ISSN 1682-8356
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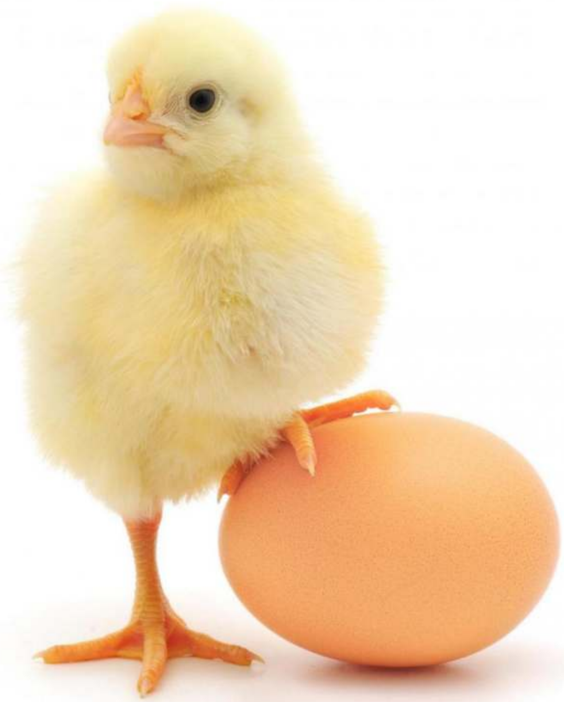

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 13 (6): 308-315, 2014
ISSN 1682-8356

Influence of Steam-Pelleting Temperatures and Grain Variety of Finely-Ground, Sorghum-Based Broiler Diets on Small Intestinal Starch and Nitrogen Digestion Dynamics in Broiler Chickens

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Abstract: The effects of sorghum grain variety and conditioning temperature at which broiler diets were steam-pelleted on small intestinal digestion dynamics of starch and nitrogen were assessed. A 2×3 factorial array of treatments comprised two sorghum varieties, with white (Liberty) and red (Venture) pericarps and three conditioning temperatures (70, 80, 90°C). Both sorghums were finely-ground through a 2.0 mm hammer-mill screen prior to incorporation into the diets. Each of the dietary treatments was offered to six replicate cages (6 birds per cage) from 7 to 28 days post-hatch. Digesta samples were collected at day 28 for determination of starch and nitrogen digestibilities and mean retention time in the proximal jejunum, distal jejunum, proximal ileum and distal ileum. The digestion dynamics of starch and nitrogen were determined using an exponential mathematical model to relate digestion coefficients with mean retention times in each small intestinal segment. Increasing conditioning temperatures generated a 'concave response' in starch digestion rates in white sorghum diets; whereas, there was a 'convex response' in red sorghum diets. Thus, at the intermediate 80°C conditioning temperature, starch digestion rates were at a minimum value for white, but at a maximum value for red sorghum-based diets, which is consistent with the significant sorghum type×temperature interaction ($p<0.05$). Conditioning temperatures increased potential digestible starch ($r = 0.336$, $p<0.05$), predicted glucose absorption ($r = 0.468$, $p<0.01$), retention time in the distal ileum ($r = 0.362$, $p<0.05$) and the entire small intestine ($r = 0.371$, $p<0.05$). There was a quadratic relationship between potential digestible starch ($p<0.05$) and Feed Conversion Ratio (FCR), which indicated that the optimal FCR was attained with a potential digestible starch value of 0.862. These results suggested that starch and protein digestion may influence feed conversion efficiency.

Key words: Digestion rates, dynamics, feed efficiency, protein, retention time, starch

INTRODUCTION

Starch and protein are two pivotally important macro-components of poultry diets and the extents of their utilization are major determinants of the efficiency of chicken-meat production. The *in vitro* digestibility of sorghum starch is inferior to other cereals (Giuberti *et al.*, 2012; Black *et al.*, 2005) suggested that synchronies between starch and protein digestion and glucose and amino acid absorption may be contributing to the shortcomings of sorghum. Efficient protein deposition and growth requires an appropriate balance of glucose and amino acids at the sites of skeletal muscle synthesis (Pelley and Goljan, 2011). Thus sorghum-based diets are considered as a suitable model to evaluate the importance of starch and protein digestion dynamics on broiler performance and nutrient utilization.

Several intrinsic factors in various sorghums could influence digestion rates of starch and protein including phenolic compounds (other than condensed tannin), phytate and kafirin, the dominant protein fraction in sorghum (Selle *et al.*, 2010). Although there is the possibility that grain sorghum contains condensed tannin which is a potent anti-nutritional factor (Nyachoti *et al.*, 1997), a pigmented testa was not detected in the two sorghum varieties evaluated in this study by the Clorox bleach test (Waniska *et al.*, 1992). This is a quantal assessment of the presence or absence of a pigmented testa and sorghums without a pigmented testa fall into the Type I category that does not contain condensed tannin (Dykes and Rooney, 2006). Thus condensed tannin is not a factor in the present study; however, the perception remains that condensed tannin may be present in sorghum.

Extrinsic factors influencing starch and protein digestion in sorghum include sorghum particle size, grain texture and conditioning temperatures at which sorghum-based diets are steam-pelleted (Liu *et al.*, 2013a). In a previous study with coarsely-ground sorghum-based diets, Liu *et al.* (2013b) showed that sorghum varieties interacted with feed form (mash, intact pellets, reground pellets) to influence starch and protein digestion dynamics

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including nitrogen digestion rates, which partially contributed to variations in feed conversion efficiency. Subsequently, Liu *et al.* (2013c) reported that white sorghum-based diet had significantly higher starch digestion rate which may have contribute to better feed conversion efficiency in white than red sorghum-based diet.

Moreover, conditioning temperatures in steam-pelleting process may influence degrees of starch gelatinization (Abdollahi *et al.*, 2010) and break down anti-nutritional factors including protease inhibitors (Svihus and Zimonja, 2011); therefore, manipulating conditioning temperatures may alter starch and protein digestibility in feedstuffs. However, Liu *et al.* (2013a) showed that increasing conditioning temperatures from 65 to 80°C and 95°C did not influence potential digestible starch and nitrogen and did not alter starch and nitrogen digestion rates. Sorghum grains were mediumly ground through 3.2 mm hammer-miller screen in the Liu *et al.* (2013a) study and Al-Rabadi *et al.* (2011) demonstrated that particle size of milled sorghum flour influenced *in vitro* starch digestion rates. It follows that sorghum-based diets with smaller grain particle sizes may respond differently to fluctuating conditioning temperatures. To clarify this situation, the intention of the present study was to examine the effects of steam-pelleting white (Liberty) and red (Venture) sorghum varieties at three conditioning temperatures on small intestinal starch and nitrogen digestion dynamics in broiler chickens at 28 days post-hatch and the relationships of digestion dynamics with previously reported growth performance parameters (Selle *et al.*, 2013a).

MATERIALS AND METHODS

The materials and methods adopted in the present study were similar to those outlined by Selle *et al.* (2013b). The sorghum-based diets were steam-pelleted at conditioning temperatures of 70, 80 and 90°C by a Palmer PP300 pellet press with a 14 seconds residence time in the conditioner. The conditioning temperatures were automatically regulated by a computer software package (Gordyn and Palmer, Hallam, Vic, Australia). Each of the dietary treatments was offered to six replicates (6 birds per cage) or a total of 216 male Ross 308 chicks from 7 to 28 days post-hatch. On day 28, all birds were euthanized by intravenous injections of sodium pentobarbitone and digesta were collected in their entirety from proximal jejunum, distal jejunum, proximal ileum and distal ileum, which were demarcated by the end of duodenal loop, Meckel's diverticulum, the ileo-caecal junction and their midpoints. The digesta samples were freeze-dried and weighed to determine retention time and apparent digestibility coefficients of starch and nitrogen at four intestinal sites by using acid insoluble ash (Celite World Minerals, Lompoc, CA, USA) as the dietary marker. Mean retention time (MRT) was calculated using the following equation:

$$MRT\ (min) = (1440 \times AIA_{digesta} \times W)/FI_{24hr} \times AIA_{feed}$$

where, $AIA_{digesta}$ is the AIA concentration in the digesta (mg/g), W is the weight of dry gut content (g), FI_{24h} is the feed intake over 24 h before sampling (g), AIA_{feed} is the AIA concentration in the feed (mg/g) and 1440 equals min per day. The mean retention time in the duodenum was estimated to be five min (Weurding *et al.*, 2001a).

The pattern of fractional digestibility coefficients was described by relating the digestion coefficient at each site with the digestion time. The digestion time was calculated from the sum of MRT determined in each intestinal segment. The curve of digestion was described by exponential model developed by Orskov and McDonald (1979):

$$D_t = D_\infty (1 - e^{-kt}) \qquad (1)$$

where, D_t (g/g starch or nitrogen) is the proportions of starch or nitrogen that digested at time t (min), the fraction D_∞ is the amount of potential digestible starch or nitrogen (asymptote) (g/g starch or nitrogen), digestion rate constant k (per unit time/min) would mean a 100% starch, nitrogen or amino acid digestion within one minute when it is equal to one. Starch absorption was assumed not to take place proximal to the small intestine. Nitrogen digestion in this study was determined as apparent N digestibility and it is impacted by endogenous N flows, which is not the case with starch.

The Microsoft Excel Solver® was used to compute parameters of the modified first-order kinetic (Eq. 1) by minimizing the sums of squares of residuals with the constraint that $D_\infty \leq 1$. Experimental data was analyzed using the JMP® 9.0.0 (SAS Institute Inc. US) The experimental units were cage means and statistical procedures included analyses of variance using the general linear models procedure, Pearson correlations, linear and quadratic regressions. A probability level of less than 5% was considered to be statistically significant. This feeding study complied with the specific guidelines (N00/6-2010/3/5344) approved by the Animal Ethics Committee of The University of Sydney.

RESULTS

The following starch and nitrogen digestibility coefficients are restated because they are fundamental to the calculation of digestion dynamic parameters. As reported previously (Selle *et al.*, 2013a), for white sorghum the average apparent starch digestibility coefficients were 0.667, 0.789, 0.837 and 0.874 at the

Table 1: Effect of sorghum type and conditioning temperature on mean retention time (min) in the proximal jejunum, distal jejunum, proximal ileum and distal ileum in broiler chickens at 28 days post-hatch

Treatment: Basis of diets	Conditioning temperature	Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Total tract*
White sorghum	70°C	27.7	32.7	40.8	38.2	144.4
	80°C	30.7	32.1	36.5	35.8	140.1
	90°C	31.0	31.3	46.3	45.3	158.9
Red sorghum	70°C	21.8	30.2	39.8	41.8	138.6
	80°C	30.5	31.6	39.5	45.2	151.8
	90°C	35.2	42.5	41.7	55.3	179.7
SEM		5.24	4.07	4.45	4.34	12.1
Main effects: Basis of diets						
White sorghum		29.8	32.1	41.2	39.7[b]	147.7
Red sorghum		29.2	34.7	40.4	47.4[a]	156.7
Conditioning temperature						
70°C		24.7	31.4	40.3	40.0[b]	141.5
80°C		30.6	31.9	38.0	40.5[b]	145.9
90°C		33.1	36.9	44.0	50.3[a]	169.2
Significance (p =)						
Basis of diets		0.889	0.426	0.828	0.038	0.372
Conditioning temperature		0.278	0.341	0.407	0.041	0.064
Interaction		0.633	0.208	0.698	0.724	0.544
Linear effect of conditioning temperatures						
r =		0.274	0.223	0.145	0.362	0.371
P =		0.106	0.191	0.398	0.030	0.026

*Retention time in the duodenum was not determined but were taken as 5 min (Weurding *et al*., 2001)

proximal jejunum, distal jejunum, proximal ileum and distal ileum, respectively. The corresponding values for red sorghum were 0.687, 0.830, 0.854 and 0.882 with a significant difference ($p<0.05$) being observed in the distal jejunum in favour of red sorghum. Average apparent nitrogen digestibility coefficients in four small intestinal segments for white sorghum were 0.557, 0.666, 0.746 and 0.787, respectively. The corresponding values for red sorghum were 0.589, 0.697, 0.786 and 0.809 with a significant differences ($p<0.05$) being observed in favour of red sorghum in the distal jejunum, proximal ileum and distal ileum.

The effects of sorghum type and conditioning temperature on mean retention time in the four small intestinal segments in broiler chickens at 28 days post-hatch are shown in Table 1. Average retention times in the proximal jejunum, distal jejunum, proximal ileum and distal ileum were 29.5, 33.4, 40.8 and 43.6 min, respectively, with an average retention time in the entire small intestine of 152.3 min. Red sorghum-based diets had a significantly longer retention time in the distal ileum by 19.4% (47.4 versus 39.7 min, $p<0.05$). Diets conditioned at 90°C (50.3 min) had significantly longer retention times ($p<0.05$) in the distal ileum than diets conditioned at 70 (40.0 min) and 80°C (40.5 min) by 25.8 and 24.2%, respectively. Increasing conditioning temperatures linearly increased retention times in the distal ileum ($r = 0.362$; $p<0.05$) and along the entire small intestinal tract ($r = 0.371$; $p<0.05$). There were no significant differences for sorghum type and conditioning temperature in retention times in the three anterior small intestinal segments.

Effects of sorghum type and conditioning temperature on starch and N digestion dynamics in broiler chickens are shown in Table 2. There was an interaction between sorghum type and conditioning temperature for starch digestion rates ($p<0.05$). In white sorghum-based diets, the highest starch digestion rate was observed at a conditioning temperature of 90°C (7.40×10^{-2}/min), while in red sorghum-based diets, the highest starch digestion rate was in diets conditioned at 80°C (7.10×10^{-2}/min). In white sorghum-based diets, increasing conditioning temperature from 80 to 90°C significantly increased starch digestion rates (3.92 versus 7.40×10^{-2}/min); whereas, with red sorghum-based diets, there was a numerical decrease (7.10 versus 5.55×10^{-2}/min). Increasing conditioning temperatures linearly increased ($r = 0.336$; $p<0.05$) potential digestible starch.

Increasing conditioning temperatures tended to increase the rate of N digestion in a linear manner ($r = 0.349$; $r<0.06$). However, sorghum type and conditioning temperature did not influence the quantity of potential digestible N. The predicted digestion curves derived from average starch and nitrogen digestion dynamics in white and red sorghum-based broiler diets with different conditioning temperatures are shown in Fig. 1.

The quadratic relationships between potential digestible starch and previously reported FCR (Selle *et al*., 2013a)

Table 2: Effects of sorghum type and conditioning temperature on digestion kinetics in broiler chickens at 28 days post-hatch [starch digestion rate (K_{starch}), nitrogen digestion rate ($K_{nitrogen}$), potential digestible starch (PDS), potential digestible nitrogen (PDN)]

Treatment					
Basis of diets	Conditioning temperature	K_{starch} ($\times 10^{-2}$/min)	$K_{nitrogen}$ ($\times 10^{-2}$/min)	PDS (g/g)	PDN (g/g)
White sorghum	70°C	5.09^{ab}	4.16	0.846	0.766
	80°C	3.92^{b}	4.35	0.893	0.790
	90°C	7.40^{a}	4.80	0.864	0.789
Red sorghum	70°C	5.31^{ab}	3.81	0.856	0.793
	80°C	7.10^{a}	5.57	0.873	0.794
	90°C	5.55^{ab}	5.88	0.896	0.807
SEM		0.777	0.698	0.0149	0.0132
Main effect. Basis of diets					
White sorghum		5.47	4.44	0.875	0.781
Red sorghum		5.99	5.09	0.868	0.798
Conditioning temperature					
70°C		5.20	3.98	0.851	0.779
80°C		5.51	4.96	0.883	0.792
90°C		6.48	5.34	0.880	0.797
Significance (P =)					
Sorghum type		0.468	0.313	0.553	0.176
Conditioning temperature		0.289	0.185	0.068	0.419
Interaction		0.031	0.526	0.245	0.753
Linear effect of conditioning temperatures					
r =		0.251	0.349	0.336	0.254
P =		0.173	0.059	0.045	0.136

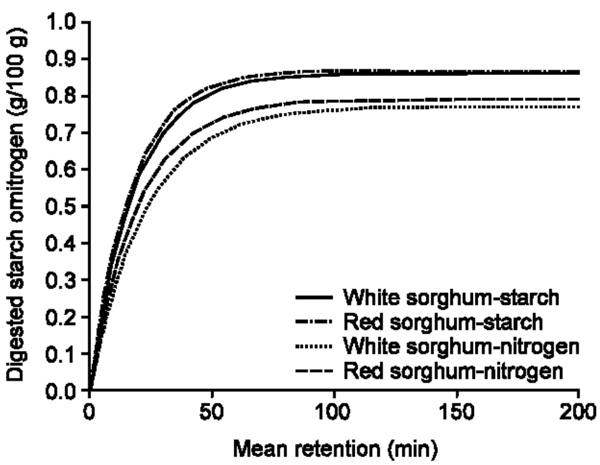

Fig. 1: Predicted digestion curves of starch and nitrogen in white and red sorghum-based broiler diets at 28 days post-hatch

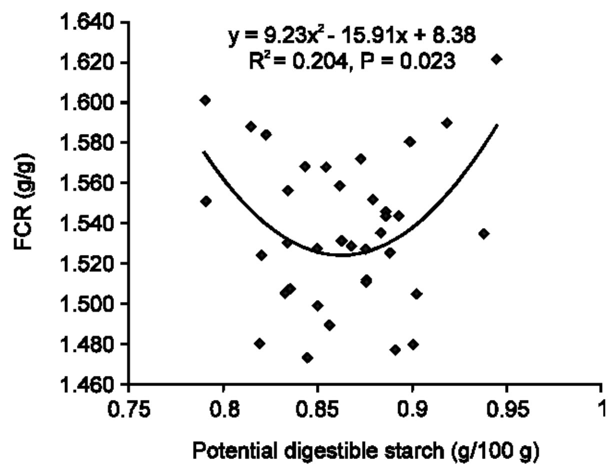

Fig. 2: Quadratic relationship between potential digestible starch and FCR at 28 days post-hatch in broiler chickens offered white and red sorghum-based diets

are shown in Fig. 2. There was a significant relationship between PDS and FCR (r = 0.452, $p<0.05$); whereas, in contrast, the relationship between PDN and FCR was not significant. It may be deduced that the most efficient FCR of 1.524 corresponds to a PDS value 0.862. As shown in Fig. 3, there was a significant linear relationship (r = 0.593; $p<0.05$) between starch digestion rates and FCR in red sorghum-based diets such that efficiency of feed conversion deteriorates with increasing starch digestion rates; however, N digestion rates did not influence FCR to any extent. In contrast, in white sorghum-based diets FCR were not correlated with rates of starch and N digestion.

DISCUSSION

Anecdotally, white sorghums are considered to be superior to red varieties (Selle *et al*., 2010) and a similar outcome was anticipated in this study. However, red sorghum was not inferior as indicated by starch and N digestibility coefficient comparisons included under Results and reported growth performance data (Selle *et al*., 2013a). In the present study, Venture red sorghum contained more protein, phytate, total phenolic compounds and almost certainly more kafirin, but less starch, than Liberty white sorghum as quantified by Selle *et al*. (2013a). These indicate that the nutritive value of

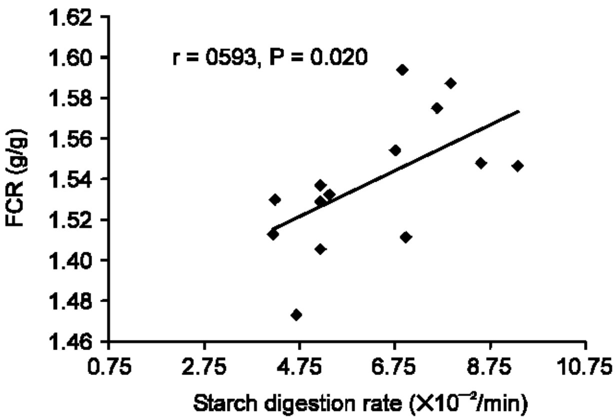

Fig. 3: Linear relationship between digestion rates of starch and FCR at 28 days post-hatch in broiler chickens offered red sorghum-based diets

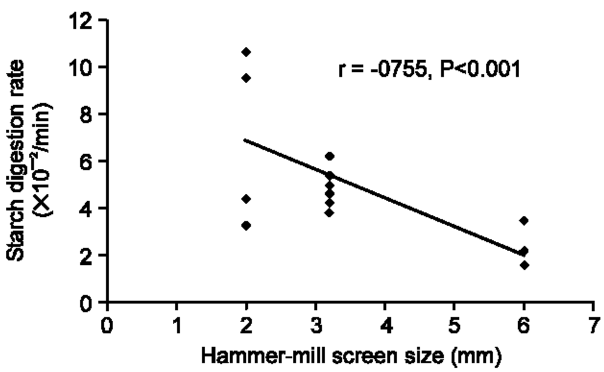

Fig. 4: Linear relationship between hammer-mill screen size and starch digestion rate at 28 days post-hatch in broiler chickens offered white (Liberty) sorghum-based diets (Liu *et al.*, 2013a,c)

Venture would be inferior to Liberty. Moreover, from their Symes' particle size indices, the grain texture of Venture (7 or 'extra very hard') was harder than Liberty (12 or 'very hard'). Cabrera (1992) demonstrated that finely-ground hard sorghums supported better weight gains in broilers than finely-ground soft sorghums and the reverse was true when sorghums were coarsely-ground. Thus the fine-grinding employed in the present study probably advantaged the harder red sorghum and compromised the performance of broilers offered white-sorghum based diets.

Pellet quality was not objectively assessed in the present study but there did not appear to be differences with increasing conditioning temperatures from visual inspection. This subjective assessment is supported by the facts that increasing conditioning temperatures did not influence feed intakes nor relative gizzard weights; whereas, enhanced pellet quality would be expected to increase both parameters. This suggests that the potential impact of pellet quality may not have had a real bearing in this study.

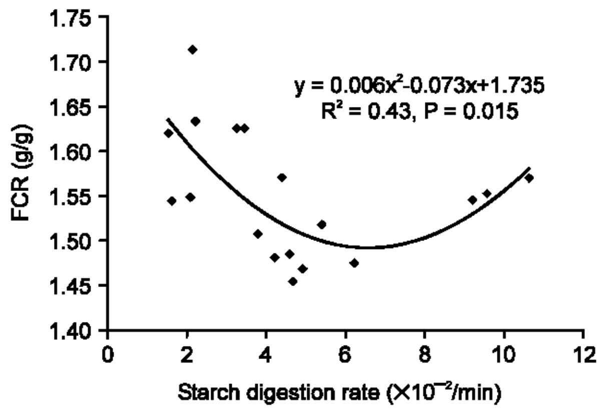

Fig. 5: Quadratic relationship between FCR and starch digestion rates at 28 days post-hatch in broiler chickens offered white (Liberty) sorghum-based diets (Liu *et al.*, 2013a,c)

Increasing conditioning temperatures linearly increased retention times in the distal ileum and the entire small intestinal tract. This is consistent with a previous study (Liu *et al.*, 2013a), where increasing conditioning temperatures from 65 to 80 and 95°C linearly increased retention time in the distal ileum. However, in the present study the retention time of red sorghum was 19.4% greater longer than white-sorghum based diets. That distal ileal N digestibility was significantly higher by 2.80% in birds offered red sorghum-based diets was probably associated with the longer retention time in that segment.

At the intermediate conditioning temperature of 80°C, white sorghum had its lowest starch digestion rate observed (3.92×10^{-2}/min); conversely, red sorghum its highest rate (7.1×10^{-2}/min). Moreover, the 'shape' of starch digestion rates across the three conditioning temperatures was markedly different ('convex versus concave') between the two sorghums. Nevertheless, there does not appear to be a straightforward explanation for the significant interaction between sorghum type and conditioning temperature for starch digestion rates.

Potential digestible starch quadratically influenced FCR to a significant extent but this was not the case for potential digestible N (Fig. 2). Moreover, it may be deduced from the quadratic relationship that 0.862 potential digestible starch generates the optimal FCR of 1.524 so that either more or less potential digestible starch depresses feed efficiency. It appears there is an ideal balance between potential digestible starch and other nutrients, notably protein, in order to achieve the most favourable FCR.

In red sorghum-based diets starch digestion rates were linearly correlated with FCR as shown in Fig. 3, but this was not the case for N digestion rates. In contrast, both starch and N digestion rates did not influence FCR in

Table 2: Effects of sorghum type and conditioning temperature on digestion kinetics in broiler chickens at 28 days post-hatch [starch digestion rate (K_{starch}), nitrogen digestion rate ($K_{nitrogen}$), potential digestible starch (PDS), potential digestible nitrogen (PDN)]

Treatment: Basis of diets	Treatment: Conditioning temperature	K_{starch} ($\times 10^{-2}$/min)	$K_{nitrogen}$ ($\times 10^{-2}$/min)	PDS (g/g)	PDN (g/g)
White sorghum	70°C	5.09^{ab}	4.16	0.846	0.766
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	90°C	7.40^{a}	4.80	0.864	0.789
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Interaction		0.031	0.526	0.245	0.753
Linear effect of conditioning temperatures					
r =		0.251	0.349	0.336	0.254
P =		0.173	0.059	0.045	0.136

Table 3: Impact of hammer-milling white sorghum (Liberty) through different screen sizes prior to incorporation into diets steam-pelleted at conditioning temperatures of 90–95 ºC on starch and nitrogen digestion kinetics [potential digestible starch (PDS), starch digestion rate (K_{starch}), potential digestible nitrogen (PDN), nitrogen digestion rate ($K_{nitrogen}$)] and relative gizzard weights at 28 days post-hatch

Screen size	6.0 mm	3.2 mm	2.0 mm
Conditioning temperature	95°C[1]	95°C[2]	90°C
Item			
K_{starch} ($\times 10^{-2}$/min)	2.18	4.83	7.40
$K_{nitrogen}$ ($\times 10^{-2}$/min)	2.27	2.78	4.80
PDS (g/g)	0.961	0.892	0.864
PDN (g/g)	0.838	0.789	0.789
Relative gizzard weight (g/kg)	19.4[3]	17.8[4]	16.9[5]

Liu *et al*. (2013a,c), Selle *et al*. (2012), Selle *et al*. (2013a,b)

white sorghum-based diets. Importantly, feed conversion efficiency was depressed in broilers offered red-sorghum-based diets with increasing starch digestion rates. This is reciprocally consistent with reported data (Weurding *et al*., 2001a,b; Weurding *et al*., 2003a,b) where the provision of slowly digestible starch in broiler diets had a positive impact on efficiency of feed conversion. The underlying mechanisms are almost certainly complex; however, one suggestion is that slowly digestible starch may have a 'protein-sparing' effect. Fleming *et al*. (1997) found that glucose and glutamic acid are approximately equally important energy substrates for small intestinal mucosal cells in the rat. However, they found that energy is derived from glucose noticeably more efficiently than glutamic acid and net ATP production was greatest with both substrates present. It follows that glucose derived from sources with rapid starch digestion rates would be absorbed in the upper small intestine, effectively forcing mucosal cells in the lower small intestine to oxidize amino acids to provide energy. Thus, sources with slow starch digestion rates would provide more glucose to the lower small intestine thereby sparing protein from oxidation and energy generation from glucose would be more efficient than from amino acids, which, importantly, would be spared to enter the systemic circulation. That this is the case in pigs was demonstrated by Van der Meulen *et al*. (1997) where a more slowly digestible starch source generated substantially greater entries of essential amino acids, including lysine, into the systemic circulation.

The dynamics of starch and N digestion shown in Table 3 are derived from this and two very similar studies (Liu *et al*., 2013b,c). This approach is unorthodox because it considers separate experiments collectively, but it is instructive given that the three studies were conducted at the same site with similar husbandry conditions and experimental design. Reducing hammer-mill screen size and consequently sorghum particle size, accelerated starch (Fig. 4) and N digestion rates but the effect on starch digestion rates was more profound. The relative gizzard weights are also tabulated where it is evident that reducing the size of the hammer-mill screen reduced gizzard weights. The likelihood is that coarser sorghum particle sizes stimulated gizzard development with increased secretions of pepsin and hydrochloric acid to enhance protein digestion (Svihus *et al*., 2004). Thus, heavier relative gizzard weights, stemming from coarser grain particle size, may impact on N digestive dynamics. In addition, hammer-mill screen size had a pronounced impact (r = -0.755; p<0.001) on starch digestion rates. Moreover, there is a significant quadratic relationship (r = 0.656; p<0.05) between starch digestion rate and FCR (Fig. 5). This suggests that there is an ideal balance between digestion of starch and protein and their absorptions to maximize feed conversion efficiency and changing hammer-mill screen sizes which manipulate sorghum particle sizes may alter starch digestion rates even in pelleted diets.

Conclusions: Increasing conditioning temperatures linearly increased retention times in the distal ileum and the small intestine irrespective of sorghum variety. However, the influence of conditioning temperatures on starch digestion rates was quite different dependent upon sorghum variety. In red sorghum-based diets increasing starch digestion rates were correlated with depressions in feed efficiency, which was not the case in white sorghum-based diets. Conditioning temperatures linearly increased predicted glucose absorption, which tended to be correlated with improved FCR. The effects of starch digestion dynamics had a more potent influence on efficiency of feed conversion than nitrogen. From three related feeding studies, starch digestion rates influenced FCR in a quadratic manner. The quadratic nature of this relationship indicates that there is an optimal starch digestion rate for FCR, which presumably provides the most appropriate blood glucose levels with other important nutrients including amino acids. The influence of sorghum particle size, as dictated by hammer-mill screen sizes, on starch and nitrogen digestion dynamics and, in turn, their impact on feed conversion efficiency in broiler chickens merits further research.

ACKNOWLEDGEMENTS

We acknowledge the financial support received from RIRDC Chicken-meat for the sorghum steam-pelleting temperatures project (PRJ-003810) and the post-graduate scholarship awarded by the Poultry Cooperative Research Centre to Ms. Sonia Yun Liu. Also, we are very appreciative of the competent technical assistance so willingly provided by Ms Joy Gill.

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