Kafirin in sorghum

Just how big a villain is this protein fraction?

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Kafirin is the dominant protein fraction in grain sorghum; classically 55% of total sorghum protein is kafirin but this proportion will fluctuate in accordance with total sorghum protein. As sorghum protein increases so does the proportion of kafirin, at the expense of the second protein fraction glutelin (Taylor et al., 1984). Kafirin is located in discrete protein bodies in sorghum endosperm which, in close proximity with starch granules, are embedded in a glutelin protein matrix. Protein bodies are more numerous in the ‘hard’ (corneous) endosperm than in the ‘soft’ (floury) endosperm of grain sorghum and increasing kafirin proportions (and sorghum protein levels) are associated with harder sorghum grain textures. This is illustrated in Figure 1 (De Mesa-Stonestreet et al., 2010) and Figure 2 is an electron micrograph of sorghum endosperm (Black, 2001).

Kafirin and the $64 million sorghum question

In our view this conundrum represents the $64 million sorghum question. Do biophysical and/or biochemical interactions between kafirin protein bodies and starch granules within the endosperm tangibly compromise starch utilisation in sorghum-based broiler diets – or not? There may be other factors inherent in grain sorghum that have more potent negative effects on starch utilisation but it
is quite evident that the ileal digestibility of starch in sorghum-based diets is inferior to that of maize–based broiler diets despite the similarities of the two grains. Kafirin may or may not be contributing to this discrepancy.

**Figure 1** Kafirin protein bodies and starch granules embedded in the glutelin protein matrix of sorghum endosperm (De Mesa-Stonestreet *et al.*, 2010)

There is the distinct likelihood is that kafirin proportions of total protein are increasing in Australian sorghum crops, probably as an inadvertent consequence of breeding programs. This is supported by changing amino acid profiles over the past two decades to patterns that are more consistent with kafirin. The amino acid profile of kafirin is quite distinctive as it is rich in leucine but contains a paucity of basic amino acids (arginine, histidine and, importantly, lysine). Also local sorghums appear to have harder grain textures than the global average which is indicative of relatively high kafirin contents (*Selle, 2011*). However, this trend probably can be accommodated
in terms of protein quality of sorghum-based diets for poultry and pigs given appropriate allowances are built into their formulations. However, if kafrin is impeding starch utilisation these food-producing industries are faced with a far more fundamental problem as dietary inclusions of sorghum are primarily to provide energy, mainly as starch. Thus the purpose of this TechNote is to consider the $64 million sorghum question.

It is generally accepted that kafrin negatively influences starch digestion but Gidley et al. (2011a) expressed reservations on the basis of scanning electron micrographs of ileal digesta in pigs offered cold-pelleted, sorghum-based diets. Their conclusion was that these micrographs do not support the concept of the ‘encapsulation’ of sorghum starch by protein bodies. Alternatively, Bach Knudsen et al. (1988) opined that kafrin and resistant starch form physicochemical complexes in the gut. The implication is that kafrin impedes starch digestion such that it is not digested in the small intestine; however, this proposal might overlooks the possibility that interactions may take place between starch and the glutelin protein matrix in which starch granules are embedded.

Importantly, Taylor (2005) argued there is clear evidence that disulphide cross-linkages in β- and γ-kafrin protein fractions in the periphery of protein bodies is a major factor in the reduced digestibility of sorghum protein and starch. Moreover, hydrothermal processes, including ‘wet-cooking’ and steam-pelleting, amplify disulphide cross-linkages in kafrin and reduce protein solubility. Instructively, Emmambux and Taylor (2009) reported that wet-cooking reduced in vitro kafrin digestibility by 24% and reduced the digestibility of total sorghum protein by 27%. In one local study (Selle et al., 2010b), sorghum was incorporated into sorghum-casein diets either unprocessed or following “wet-cooking”. In this case the hydrothermal process involved mixing a 1:1 slurry of finely-ground sorghum and distilled water for 2 hours at 45°C followed by drying at 60°C for 70 hours. As shown in Table 1, this process depressed growth performance and N retention of broilers offered these diets from 7 to 21 days post-hatch to remarkable extents. Alternatively, AME was only numerically depressed by 0.2 MJ in this study. It could be argued that the amplification of disulphide cross-linkages in kafrin by wet-cooking compromised protein digestibility and growth rates but its negative impact on energy utilisation and presumably starch digestibility and was relatively limited. This is contrary to the conclusion of Ezeogu et al. (2008) that disulphide cross-linkages in sorghum proteins induced by wet-cooking limit expansion of starch granules and deny amylase access its substrate thereby compromising starch digestibility.
Table 1  The effects of unprocessed or “wet-cooked” sorghum in sorghum-casein diets on growth performance and N retention of broilers from 7 to 21 days post-hatch (Selle et al., 2010)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight gain (g/bird)</th>
<th>Feed intake (g/bird)</th>
<th>FCR (g/g)</th>
<th>N retention (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unprocessed sorghum</td>
<td>490</td>
<td>822</td>
<td>1.69</td>
<td>62.7</td>
</tr>
<tr>
<td>‘Wet-cooked’ sorghum</td>
<td>352</td>
<td>650</td>
<td>1.86</td>
<td>57.4</td>
</tr>
<tr>
<td>Significance (P)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.025</td>
</tr>
<tr>
<td>Difference (Difference)</td>
<td>-28.2%</td>
<td>-21.4%</td>
<td>+10.1%</td>
<td>-8.45%</td>
</tr>
</tbody>
</table>

Six red sorghums from the 2009 Liverpool Plains harvest were compared in broiler bioassays using sorghum-casein diets. Two varieties [sorghums #3 (99.4 g/kg protein; 712 g/kg starch) and #5 (116.3 and 700 g/kg)] were selected and compared in nutritionally equivalent, conventional diets fed as unprocessed mash or as intact pellets and reground mash following steam-pelleting at conditioning temperatures of 65 and 97°C (Selle et al., 2013). There were treatment interactions but, as main effects, sorghum #3 supported superior weight gains, feed conversion ratios, energy utilisation (AME and AMEn) and N retention as shown in Table 2. The protein contents and amino acid profiles of the two sorghums suggest that inferior sorghum #5 contained more kafirin. Leucine is plentiful in kafirin and sorghum #5 contained 25.2% more leucine (16.4 versus 13.1 g/kg) than sorghum #3 and possessed a higher kafirin index (6.8 versus 4.3). The kafirin index is only a guide and is calculated from the concentrations of leucine less those of the basic amino acids which is consistent with the amino acid profile of kafirin. The phytate levels in the two sorghums were both typical and very similar at 2.35 and 2.40 g/kg phytate-P so the superiority of sorghum #3 cannot be attributed to phytate levels.

Somewhat surprisingly, there were no differences in protein (N) digestibility coefficients between the two sorghums (data not shown) at the distal jejunum and distal ileum. It is tempting to associate ‘high-kafirin’ sorghums with depressed protein digestibility coefficients. Alternatively, there were profound differences in energy utilisation of more than 1.00 MJ in both AME and AMEn in favour of sorghum #3 which suggests that the utilisation of starch/energy was inferior in sorghum #5 with its probably higher kafirin concentrations.
Table 2  
Main effects of conventional diets based on two red sorghums (#3 and #5) offered to broiler chicks from 7 to 28 days post-hatch on growth performance and nutrient utilisation (Selle et al., 2013)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gain (g/bird)</th>
<th>Intake (g/g)</th>
<th>FCR</th>
<th>AME (MJ/kg)</th>
<th>N retention (%)</th>
<th>AMEn (MJ/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red #3</td>
<td>1302</td>
<td>1983</td>
<td>1.524</td>
<td>13.61</td>
<td>63.6</td>
<td>12.38</td>
</tr>
<tr>
<td>Red #5</td>
<td>1255</td>
<td>2009</td>
<td>1.601</td>
<td>12.55</td>
<td>58.0</td>
<td>11.35</td>
</tr>
<tr>
<td>Significance</td>
<td>0.003</td>
<td>0.258</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>#3 vs #5</td>
<td>+3.74%</td>
<td>-1.29%</td>
<td>-4.81%</td>
<td>+1.06 MJ</td>
<td>+9.66%</td>
<td>+1.03 MJ</td>
</tr>
</tbody>
</table>

Pivotaly, the Australian Proteome Analysis Facility at Macquarie University has successfully developed the methodology to quantify kafirin concentrations in sorghum. Such analyses have not been previously completed in Australia to the best of our knowledge. Therefore, retrospective analyses are currently being completed to determine concentrations of kafirin in the six Liverpool Plains sorghums. The Poultry Research Foundation has retrospectively determined distal jejunal and distal ileal starch digestibility coefficients from the broiler bioassay involving sorghums #3 and #5. The outcomes of these analyses for kafirin concentrations and starch digestibility should be highly instructive.

![Figure 3](attachment:image.png)

Figure 3  
Correlation between sorghum kafirin content as a proportion of protein and apparent metabolisable energy density of sorghum–based broiler diets (adapted from Salinas et al. 2006)
Salinas et al. (2006) reported significant (P < 0.01), negative correlations between kafirin proportions in sorghum protein and energy utilisation (AME: r = -0.61; TMEn: r = -0.63). These negative correlations suggest that kafirin is impeding energy utilisation and, by inference, starch digestion as shown in Figure 3. Therefore, it will be fascinating to correlate the kafirin concentrations of the six Liverpool Plains sorghums with the AME and AMEn values they supported in broiler chickens when the kafirin data comes to hand. With any luck these retrospective analyses of kafirin concentrations in the six sorghums will go some of the way to answering the $64 million sorghum question.

**Concluding remarks**

In a very recent Poultry Research Foundation feeding study broiler diets based on six diverse grain sorghum samples were assessed. Not surprisingly, all six sorghums do not possess pigmented testa and are, therefore, ‘tannin-free’. We have established the kafirin, amino acid and protein contents of these sorghums (1 white and 5 red varieties) and also their concentrations of phytate, polyphenolics compounds and phenolic acids. Consequently we are optimistic that, when all the results are to hand, this study will define how important a negative factor kafirin is in respect of sorghum starch/energy utilisation. If kafirin really is the villain that many experts believe to be the case then our sorghum breeders and geneticists should be persuaded to select for ‘low-kafirin’ sorghums.
References