11.36ab
Pre-pellet	7.5	11.59a	0.670a	56.08ab	11.30a
	15.0	12.05a	0.693a	58.69bc	11.83ab
	30.0	12.10a	0.693a	58.58bc	11.93b
Post-pellet blended	7.5	12.70b	0.725b	61.21cd	12.57c
	15.0	12.99b	0.742bc	61.02bcd	12.89cd
	30.0	13.25b	0.755c	63.41cde	13.14cd
Post-pellet separated	7.5	13.05b	0.744bc	63.91de	12.97cd
	15.0	13.18b	0.752bc	67.47e	13.09cd
	30.0	13.25b	0.756c	65.76e	13.18d
SEM		0.2086	0.0119	1.6420	0.2090
Significance ( $P =$ )		<0.001	<0.001	<0.001	<0.001
LSD ( $P < 0.05$ )		0.592	0.0293	4.664	0.594
Linear effects					
Pre-pellet		$r = 0.229$ $P = 0.281$	$r = 0.170$ $P = 0.428$	$r = 0.437$ $P = 0.033$	$r = 0.463$ $P = 0.023$
Post-pellet blended		$r = 0.566$ $P = 0.004$	$r = 0.532$ $P = 0.007$	$r = 0.549$ $P = 0.005$	$r = 0.635$ $P = 0.001$
Post-pellet separated		$r = 0.629$ $P = 0.001$	$r = 0.615$ $P = 0.001$	$r = 0.598$ $P = 0.002$	$r = 0.659$ $P < 0.001$

<sup>abcde</sup>Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

( $r = 0.566$ ;  $P < 0.001$ ) and separated ( $r = 0.629$ ;  $P < 0.001$ ) post-pellet whole grain ratios linearly increased AME. The pattern of responses in ME:GE ratios to dietary treatments was effectively identical. All three modes of whole grain incorporation linearly increased N retention to significant extents and the 7.5% pre-pellet whole grain treatment was the only WG treatment that failed to increase N retention significantly relative to the control ration. Collectively, pre-pellet WGF increased N retention by an average of 4.13 percentage units (57.78 versus 53.68%), post-pellet blended WGF increased N retention by 8.23 percentage units (61.88 versus 53.68%) and pre-pellet separated WGF increased N retention by 12.06 percentage units (65.71 versus 53.68%). All three modes of whole grain incorporation linearly increased AMEn to significant extents. Pre-pellet WG increased AMEn by an average of 0.33 MJ (11.69 versus 11.36 MJ/kg), post-pellet blended WG by 1.51 MJ (12.87 versus 11.36 MJ/kg) and post-pellet separated WG

by 1.72 MJ (13.08 versus 11.36 MJ/kg) relative to the ground-grain control.

Table 5 shows the effects of dietary treatments on starch and protein (N) digestibility coefficients where there was a significant influence of treatment on starch digestibility coefficients in the distal jejunum ( $P < 0.05$ ) and both ileal segments ( $P < 0.001$ ). In the distal ileum, 15 and 30% pre-pellet WG grain inclusions significantly increased starch digestibility by 16.1% (0.842 versus 0.725) and 18.8% (0.861 versus 0.725), respectively, relative to the ground-grain control. Collectively, post-pellet blended WG inclusions significantly increased distal ileal starch digestibility by an average of 30.5% (0.946 versus 0.725) and post-pellet separated WG inclusion by an average of 31.4% (0.953 versus 0.725). Pre-pellet WG ( $r = 0.475$ ;  $P < 0.05$ ), post-pellet blended WG ( $r = 0.704$ ;  $P < 0.001$ ) and post-pellet separated WG

**Table 5.** Effects of dietary treatments on apparent starch and protein (N) digestibility coefficients at 28 d post-hatch.

Treatment	Whole grain inclusion (%)	Starch digestibility coefficients (g/g)				Protein (N) digestibility coefficients (g/g)			
		Proximal Jejunum	Distal Jejunum	Proximal Ileum	Distal Ileum	Proximal Jejunum	Distal Jejunum	Proximal Ileum	Distal Ileum
Control	0.0	0.403	0.656ab	0.649a	0.725a	0.492cd	0.622b	0.650ab	0.706b
Pre-pellet	7.5	0.537	0.683ab	0.685a	0.703a	0.279a	0.584a	0.623a	0.666a
	15.0	0.642	0.728ab	0.752ab	0.842b	0.404bc	0.656c	0.717def	0.751cd
	30.0	0.549	0.727ab	0.672a	0.861bc	0.542d	0.697d	0.666bc	0.766cde
Post-pellet blended	7.5	0.578	0.770bc	0.848bcd	0.942bcd	0.391bc	0.687d	0.735ef	0.777de
	15.0	0.467	0.646ab	0.846bc	0.945cd	0.313ab	0.652c	0.707cdef	0.750cd
	30.0	0.516	0.727ab	0.889cd	0.981d	0.558d	0.720e	0.747f	0.788e
Post-pellet separated	7.5	0.532	0.720ab	0.641a	0.937bcd	0.314ab	0.652c	0.697cde	0.746c
	15.0	0.534	0.631a	0.822bc	0.977d	0.388bc	0.644c	0.729ef	0.770cde
	30.0	0.692	0.893c	0.966d	0.944cd	0.377ab	0.616b	0.685bcd	0.747c
SEM		0.0948	0.0469	0.0420	0.0356	0.0382	0.0169	0.0146	0.0105
Significance ( $P =$ )		0.672	0.015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD ( $P < 0.05$ )			0.1332	0.1193	0.1012	0.1085	0.0479	0.0415	0.0298
Linear effects									
Pre-pellet		$r = 0.283$ $P = 0.180$	$r = 0.374$ $P = 0.072$	$r = 0.125$ $P = 0.562$	$r = 0.475$ $P = 0.019$	$r = 0.385$ $P = 0.063$	$r = 0.592$ $P = 0.002$	$r = 0.222$ $P = 0.296$	$r = 0.614$ $P = 0.001$
Post-pellet blended		$r = 0.087$ $P = 0.685$	$r = 0.095$ $P = 0.659$	$r = 0.631$ $P < 0.001$	$r = 0.704$ $P < 0.001$	$r = 0.258$ $P = 0.223$	$r = 0.601$ $P = 0.002$	$r = 0.646$ $P < 0.001$	$r = 0.579$ $P = 0.003$
Post-pellet separated		$r = 0.291$ $P = 0.168$	$r = 0.438$ $P = 0.032$	$r = 0.776$ $P < 0.001$	$r = 0.531$ $P = 0.008$	$r = 0.200$ $P = 0.348$	$r = -0.114$ $P = 0.596$	$r = 0.288$ $P = 0.172$	$r = 0.392$ $P = 0.058$

<sup>abcdef</sup>Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

( $r = 0.531$ ;  $P < 0.01$ ) all linearly improved starch digestibility coefficients in the distal ileum.

Protein (N) digestibility coefficients were significantly influenced ( $P < 0.001$ ) by dietary treatment in 4 small intestinal segments and post-pellet blended WG linearly increased protein (N) digestibility in the distal jejunum ( $r = 0.601$ ;  $P < 0.01$ ), proximal ileum ( $r = 0.646$ ;  $P < 0.001$ ) and distal ileum ( $r = 0.579$ ;  $P < 0.001$ ). Collectively, post-pellet blended WG inclusions significantly increased distal jejunal protein (N) digestibility coefficients by an average of 10.3% (0.686 versus 0.622), proximal ileal digestibility by 12.3% (0.730 versus 0.650) and distal ileal digestibility by 9.35% (0.772 versus 0.706) relative to the ground-grain control.

The effects of dietary treatments on starch and protein (N) disappearance rates are shown in Table 6. There was a significant influence of treatment on starch disappearance rates in the distal jejunum ( $P = 0.001$ ) and both ileal

segments ( $P < 0.001$ ). The three modes of whole grain incorporation linearly increased starch disappearance rates in the distal ileum; pre-pellet WGF ( $r = 0.450$ ;  $P < 0.05$ ), post-pellet bended WGF ( $r = 0.494$ ;  $P < 0.05$ ) and post-pellet separated WGF ( $r = 0.649$   $P < 0.001$ ). While there were significant treatment influences on protein (N) disappearance rates in the proximal jejunum ( $P < 0.001$ ), distal jejunum ( $P < 0.01$ ), proximal ileum ( $P < 0.015$ ) and distal ileum ( $P < 0.001$ ); the transition from ground-grain to WGF did not generate clear-cut differences. However, pre-pellet WGF linearly accelerated protein (N) disappearance rates in the distal jejunum ( $r = 0.576$ ;  $P < 0.001$ ) and distal ileum ( $r = 0.622$ ;  $P < 0.001$ ) and post-pellet separated WGF linearly accelerated protein (N) disappearance rates in the proximal ( $r = 0.492$ ;  $P < 0.05$ ) and distal ( $r = 0.554$ ;  $P < 0.01$ ) ileum.

The effects of choice feeding on apparent starch and protein (N) digestibility coefficients are shown in Table 7

**Table 6.** Effects of dietary treatments on apparent starch and protein (N) disappearance rates at 28 d post-hatch.

Treatment	Whole grain inclusion (%)	Starch disappearance rate (g/bird/d)				Protein (N) disappearance rate (g/bird/d)			
		Proximal Jejunum	Distal Jejunum	Proximal Ileum	Distal Ileum	Proximal Jejunum	Distal Jejunum	Proximal Ileum	Distal Ileum
Control	0.0	14.32	23.08abc	22.92a	25.53ab	19.08cd	24.07ab	25.18ab	27.33ab
Pre-pellet	7.5	18.99	24.21abc	24.31ab	24.92a	11.05a	23.23a	24.75a	26.52a
	15.0	22.52	25.47bc	26.35abc	29.49bc	15.38bc	24.97abc	27.29cde	28.59bc
	30.0	19.07	25.11abc	23.20a	29.79c	21.21d	27.23c	25.87abcd	29.88c
Post-pellet blended	7.5	20.11	26.91c	29.61c	32.89cd	14.85ab	26.21bc	28.04e	29.62c
	15.0	15.67	21.43ab	28.07bc	31.36cd	11.11a	23.46a	25.39abc	26.89ab
	30.0	16.93	23.64abc	28.98c	32.03cd	20.15d	25.90bc	26.91bcde	28.43bc
Post-pellet separated	7.5	18.66	25.22abc	22.43a	32.58cd	11.56ab	24.09ab	25.73abc	27.53ab
	15.0	17.33	20.55a	26.49abc	31.64cd	13.78ab	22.86a	25.86abcd	27.29ab
	30.0	25.69	33.18d	35.77d	35.00d	15.29bc	24.94abc	27.74de	30.25c
SEM		3.360	1.726	1.530	1.421	1.446	0.821	0.701	0.650
Significance ( $P =$ )		0.476	0.001	<0.001	<0.001	<0.001	0.006	0.014	<0.001
LSD ( $P < 0.05$ )			4.903	4.345	4.037	4.106	2.333	1.990	1.845
Linear effects									
Pre-pellet		$r = 0.255$ $P = 0.229$	$r = 0.329$ $P = 0.116$	$r = 0.032$ $P = 0.882$	$r = 0.450$ $P = 0.027$	$r = 0.384$ $P = 0.064$	$r = 0.576$ $P = 0.003$	$r = 0.237$ $P = 0.266$	$r = 0.622$ $P = 0.001$
Post-pellet blended		$r = 0.034$ $P = 0.873$	$r = -0.075$ $P = 0.729$	$r = 0.444$ $P = 0.030$	$r = 0.494$ $P = 0.014$	$r = 0.137$ $P = 0.522$	$r = 0.203$ $P = 0.342$	$r = 0.154$ $P = 0.474$	$r = 0.062$ $P = 0.774$
Post-pellet separated		$r = 0.453$ $P = 0.026$	$r = 0.547$ $P = 0.006$	$r = 0.724$ $P < 0.001$	$r = 0.649$ $P < 0.001$	$r = -0.143$ $P = 0.504$	$r = 0.127$ $P = 0.554$	$r = 0.492$ $P = 0.015$	$r = 0.554$ $P = 0.005^{11}$

<sup>abcde</sup>Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

**Table 7.** Effects of choice feeding on apparent starch and protein (N) digestibility coefficients at 28 d post-hatch.

Treatment		Starch digestibility coefficients (g/g)				Protein (N) digestibility coefficients (g/g)			
Whole wheat form	Whole grain inclusion (%)	Proximal Jejunum	Distal Jejunum	Proximal Ileum	Distal Ileum	Proximal Jejunum	Distal Jejunum	Proximal Ileum	Distal Ileum
Pre-pellet	7.5	0.537	0.683a	0.685ab	0.703	0.279	0.584a	0.623	0.666a
	15.0	0.642	0.728a	0.752ab	0.842	0.404	0.656bc	0.717	0.751b
	30.0	0.549	0.727a	0.672a	0.861	0.542	0.697c	0.666	0.766b
Post-pellet separated	7.5	0.532	0.720a	0.641a	0.937	0.314	0.652bc	0.697	0.746b
	15.0	0.534	0.631a	0.822b	0.977	0.388	0.644b	0.729	0.770b
	30.0	0.692	0.893b	0.966c	0.944	0.377	0.616ab	0.685	0.747b
SEM		0.0767	0.0417	0.0475	0.0423	0.0422	0.0178	0.0168	0.0091
Main effects: Wheat form									
Pre-pellet		0.576	0.712	0.703	0.802a	0.408	0.646	0.668a	0.728
Post-pellet separated		0.586	0.748	0.810	0.953b	0.360	0.637	0.704b	0.754
Inclusion (%)									
7.5		0.535	0.701	0.663	0.820	0.296a	0.618	0.660a	0.706
15.0		0.588	0.680	0.787	0.910	0.396b	0.650	0.723b	0.761
30.0		0.621	0.810	0.819	0.903	0.460b	0.657	0.675a	0.757
Significance ( $P =$ )									
Wheat form (WF)		0.875	0.303	0.010	< 0.001	0.170	0.576	0.015	0.001
Inclusion (%)		0.534	0.009	0.006	0.078	0.002	0.086	0.002	<0.001
Interaction		0.274	0.014	0.004	0.211	0.063	< 0.001	0.153	<0.001

<sup>abc</sup>Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

where pre-pellet WGF regimes are compared with post-pellet separated WGF regimes as a  $2 \times 3$  factorial array of treatments. Most notably, choice feeding significantly increased distal ileal starch digestibility by 18.8% (0.953 versus 0.802;  $P < 0.001$ ). Significant treatment interactions for starch digestibility were observed in the distal jejunum and proximal ileum, while there were no significant treatment effects in the proximal jejunum. Increasing whole grain inclusions from 7.5 to 15 and 30% significantly ( $P < 0.005$ ) enhanced protein (N) digestibility coefficients by 33.8% (0.396 versus 0.296) and 55.4% (0.460 versus 0.296), respectively, in the distal jejunum. Significant treatment interactions for protein (N) digestibility were observed in the distal jejunum where digestibility was significantly enhanced by increasing whole grain inclusions by pre-pellet diets but numerically depressed by post-pellet separated diets. In the proximal ileum choice feeding increased protein (N) digestibility by 5.39% (0.704 versus 0.668;  $P < 0.05$ ) and 15% whole grain inclusion supported significantly superior ( $P < 0.005$ ) digestibility coefficients by an average of 8.23% (0.723 versus 0.668). Birds offered the 7.5% pre-pellet whole grain inclusion diet had statistically inferior distal ileal protein (N) digestibility coefficients by an average of 11.9% (0.666 versus 0.756).

The effects of offering the pelleted concentrate and whole grain components separately on feed intakes of both components from 7 to 28 d post-hatch are shown in Table 8. Birds offered the notionally 7.5% whole grain diet actually

consumed 8.39% wheat, which is in quite good agreement with their 'target'. In contrast, birds offered notionally 15 and 30% whole grain diets consumed 8.18 and 16.43%, respectively, or approximately 45% less than their targets. This pattern of consumption under choice feeding linearly increased protein intakes and decreased starch intakes to significant extents such that starch-to-protein-intake ratios were linearly condensed ( $r = -0.727$ ;  $P < 0.001$ ).

Table 9 shows Pearson correlations between gizzard and pancreas characteristics with parameters of nutrient utilisation. The correlations are highly significant in the majority of cases; for example, relative gizzard weights are correlated ( $P < 0.001$ ) with relative gizzard contents ( $r = 0.632$ ), gizzard pH ( $r = -0.527$ ), relative pancreas weights ( $r = 0.640$ ), AME ( $r = 0.701$ ), ME:GE ratios ( $r = 0.679$ ), N retention ( $r = 0.624$ ) and AMEn ( $r = 0.745$ ). Pearson correlations between gizzard and pancreas characteristics with starch digestibility coefficients along the small intestine are shown in Table 10. Distal ileal starch digestibility is significantly correlated with relative gizzard weights ( $r = 0.608$ ;  $P < 0.001$ ), relative gizzard contents ( $r = 0.575$ ;  $P < 0.001$ ), gizzard pH ( $r = -0.345$ ;  $P < 0.01$ ) and relative pancreas weights ( $r = 0.421$ ;  $P < 0.001$ ). Similarly, distal ileal protein (N) digestibility coefficients are significantly correlated with relative gizzard weights ( $r = 0.462$ ;  $P < 0.001$ ), relative gizzard contents ( $r = 0.616$ ;  $P < 0.001$ ), gizzard pH ( $r = -0.283$ ;  $P < 0.05$ ) and relative pancreas weights ( $r = 0.457$ ;  $P < 0.001$ ) as shown in Table 11.

**Table 8.** Effects of offering pelleted concentrate and whole grain (notional whole grain inclusions) separately on absolute and relative ration components and starch and protein intakes from 7 to 28 d post-hatch.

Treatment		Pelleted concentrate (g/bird)	Whole wheat (g/bird)	Proportion of wheat (%)	Protein intake (g/bird/d)	Starch intake (g/bird/d)	Starch to protein intake ratio
Description	Whole grain inclusion (%)						
Post-pellet separated	7.5	1981a	165b	8.39b	23.07b	19.15b	0.83c
	15.0	1779b	147b	8.18b	22.15b	14.58a	0.66b
	30.0	1751b	290a	16.43a	25.32a	13.92a	0.55a
SEM		55.7579	39.9890	2.1875	0.4873	1.1060	0.0428
Significance ( $P =$ )		0.021	0.046	0.027	0.001	0.009	0.001
LSD ( $P < 0.05$ )		168.1	120.5	6.594	1.4690	3.3338	0.129
Linear effects		$r = 0.581$	$r = 0.466$	$r = 0.528$	$r = 0.534$	$r = -0.629$	$r = -0.727$
		$P = 0.011$	$P = 0.051$	$P = 0.024$	$P = 0.022$	$P = 0.005$	$P < 0.001$

<sup>abc</sup>Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

**Table 9.** Pearson correlations between gizzard and pancreas characteristics with parameters of nutrient utilisation.

	Relative gizzard weight	Relative gizzard content	Gizzard pH	Relative pancreas weight	AME (MJ/kg DM)	ME:GE ratio	N retention (%)	AMEn (MJ/kg DM)
Gizzard weight	1.000							
Gizzard content	$r = 0.632$ $P < 0.001$	1.000						
Gizzard pH	$r = -0.527$ $P < 0.001$	$r = -0.103$ $P = 0.432$	1.000					
Pancreas weight	$r = 0.640$ $P < 0.001$	$r = 0.477$ $P < 0.001$	$r = -0.284$ $P = 0.028$	1.000				
AME	$r = 0.701$ $P < 0.001$	$r = 0.451$ $P = 0.001$	$r = -0.300$ $P = 0.020$	$r = 0.550$ $P < 0.001$	1.000			
ME:GE ratio	$r = 0.679$ $P < 0.001$	$r = 0.427$ $P < 0.001$	$r = -0.447$ $P < 0.001$	$r = 0.540$ $P < 0.001$	$r = 0.998$ $P < 0.001$	1.000		
N retention	$r = 0.624$ $P < 0.001$	$r = 0.263$ $P = 0.043$	$r = -0.399$ $P = 0.002$	$r = 0.543$ $P < 0.001$	$r = 0.809$ $P < 0.001$	$r = 0.804$ $P < 0.001$	1.000	
AMEn	$r = 0.745$ $P < 0.001$	$r = 0.480$ $P < 0.001$	$r = -0.338$ $P = 0.003$	$r = 0.542$ $P < 0.001$	$r = 0.985$ $P < 0.001$	$r = 0.978$ $P < 0.001$	$r = 0.816$ $P < 0.001$	1.000

**Table 10.** Pearson correlations between gizzard and pancreas characteristics with starch digestibility coefficients in the proximal jejunum, distal jejunum, proximal ileum and distal ileum.

	Relative gizzard weight	Relative gizzard content	Gizzard pH	Relative pancreas weight	Starch digestibility PJ	Starch digestibility DJ	Starch digestibility PI	Starch digestibility DI
Gizzard weight	1.000							
Gizzard content	$r = 0.632$ $P < 0.001$	1.000						
Gizzard pH	$r = -0.527$ $P < 0.001$	$r = -0.103$ $P = 0.432$	1.000					
Pancreas weight	$r = 0.640$ $P < 0.001$	$r = 0.477$ $P < 0.001$	$r = -0.284$ $P = 0.028$	1.000				
Starch PJ	$r = 0.017$ $P = 0.900$	$r = 0.040$ $P = 0.764$	$r = -0.074$ $P = 0.576$	$r = 0.008$ $P = 0.954$	1.000			
Starch DJ	$r = 0.003$ $P = 0.980$	$r = 0.092$ $P = 0.486$	$r = 0.033$ $P = 0.803$	$r = 0.015$ $P = 0.911$	$r = 0.233$ $P = 0.073$	1.000		
Starch PI	$r = 0.675$ $P < 0.001$	$r = 0.423$ $P = 0.001$	$r = -0.525$ $P < 0.001$	$r = 0.502$ $P < 0.001$	$r = 0.146$ $P = 0.267$	$r = 0.354$ $P = 0.006$	1.000	
Starch DI	$r = 0.608$ $P < 0.001$	$r = 0.575$ $P < 0.001$	$r = -0.345$ $P = 0.007$	$r = 0.421$ $P = 0.001$	$r = 0.096$ $P = 0.466$	$r = 0.118$ $P = 0.371$	$r = 0.710$ $P < 0.001$	1.000

**Table 11.** Pearson correlations between gizzard and pancreas characteristics with protein (N) digestibility coefficients in the proximal jejunum, distal jejunum, proximal ileum and distal ileum.

	Relative gizzard weight	Relative gizzard content	Gizzard pH	Relative pancreas weight	Protein (N) digestibility PJ	Protein (N) digestibility DJ	Protein (N) digestibility PI	Protein (N) digestibility DI
Gizzard weight	1.000							
Gizzard content	$r = 0.632$ $P < 0.001$	1.000						
Gizzard pH	$r = -0.527$ $P < 0.001$	$r = -0.103$ $P = 0.432$	1.000					
Pancreas weight	$r = 0.640$ $P < 0.001$	$r = 0.477$ $P < 0.001$	$r = -0.284$ $P = 0.028$	1.000				
Protein PJ	$r = -0.042$ $P = 0.752$	$r = 0.424$ $P = 0.001$	$r = 0.452$ $P < 0.001$	$r = 0.012$ $P = 0.927$	1.000			
Protein DJ	$r = 0.218$ $P = 0.752$	$r = 0.311$ $P = 0.016$	$r = -0.147$ $P = 0.262$	$r = 0.299$ $P = 0.020$	$r = 0.283$ $P = 0.029$	1.000		
Protein PI	$r = 0.461$ $P < 0.001$	$r = 0.396$ $P = 0.002$	$r = -0.376$ $P = 0.003$	$r = 0.401$ $P = 0.001$	$r = 0.075$ $P = 0.572$	$r = 0.486$ $P < 0.001$	1.000	
Protein DI	$r = 0.462$ $P < 0.001$	$r = 0.616$ $P < 0.001$	$r = -0.283$ $P = 0.029$	$r = 0.457$ $P < 0.001$	$r = 0.351$ $P = 0.006$	$r = 0.536$ $P < 0.001$	$r = 0.683$ $P < 0.001$	1.000

The effects of dietary treatments on free amino acid concentrations in the portal circulation in birds offered the control, pre-pellet WG and post-pellet blended WG are shown in Table 12. Post-pellet blended WG significantly decreased ( $P < 0.05$ ) lysine concentrations and both WG regimes significantly increased ( $P < 0.05$ ) tyrosine concentrations relative to the ground-grain control but the balance of amino acids were not influenced by dietary treatments.

Pearson correlations between apparent starch digestibility coefficients in 4 small intestinal segments and parameters of nutrient utilisation are shown in Table 13. Proximal ileal starch digestibility was significantly correlated with AME ( $r = 0.747$ ;  $P < 0.001$ ), ME:GE ratio ( $r = 0.733$ ;  $P < 0.001$ ), N retention ( $r = 0.631$ ;  $P < 0.001$ ) and AMEn ( $r = 0.784$ ;  $P < 0.001$ ). Similarly, distal ileal starch digestibility was significantly correlated with AME ( $r = 0.684$ ;  $P < 0.001$ ), ME:GE



Table 12. Effects of dietary treatments on free amino acid concentrations (mg/mL) in plasma taken from the anterior mesenteric vein at 28 d post-hatch.

Description	WG inclusion (%)	Treatment															
		Arg	His	Iso	Leu	Lys	Met	Phe	Thr	Val	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
Control	0.0	70.83	12.52	19.73	28.69	40.46b	12.18	20.78	116.93	27.58	83.32	21.14	184.65	49.15	67.24	50.98	30.82a
Pre-pellet	30.0	75.19	13.91	21.16	29.54	36.98ab	12.10	21.93	131.37	30.01	96.20	28.07	189.58	59.16	71.29	56.23	37.72b
Post-pellet blended	30.0	71.73	13.45	21.17	30.04	27.16a	12.15	23.05	130.70	31.05	98.26	32.61	207.86	61.73	80.88	59.91	37.93b
SEM		4.2735	1.4431	0.2027	2.1873	3.5726	1.1244	1.2619	10.9772	2.2730	9.0629	3.0305	15.0714	4.3477	5.7082	4.6275	2.4495
Significance ( $P =$ )		0.752	0.789	0.785	0.908	0.049	0.999	0.463	0.587	0.555	0.469	0.052	0.532	0.131	0.253	0.412	0.043
LSD ( $P < 0.05$ )		-	-	-	-	10.769	-	-	-	-	-	-	-	-	-	-	6.1639

<sup>ab</sup>Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

ratio ( $r = 0.665$ ;  $P < 0.001$ ), N retention ( $r = 0.593$ ;  $P < 0.001$ ) and AMEn ( $r = 0.707$ ;  $P < 0.001$ ).

## Discussion

The transition from a conventional ground-grain diet to WGF regimes did not significantly influence weight gain but feed intake was depressed by an average of 4.52% (2150 versus 2252 g/bird) and feed conversion was enhanced by 1.83% (1.340 versus 1.365) in the present study. These patterns were similar to a review of 17 studies where WGF maintained weight gain, reduced feed intake by 2.97% and enhanced feed conversion by 2.55% (Liu et al. 2015). Remarkably, the 30% post-pellet separated whole grain treatment generated an improvement of 7.69% (1.260 versus 1.365) in FCR (Table 2); however, this was the only WGF regime to outperform the ground-grain control diet in feed efficiency to a significant extent.

The hallmark response to WGF regimes is increases in relative gizzard weights; however, it should be noted that the magnitude of these responses is variable due to a multiplicity of factors including the proportion of whole grain incorporated into the ration and its characteristics. In the present study, maximum responses to WGF included increases in relative gizzard weights of 52.7% (21.39 versus 14.01 g/kg), in relative gizzard contents of 73% (11.21 versus 6.48 g/kg), reductions in gizzard pH of 0.81 (2.15 versus 2.96) and increases in relative pancreas weights of 18% (2.69 versus 2.28 g/kg) relative to the ground grain, control diet. Importantly, pronounced responses in energy utilisation and N retention were generated by the 9 WGF regimes, especially with post-pellet whole grain application. The 9 WGF dietary treatments generated mean increases in AME of 0.74 MJ (12.68 versus 11.94 MJ/kg), improvements in ME:GE ratios of 5.52% (0.726 versus 0.688), increases in AMEn of 1.18 MJ (12.54 versus 11.36 MJ/kg). While positive energy responses to WGF were anticipated, the average improvement in N retention of 8.11 percentage units (61.79 versus 53.68%) is not as frequently observed.

Relative gizzard weights were highly correlated ( $P < 0.001$ ) with relative gizzard contents, gizzard pH and relative pancreas weights; moreover, relative gizzard weights were significantly correlated with AME, ME:GE ratios, N retention and AMEn. In addition, relative gizzard contents, gizzard pH and relative pancreas weights were all significantly correlated with these parameters of nutrient utilisation. Thus, the implication is that heavier, and presumably more functional, gizzards advantaged nutrient utilisation in broiler chickens.

These nutrient utilisation outcomes suggest that WGF regimes have the potential to enhance the digestion of protein and starch. However, the accurate determination of apparent digestibility coefficients is thwarted by the fact that inert dietary markers can only be incorporated into the pelleted concentrate component of a ration following post-pelleting whole grain incorporation. This caveat does not apply to pre-pellet whole grain inclusion, so it is relevant that the addition of 30% whole grain via this mode increased distal ileal protein (N) digestibility coefficients by 8.50% (0.766 versus 0.706) and distal ileal starch digestibility by 18.8% (0.861 versus 0.725). Also, pre-pellet whole grain inclusions linearly increased protein (N) digestibility

**Table 13.** Pearson correlations between apparent starch digestibility coefficients in 4 small intestinal segments and parameters of nutrient utilisation.

	Starch digestibility PJ	Starch digestibility DJ	Starch digestibility PI	Starch digestibility DI	AME (MJ/kg DM)	ME:GE ratio	N retention (%)	AMEn (MJ/kg DM)
Starch PJ	1.000							
Starch DJ	$r = 0.233$ $P < 0.073$	1.000						
Starch PI	$r = 0.354$ $P = 0.006$	$r = 0.146$ $P = 0.267$	1.000					
Starch DI	$r = 0.118$ $P < 0.371$	$r = 0.096$ $P = 0.466$	$r = 0.710$ $P < 0.001$	1.000				
AME	$r = 0.242$ $P = 0.062$	$r = 0.069$ $P = 0.599$	$r = 0.747$ $P < 0.001$	$r = 0.684$ $P < 0.001$	1.000			
ME:GE ratio	$r = 0.246$ $P = 0.059$	$r = 0.074$ $P = 0.575$	$r = 0.733$ $P < 0.001$	$r = 0.665$ $P < 0.001$	$r = 0.998$ $P < 0.001$	1.000		
N retention	$r = 0.149$ $P = 0.255$	$r = 0.075$ $P = 0.571$	$r = 0.631$ $P < 0.001$	$r = 0.593$ $P < 0.001$	$r = 0.809$ $P < 0.001$	$r = 0.804$ $P < 0.001$	1.000	
AMEn	$r = 0.221$ $P = 0.089$	$r = 0.082$ $P = 0.533$	$r = 0.784$ $P < 0.001$	$r = 0.707$ $P < 0.001$	$r = 0.985$ $P < 0.001$	$r = 0.978$ $P < 0.001$	$r = 0.816$ $P < 0.001$	1.000

( $r = 0.614$ ;  $P < 0.01$ ) and starch digestibility ( $r = 0.475$ ;  $P < 0.05$ ) to significant extents.

In this study, the provision of whole wheat and pelleted concentrate in separate feeding trays permitted the monitoring of feed intakes of both components and the accurate calculation of dietary marker concentrations. In this context, 15% whole wheat increased distal ileal starch and protein (N) digestibility coefficients by 34.8% (0.977 versus 0.725) and 9.07% (0.770 versus 0.706), respectively, in comparison to the ground grain control. These outcomes demonstrate the capacity of WGF to enhance starch and protein digestibility, although the starch improvement is almost certainly inflated by the poor inherent starch digestibility of the control wheat-based diet. The procurement of the particular wheat used in the present study may have been serendipitous in that it contained 6.4 g/kg soluble arabinoxylans and a 6-h *in vitro* starch digestibility of 78.3% on the basis of near-infrared spectroscopy (NIR) calibrations. The distal ileal starch digestibility coefficient of 0.725 in broilers offered the ground-grain control diet was remarkably low which provided WGF regimes with considerable scope to enhance starch digestibility.

WGF regimes provide birds the opportunity of choice feeding in that they can select between the whole grain (low protein) and the pelleted concentrate (high protein) and broiler chickens will enhance their growth performance by selecting the best possible combination of protein sources (Gous and Swatson 2000). The reviews by both Forbes and Shariatmadari (1994) and Forbes and Covasa (1995) support the concept that choice feeding is contributing to the responses observed in broiler chickens under WGF regimes. Alternatively, some studies have not reported performance advantages stemming from choice feeding under WGF regimes (Olver and Jonker 1997; Delezie et al. 2009). Broiler chickens were given the unfettered opportunity to choice feed when the whole grain and pelleted concentrate components were offered in separate feeding trays in the present study and birds displayed a preference for the relatively high protein pelleted concentrate with increasing whole grain inclusions. The protein content of the wheat was 130.7 g/kg (DM) and the protein contents of the pelleted concentrates increased from 233.6 to 246.1 and 273.4 g/kg with increasing WG inclusions. Thus, greater the protein concentration differential between the two components of the ration, greater was the propensity of birds to select the relatively high protein pelleted concentrate. The transition from 4.5 to 30% whole

grain regimes increased protein intakes by 9.8% (25.32 versus 23.07 g/bird/d) but decreased starch intakes by 27.3% (13.92 versus 19.15 g/bird/d). This could reflect either over-consumption of protein and/or an under-consumption of starch or whole grain. While this outcome was not anticipated, it should be noted that Amerah and Ravindran (2008) observed essentially similar findings with birds displaying a preference for the pelleted concentrate. Nevertheless, the most efficient FCR of 1.260 was observed in birds offered the 30% post-pellet WG ration separately which represented an improvement of 7.69% (1.260 versus 1.365) relative to the ground grain control treatment. Also, it is interesting to consider starch to protein intake ratios in the control, pre-pellet and post-pellet separated dietary treatments. Increases in protein relative to starch intakes were correlated with improvements in FCR ( $r = 0.664$ ;  $P < 0.01$ ) and AME ( $r = -0.869$ ;  $P < 0.001$ ).

The effects of choice feeding on starch and protein (N) digestibility coefficients should be interpreted with caution as choice-fed birds consumed proportionally less starch so starch digestibility could be expected to increase as a consequence. Feed intakes were negatively correlated with starch digestibility in both proximal ileum ( $r = -0.474$ ;  $P < 0.001$ ) and distal ileum ( $r = -0.433$ ;  $P < 0.001$ ) across all dietary treatments in the present study so it appears that lower feed intakes facilitated starch digestion. The average feed intake for pre-pellet whole grain were 2238 g/bird which reduced to 2068 g/bird in the post-pellet/separated treatments and the corresponding average distal ileal starch digestibility coefficients were 0.802 and 0.953.

This study suggests that the opportunity of choice feeding provided by WGF regimes contributes to positive FCR and energy utilisation responses and also emphasises the 'power of the gizzard' in relation to enhanced nutrient utilisation. A well-developed gizzard is a powerful grinding organ with an estimated capacity to exert forces of 585 kg/cm<sup>2</sup> in pressure (Cabrera 1994) so that the gizzard can disrupt physical structures within feedstuffs and reduce particle size in digesta flowing into the duodenum (Amerah et al. 2007). Also, the gizzard has been described as the pace-maker of gut motility which includes episodes of reverse peristalsis (Duke 1982; Ferket 2000). Reverse peristalsis increases the exposure of digesta in the gizzard to proventricular secretions of pepsin and hydrochloric acid and pancreatic secretions of amylase and several other digestive enzymes.

Pepsin (and HCl) both initiate the protein digestive process and also play an overall regulatory role via the release of enteric hormones, including gastrin and cholecystokinin (CCK), which are triggered by peptide end-products of pepsin digestion (Krehbiel and Matthews 2003). Thus, the relationships between heavier gizzards containing increased amounts of digesta at more acidic pH and heavier pancreas weights are entirely consistent with the concept of WGF generating a more functional gizzard and amplified pepsin activity. The digestion of starch in poultry is achieved by the pancreatic secretion of amylase into the duodenum (Moran 1982). Rogel et al. (1987) investigated the effects of oat hulls on relative gizzard weights and total-tract digestibility of raw potato starch in broilers. Oat hulls increased gizzard weights by 49% (22.2 versus 14.9 g/kg and improved starch digestibility coefficients by 69% (0.926 versus 0.547) and significant correlation between gizzard weights and starch digestibility coefficients were observed. The clear implication of the Rogel et al. (1987) study is that heavier gizzards generated by WGF have the potential to increase the extent of starch digestion.

Additionally, it has been suggested that some of the WGF benefits stem from its generation of slowly digestible starch because the starch digestion rate of whole grain is inherently slower than the same feed grain that has been hammer-milled and steam-pelleted (Truong et al. 2016). Slowly digestible starch may be defined as that which is digested in the three posterior segment of the small intestine as opposed to the proximal jejunum (rapidly digestible starch). However, in this study the three WGF regimes did not generate slowly digestible starch. There is the possibility that slowly digestible starch spares amino acids from catabolism in the gut mucosa (Weurding et al. 2003; Enting et al. 2005) and it is for this reason that concentrations of free amino acids in plasma taken from the anterior mesenteric vein were determined. Given the above, it is perhaps not surprising that there were no significant differences in concentrations of the majority of amino acids in the portal circulation.

In conclusion, WGF regimes generate larger, and presumably, more functional gizzards to the advantage of energy utilisation. Additionally, the opportunity of choice feeding provided by WGF regimes contributed to enhanced feed conversion efficiency and energy utilisation on the basis of the present study. Also, differential impacts of inclusion levels and modes of whole grain incorporation were observed. On average, post-pellet whole grain inclusions increased relative gizzard weights by 35.3% (18.95 versus 14.01 g/kg) as opposed to a 9.3% increase (15.31 versus 14.01 g/kg) for pre-pellet whole grain inclusions relative to the ground grain control. Post-pellet whole grain inclusions increased AME by an average of 1.03 MJ (12.97 versus 11.94 MJ/kg); whereas, there was a numerical negative response of 0.03 MJ with pre-pellet WG. Finally, when offered separately, post-pellet whole grain inclusions enhanced FCR by 5.01% (1.297 versus 1.365) in comparison to modest average improvements of 0.34 and 0.17% for pre-pellet WG and post-pellet WG blended, respectively.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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