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# **Retrospective analyses of retained PGLP sorghum samples**

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## Executive Summary

Nineteen grain sorghum samples for which apparent metabolisable energy (AME) in broiler chickens and apparent metabolisable energy (ADE) in pigs had been previously established by the Premium Grains for Livestock Program (PGLP) were retrospectively analysed. The retrospective sorghum analyses included gross energy, crude protein, texture (Symes PSI indices), quantification of kafirin, amino acid profiles of both sorghum protein and kafirin per se, Clorox bleach tests, quantification of polyphenols (total phenolic compounds, anthocyanins, flavan-4-ols) and phenolic acids (soluble and insoluble p-hydroxybenzoic, syringic, p-coumaric and ferulic acids) and quantification of phytate, phytate-P and ten minerals (Ca, Cu, Fe, Mg, Mn, Na, P, Sr, Zn). In addition, rapid-visco analyses (RVA) were completed to monitor starch pasting profiles of the nineteen sorghum samples and Cielab colour values were determined.

The primary objective was to analyse retained PGLP sorghum samples for selected parameters that are believed to negatively influence energy utilisation in pigs and broiler chickens offered sorghum-based diets. It was anticipated that these parameters could be correlated to both ADE in pigs and AME in poultry and that their relative importance could be established. The secondary objective was to ascertain if RVA starch pasting profiles and Cielab colour values of the retained sorghum samples are indicative of the quality of sorghum as a feed grain for pigs and poultry.

The mean energy values of the 19 PGLP sorghums were 15.98 MJ/kg AME in broiler chickens and 14.56 MJ/kg ADE in pigs with limited variation across observations. However, there was not any relationship between AME in poultry and ADE in pigs ( $r = -0.086$ ;  $P > 0.70$ ). Thus, a sorghum that would be highly suitable for one species may not be at all suitable for the other species, which is a real impediment to the development of appropriate sorghum breeding programs.

Kafirin, the dominant protein fraction in sorghum, has been shown to compromise energy utilisation in poultry, which is probably a consequence of starch-protein interactions in sorghum endosperm. However, kafirin concentrations in PGLP sorghums were not correlated ( $P > 0.20$ ) with AME in poultry or ADE in pigs. This outcome was not as anticipated and the mean kafirin concentration of 46.7 g/kg represented 48.3% of total sorghum protein. Nevertheless, the kafirin amino acid profiles of 19 sorghums are valuable data from which the case is made that the kafirin proportion of sorghum protein in local crops is escalating. This becomes evident when sorghum amino acid data are compared with two RIRDC reports published in 1998 and 2009.

Concentrations of total phenolic compounds, anthocyanins and flavan-4-ols were not correlated with AME ( $P > 0.25$ ) or ADE ( $P > 0.30$ ) across the PGLP sorghums and correlations across the phenolic acids were limited to a negative correlation ( $r = -0.616$ ;  $P < 0.01$ ) between insoluble p-hydroxybenzoic acid and AME in poultry. The overall lack of significant correlations between retrospectively analysed parameters and AME in chickens and ADE in pigs may be attributed to the high energy densities recorded in atypical, 'all-sorghum' diets coupled with low coefficients of variation.

On the other hand, Cielab colour values of sorghum were found to be strong predictors of AME in poultry (but not of ADE in pigs). Obviously, white and yellow sorghums will have quite different Cielab colour values to red sorghums. However, when only the red PGLP sorghums are considered a highly significant ( $R^2 = 0.70$ ;  $P < 0.005$ ) equation remains, in which the independent variables are all significant, as follows:

$$\text{AME}_{(\text{MJ/kg dry matter})} = 21.41 - 0.182L^* - 0.307a^* + 0.382b^*.$$

Thus, determinations of sorghum Cielab colour values may well prove to be a rapid, inexpensive means to assess the quality of sorghum as a feed grain for broiler chickens. It is almost certainly relevant that anthocyanins are red pigments and flavan-4-ols are precursors of pigmented polyphenols and the implication is that these polyphenols are compromising energy utilisation in poultry offered sorghum-based diets by negatively impacting on starch digestion and glucose absorption.

RVA starch pasting profiles of sorghum may also be predictive of energy utilisation in pigs and poultry. Across the PGLP sorghums peak RVA viscosity ( $r = -0.503$ ;  $P < 0.04$ ) and breakdown RVA viscosity ( $r = -0.472$ ;  $P < 0.05$ ) were negatively correlated with ADE in pigs. In contrast, however, peak RVA viscosity was positively correlated ( $r = 0.522$ ;  $P < 0.04$ ) with ME:GE ratios, or efficiency of energy utilisation, in broiler chickens, which is an instructive dichotomy.

Finally, it is our belief that the approach this project adopted was entirely viable but the outcomes were somewhat thwarted by the way in which the basic AME and ADE data was determined. Our contention is that the animal data should be determined by offering pigs and poultry typical, complete sorghum-based diets and that the assessed parameters should be extended to include at least growth performance in addition to energy utilisation. Such an approach should lead to far more meaningful and instructive outcomes and facilitate the selection of better sorghum varieties for animal production in Australia.

## Table of Contents

Acknowledgements	2
Executive Summary	3
1. Background to Research	9
2. Objectives of the Research Project	10
3. Introductory Technical Information	11
4. Research Methodology	12
4.1 General methodology	12
4.2 Methodology of retrospective analyses	13
5. Results	15
5.1 Results of multiple regressions	17
6. Discussion	19
6.1 RVA starch pasting profiles	19
6.2 Cielab colour values	19
6.3 Phenolic compounds	20
6.4 Kafirin	20
6.5 Phytate	21
6.6 Red versus white sorghums	22
7. Implications & Recommendations	24
8. Intellectual Property	25
9. Technical Summary	26
10. Literature cited	27
10.1 Previous publications relating to grain sorghum by the Poultry Research Foundation	30
11. Publications Arising	34
12. Appendix 1 – Tables	35
13. Appendix 2 – Figures	58

## List of Tables

Table 1 Apparent metabolisable energy (AME), ME:GE ratios in poultry and apparent digestible energy (ADE) in pigs of 19 retained PGLP sorghum samples	35
Table 2 Gross energy densities, starch and protein (N) concentrations, amylose proportions and Symes PSI textures of 19 retained PGLP sorghum samples	36
Table 3 kafirin concentrations, protein (N) concentrations, kafirin proportions of sorghum protein and kafirin index in 19 retained PGLP sorghum samples	37
Table 4 Amino acid concentrations in kafirin (g/kg) of essential amino acids in 19 retained PGLP sorghum samples	38
Table 5 Amino acid concentrations in kafirin (g/kg) of non-essential amino acids in 19 retained PGLP sorghum samples	39
Table 6 Concentrations (g/kg) of essential amino acids in 19 retained PGLP sorghum samples	40
Table 7 Concentrations (g/kg) of non-essential and total amino acids in 19 retained PGLP sorghum samples	41
Table 8 Concentrations of total phenolic compounds, anthocyanins and flavan-4-ols and Clorox bleach test in 19 retained PGLP sorghum samples	42
Table 9 Concentrations ( $\mu\text{g/g DM}$ ) of soluble (free and conjugated) phenolic acids in 19 retained PGLP sorghum samples	43
Table 10 Concentrations ( $\mu\text{g/g DM}$ ) of insoluble (bound) phenolic acids in 19 retained PGLP sorghum samples	44
Table 11 Concentrations of phytate, total phosphorus (P), phytate-P and proportion of phytate-P of total P in 19 retained PGLP sorghum samples	45
Table 12 Concentrations (mg/kg) of ten minerals in sorghum samples analysed by inductively coupled plasma optical emission spectrometry (ICP-OES) in 19 retained PGLP sorghum samples	46
Table 13 Rapid visco-analysis (RVA) starch pasting profiles of 19 retained PGLP sorghum samples	47
Table 14 Quantification of grain sorghum colour using the Hunterlab system giving $L^*$ , $a^*$ and $b^*$ measurements of 19 retained PGLP sorghum samples	48
Table 15 Coefficients and significance of individual rapid visco-analysis (RVA) of starch pasting variables in equations to predict pig digestible energy (DE) values for sorghum	49
Table 16 Coefficients and significance of phenolic compounds in equations to predict AME values for broiler chickens offered PGLP sorghums	50
Table 17 Coefficients and significance of phenolic compounds in equations to predict ADE values for pigs offered PGLP sorghums	51
Table 18 Pearson correlations between AME in poultry and ADE in pigs with Cielab colour values for PGP sorghums	52
Table 19 Pearson correlations between polyphenolic compounds and Cielab colour values of PGLP sorghums	53
Table 20 Pearson correlations between polyphenolic compounds and RVA starch pasting viscosities of PGLP sorghums	54

Table 21 Amino acid profiles of karfirin in sorghum (percentage of 15 amino acids) as reported by Xiao et al. (2015), Truong et al. (2017) and karfirin and total protein in PGLP sorghums	55
Table 22 Statistical comparison of amino acid profiles (percentage of 15 amino acids) in 1998 (n=6) and 2009 (n=11) grain sorghums	56
Table 23 Statistical comparison of selected parameters between red and white varieties of PGLP sorghums	57

## List of Figures

- Figure 1 Linear relationship ( $r=0.774$ ;  $P<0.001$ ) between kafirin index and kafirin concentrations in 19 retained PGLP sorghum samples 58
- Figure 2 Linear relationship ( $r=0.585$ ;  $P<0.015$ ) between Cielab L\* colour value and AME in poultry 58
- Figure 3 Quadratic relationships between Cielab L\* values of six red sorghums and crude protein (N) digestibility coefficients in (A) distal jejunum ( $r=0.776$ ;  $P=0.070$ ) and (B) distal ileum ( $r=0.774$ ;  $P=0.071$ ) in broiler chickens. Adapted from Khoddami et al. (2015) 59
- Figure 4 Linear relationship ( $r=0.552$ ;  $P=0.038$ ) between Peak RVA viscosity of sorghums and ME:GE ratios in poultry  $y(\text{ME:GE ratio}) = 0.901 + 0.00002 \cdot \text{peak viscosity}$  60



## **I. Background to Research**

Sorghum is the second feed grain to wheat in both pig and poultry diets in Australia and it is likely that these two meat-producing industries use more grain sorghum on a relative basis than any other country in the world. However, the performance of pigs and poultry offered sorghum-based diets is almost invariably considered inferior to wheat. Wheat-based diets are associated with better pellet quality and responses in animals to the inclusion of both phytate- and NSP-degrading feed enzymes in wheat-based diets are usually more pronounced. The protein content of sorghum is generally less than that of wheat and sorghum's amino acid profile is not as favourable. Nevertheless, the fundamental problem is thought to be related to inferior energy utilisation in pigs and poultry offered sorghum-based diets.

Extensive research completed by the Poultry Research Foundation within the University of Sydney has indicated that three factors contributing to this inferior energy utilisation are (i) kafirin, (ii) phenolic compounds and (iii) phytate. It appears that these three factors impede starch digestion and/or glucose absorption, thereby compromising energy utilisation in broiler chickens. Presumably, the same three factors are similarly operative in pigs. Kafirin is the dominant fraction in sorghum protein and is almost certainly involved in biophysical and biochemical starch-protein interactions in sorghum endosperm. Sorghum contains more phenolic compounds than other feed grains but Australian sorghum varieties do not now contain condensed tannin, a polyphenolic compound with potent anti-nutritive properties. However, together phenolic compounds and phytate, which is not unique to sorghum, appear capable of both compromising starch digestion in the gut lumen and retarding glucose absorption, via the Na<sup>+</sup>-dependent transporter, SGLT-1, from the small intestine.

The suggestion was made that retained sorghum samples, with energy values in pigs and poultry established by the Premium Grains for Livestock Production (PGLP) program, should be retrospectively analysed. Effectively this suggestion was put to the Feed Grain Partnership and this organisation kindly agreed to fund such a project.

## **2. Objectives of the Research Project**

The primary objective of the project was to analyse retained PGLP sorghum samples retrospectively for a number of selected parameters that have been shown to influence energy utilisation of sorghum in pigs and poultry. It was anticipated that these parameters could be correlated to both apparent digestible energy (ADE) in pigs and apparent metabolisable energy (AME) in broiler chickens and that their relative importance could be established. The secondary objective was to ascertain if RVA starch pasting profiles and Cielab colour values of the retained sorghum samples are indicative of the quality of sorghum as a feed grain for pigs and poultry.

### 3. Introductory Technical Information

The Poultry Research Foundation has completed a substantial amount of research into sorghum as a feed grain for chicken-meat production which has been largely funded by AgriFutures Chicken-meat (previously known as RIRDC Chicken-meat). As detailed in Section 10.1, the Poultry Research Foundation has published twenty-four research articles and four review papers in peer-reviewed scientific journals, two RIRDC Chicken-meat reports and one book chapter in relation to sorghum. A second book chapter is in press and probably will be published towards the end of 2018. Much of this research has been encapsulated in a 2018 review entitled “Outlook: Sorghum as a feed grain for Australian chicken-meat production”. This review is published in *Animal Nutrition* and the paper may be accessed at <https://doi.org/10.1016/j.aninu.2017.08.007>. The Abstract of this sorghum outlook review reads as follows and it demonstrates our interest in kafirin, ‘non-tannin’ phenolic compounds and phytate that were found to be compromising starch digestibility and energy utilisation in broiler chickens offered sorghum-based diets.

#### Abstract

The purpose of this review is to generate an outlook for sorghum as a feed grain for broiler chickens based on a survey of relevant stake-holders and recent research outcomes. The likelihood is that Australian grain sorghum production will continue to generate a harvest in the order of 2.5 million tonnes of which some 790,000 tonnes will be used as a feed grain for poultry and pigs. Wheat, the dominant feed grain, is considered to be about \$20 per tonne superior to sorghum and this price premium stems largely from the higher crude protein levels in wheat. Nevertheless, feed grains are included in pig and poultry diets primarily to provide energy from starch but energy utilisation by broiler chickens offered sorghum-based diets is relatively inferior, because of incomplete starch digestion. Kafirin, the dominant protein fraction, ‘non-tannin’ phenolic compounds and phytate are three ‘starch extrinsic’ factors in sorghum that were found to be compromising starch digestibility and energy utilisation in broiler chickens offered sorghum-based diets. Kafirin concentrations in six sorghum varieties were negatively correlated with ME:GE ratios ( $r = -0.891$ ;  $P < 0.02$ ) or the efficiency of energy utilisation in broiler chickens. Importantly, kafirin proportions of sorghum protein may be increasing with time in Australia on the basis of changes in sorghum amino acid profiles. If so, this represents a fundamental challenge to sorghum breeders which presumably could be met by the development of sorghum varieties with different characteristics, especially in relation to the  $\gamma$ - and  $\beta$ -kafirin fractions. White sorghum varieties axiomatically contain lower polyphenol concentrations which should be advantageous as concentrations of total phenolic compounds were negatively correlated to ME:GE ratios ( $r = -0.838$ ;  $P < 0.04$ ) in six sorghum varieties. Thus it would be desirable if more white varieties were to become available that were suited to local conditions. It is suggested that responses to exogenous phytase in birds offered sorghum-based diets would be more robust if sorghum were to contain lower concentrations of kafirin and phenolic compounds. Also in such sorghums starch gelatinisation temperatures should be lower and pellet quality may be enhanced as a consequence. Paradoxically, while better sorghum varieties almost certainly could be developed, it may not necessarily follow that they will command a price premium from poultry and pig producers.

## **4. Research Methodology**

### ***4.1 General methodology***

A total of 19 sorghum samples were retrieved from PBI Narrabri for which energy utilisation in broiler chickens and pigs had been established by the Premium Grains for Livestock Program (PGLP) as listed in Table 1.

This included apparent metabolisable energy (AME) for poultry and apparent digestible energy (ADE) for pigs. In addition it was possible to calculate metabolisable energy to gross energy ratios (ME:GE) for the majority of the retained sorghums where their gross energy values remained available. It was also possible to locate starch concentration and amylose proportion data for some of the sorghums, as shown in Table 2. All of the remaining tabulated data was generated by the present project. The majority of the sorghums were harvested in 2009. The retained sorghum samples, as listed below, were retrospectively analysed, as outlined in 4.2.

Then a series of statistical analyses were completed to detect, in the first instance, correlations between energy utilisation in broiler chickens (AME in MJ/kg) and pigs (ADE in MJ/kg) with the retrospectively analysed data. Relationships between retrospectively analysed data-sets were also completed. The relevant data were analysed using the IBM® SPSS® Statistics 24 program (IBM Corporation, Somers, NY), which included Pearson correlations.

Many of the measured variables likely to influence poultry or pig values for sorghum are correlated with one another and, therefore, likely to influence *in vivo* measurements of AME and ADE in complex ways. Multivariate regression procedures such as the maximum R square improvement technique provide a way to assess how combinations of independent variables influence the dependent variable of interest.

The maximum R square improvement technique (MAXR) within the REG procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was used to build a series of multivariate models to determine the best one-variable, two-variable, three-variable, etc, models. The SAS/STAT 14.3 User's Guide states that the MAXR method begins by finding the one-variable model producing the highest R square. Then another variable, the one that yields the greatest increase in R square, is added. Once the two-variable model is obtained, each of the variables in the model is compared to each variable not in the model. For each comparison, the MAXR method determines if removing one variable and replacing it with the other variable increases R square. After comparing all possible switches, the MAXR method makes the switch that produces the largest increase in R square. Comparisons begin again, and the process continues until the MAXR method finds that no switch could increase R square. Thus, the two-variable model achieved is considered the "best" two-variable model the technique can find. Another variable is then added to the model, and the comparing-and-switching process is repeated to find the "best" three-variable model, and so forth.

The investigation commenced by allowing all measurements of sorghum grain to enter the model in order to gauge those variables associated with largest variation in AME and ADE. In general, AME and ADE differed in the strongest combinations of measurements affecting variation. It also became clear that measurements of grain colour, rapid visco-analysis (RVA) of starch pasting and various phenolic compounds were key combinations of variables for further statistical analysis. Henceforth,

attention was focussed on how well grain colour, RVA and phenolic compounds, respectively, predicted AME and ADE.

#### ***4.2 Methodology of retrospective analyses***

Gross energy (GE) values of the sorghum samples, as shown in Table 2 were determined with a Parr 1281 adiabatic bomb calorimeter. (Parr Instrument Company IL). Nitrogen (N) concentrations in the sorghum samples were analysed with a N determinator (Leco Corporation. Saint Joseph, MI). Nitrogen concentrations were expressed as crude protein ( $N \times 5.81$ ) concentrations using the nitrogen-to-protein conversion factor of 5.81, as recommended by Mosse et al. (1988). Grain texture was assessed using the Symes particle size index (PSI) index as described in Symes (1965).

Kafirin concentrations (Table 3) were quantified by procedures developed by Dr Karlie Nielsen in the Australian Proteome Analytical Facility (Macquarie University). These quantification procedures were adapted from methodologies described in Wallace et al. (1990) and Hamaker et al. (1995) and were thoroughly outlined in Truong et al. (2015). The 'kafirin index' is derived from a basic calculation where the sum concentration of basic amino acids (arginine, histidine, lysine) in sorghum is subtracted from that of leucine. It is based on the premise that kafirin contains more leucine but less basic amino acids than total sorghum protein (Selle et al., 2010).

In this project, concentrations of sixteen amino acids in kafirin per se and total sorghum protein (Tables 4 to 7) were determined following 24 h liquid hydrolysis at 110°C in 6 M HCl and amino acids were analysed with a Waters ACQUITY Ultra Performance Liquid Chromatography system. The methodology followed is outlined in Cohen and Michaud (1993) and Cohen (2001).

Analytical methods to quantify plant phenolic compounds (Tables 8 to 10) were reviewed by Khoddami et al. (2013) and the specific methods used in this project were fully described in Khoddami et al. (2015). The presence or absence of a pigmented testa was determined by the Clorox bleach test (Waniska et al., 1992). Sorghum grain (15 g) was mixed with 7.5 g KOH and 70 ml NaOCl solution (bleach) with constant stirring at 60°C for 7 min, rinsed and washed with cold water. The grains that turned white or yellow were classified as Type I or tannin-free sorghums; whereas, a black colour would indicate the presence of condensed tannin. Total phenolic compounds were measured using the modified Folin–Ciocalteu method of Kaluza et al. (1980).

Results were expressed as gallic acid equivalents (GAE) in a mg/g dry matter basis. Anthocyanins were determined according to the method of Fuleki and Francis, 1968. The absorbance was read at 485 nm and reported as absorbance per millilitre (Abs/ml) per gram of dry weight sample. Flavan-4-ol contents were determined using the method described in Gous (1989). Again, results were expressed as absorbance per millilitre (Abs/ml) per gram of dry weight sample. Analyses of phenolic acids were completed on an Agilent 1200 series HPLC (Agilent 1200 series) equipped with photodiode array and auto-sampler as described in Chiremba et al. (2012). Analyses of phenolic acids were completed on an Agilent 1200 series HPLC (Agilent 1200 series) equipped with photodiode array and auto-sampler as described in Chiremba et al. (2012). The extraction of phenolic acids was completed in duplicate, following the procedures outlined in Li et al. (2008) HPLC analysis of phenolic acids in either soluble (free or conjugated) or insoluble (bound) forms was carried out on a Agilent 1200 series HPLC equipped with photodiode array (PDA) and auto-sampler (Agilent 1200 series) as described by Chiremba et al. (2012).

Concentrations of phytate and phytate-P (Table 11) were determined by the classic ferric chloride-precipitation method as described by Miller et al. (1980). This method is based on the principle that ferric ions form a stable complex with phytate in dilute acidic solutions. The concentration of phosphorus in IP6 phytate (myo-inositol hexaphosphate) is 282 g/kg and phytate-P was converted to phytate on this basis.

The concentrations of ten minerals, including phosphorus, calcium and sodium, were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES). These values are shown in Table 12.

The determination of starch pasting profiles by rapid visco-analysis (RVA) of starch pasting was originally developed in Australia to evaluate rain-damaged wheat (Walker et al., 1988) and the results are shown in Table 12. Descriptions of the methodology and interpretation of starch pasting profiles have been provided by Atwell et al. (1988) and Batey (2007). However, there is the contention, as advanced by Truong et al. (2017), that RVA starch pasting profiles may be a reasonably rapid and accurate means to assess the quality of sorghum as a feed grain. It is for this reason that the RVA profiles of the retained sorghum samples were determined. The starch pasting properties of sorghum were determined with a RVA-4 Rapid-Visco-Analyzer (Newport Scientific Pty Ltd. Warriewood, NSW). following procedures outlined by Beta and Corke (2004). Ground sorghum grain (4.2 g) was mixed with deionised water (23.8 g) of water in a programmed heating and cooling cycle of 13 minutes. The slurry was held at a temperature of 50°C for 1 minute and then heated to 95°C and held for 2.5 minutes prior to cooling the slurry to 50°C and holding that temperature for 2 minutes. The speed of the mixing paddle was 960 rpm for 10 seconds and then 160 rpm for the remainder of the cycle. Peak viscosity, holding viscosity, final viscosity, breakdown viscosity (peak – holding) and setback viscosity (final – peak) were recorded as were peak time and pasting temperature. Two replicates per sample were analysed.

The Cielab colour values of sorghum grain, as shown in Table 14, were determined using a Minolta CR-310 Colorimeter (Minolta Co. Ltd., Osaka, Japan) and measurements were expressed as Commission Internationale de l'Eclairage (CIELAB) L\*, a\*, and b\* values. A high L\* value is indicative of white opposed to black, a high a\* value is indicative of red opposed to green and a high b\* value is indicative of yellow opposed to blue.

## 5. Results

Apparent metabolisable energy, ME:GE ratios in poultry and apparent digestible energy in pigs are shown in Table 1. The mean values are 15.98 MJ/kg, 0.949 and 14.56 MJ/kg, respectively. However, there is little variation between observations with coefficients of variation of less than 3%. Predictably, AME and ME:GE ratios are correlated ( $r = 0.603$ ;  $P < 0.015$ ) but, somewhat surprisingly, there is no relationship between AME in poultry and ADE in pigs ( $r = -0.086$ ;  $P > 0.70$ ). It should be noted that the AME of 15.98 MJ/kg and the ME:GE ratio of 0.949 recorded with 'all-sorghum' diets are substantially higher than values that have been observed in conventional, complete sorghum-based broiler diets. For example, Truong et al. (2016) reported ranges in AME from 11.78 to 12.26 MJ/kg and in ME:GE ratios from 0.703 to 0.751 in broilers offered conventional diets based on six diverse sorghum varieties containing 620 g/kg of the feed grain.

Gross energy densities, starch and protein (N) concentrations, amylose proportions and textures of the retained sorghums are shown in Table 2. The mean crude protein content was 97.3 g/kg within a range from 81.6 to 120.9 g/kg and the mean gross energy density was 16.25 MJ/kg. The mean grain texture (Symes PSI) was 11.1 which ranged from 6 to 18 with a high 34% coefficient of variation. Under the Symes categories, four sorghums were "extra hard" (up to 7), nine sorghums were "very hard" (8-12), 5 sorghums were "hard" (13-16) and one sorghum was medium hard (17-20). In previous PRF surveys the vast majority of sorghums uniformly fell into the "very hard" category; whereas, in this survey 32% of sorghums were "softer" with PSI values of greater than 12. However, PSI values or grain sorghum texture was not correlated ( $P > 0.15$ ) with AME, ME:GE ratios or ADE. The first 9 sorghums had a mean starch concentration of 751 g/kg with an amylose proportion of 34.4% and these values are almost certainly more indicative than all 15 values. Interestingly, there was a highly significant negative correlation between the amylose proportion of starch and AME ( $r = -0.955$ ;  $P < 0.001$ ) across these nine sorghums. Gross energy of the PGLP sorghums per se were not correlated with AME in poultry ( $r = 0.032$ ;  $P > 0.85$ ) or ADE in pigs ( $r = 0.035$ ;  $P < 0.85$ ). Similarly, crude protein of the PGLP sorghums were not correlated with AME in poultry ( $r = 0.052$ ;  $P > 0.80$ ) or ADE in pigs ( $r = 0.277$ ;  $P < 0.25$ ).

Kafirin concentrations, kafirin proportions of sorghum protein and the kafirin index are recorded in Table 3. The mean kafirin concentration was 46.7 g/kg, which ranged from 33.0 to 56.7 g/kg and corresponded to 48.3% of total sorghum protein (97.3 g/kg). These outcomes are remarkably similar to those reported by Taylor et al. (1984) who found an average kafirin concentration of 54.0 g/kg in 42 sorghums with an average protein content of 110 g/kg or a kafirin proportion of 48.0%. The kafirin index is simply the leucine concentration less the sum of the basic amino acids (arginine, histidine, lysine) and may be a simple way to estimate the quantity of kafirin in sorghum. In fact, there is a linear relationship ( $r = 0.774$ ;  $P < 0.001$ ) between kafirin indices and kafirin concentrations across the PGLP sorghums as shown in Figure 1. However, kafirin concentrations were not correlated ( $P > 0.20$ ) with AME, ME:GE ratios or ADE across the PGLP sorghums.

Essential and non-essential amino acid concentrations in kafirin per se in retained PGLP sorghum samples are shown in Tables 4 and 5. The amino acid profiles of kafirin that may be deduced from 19 sorghums numerically exceed any previous surveys and are in good agreement with previously published data (Salunkhe et al., 1977; Mosse et al., 1988; Xiao et al., 2015; Truong et al., 2016).

The concentrations of essential and non-essential amino acids in the retained grain sorghum samples are shown in Tables 6 and 7. The amino acid profiles of sorghum protein in the present study are in

very close agreement with those recorded by Jambunathan and Mertz (1973) which were based on the analyses of 522 sorghum samples by Purdue University. When the amino acid profiles of kafirin and sorghum are compared in the present study there is noticeable differences in the reductions of several amino acids including lysine (0.47), methionine (0.54), serine (0.72), threonine (0.76), histidine and arginine (0.82). The importance of lysine and methionine to both pigs and poultry does not require any emphasis but underlines the inadequate protein quality of sorghum.

The concentrations of total phenolic compounds, anthocyanins and flavan-4-ols in retained sorghums are shown in Table 8. The mean value of total phenolics was 3.30 mg GAE/g, anthocyanins was 4.52 abs/ml/g and flavan-4-ols was 1.74 abs/ml/g, with considerable variation for each of the three categories. By way of comparison, Truong et al. (2016) reported average values of 3.74 mg GAE/g for total phenolics, 5.58 abs/ml/g for anthocyanins and 4.61 abs/ml/g for flavan-4-ols in six diverse sorghum varieties. Similarly, Khoddami et al. (2015) reported mean values of 3.38 mg GAE/g for total phenolics, 9.00 abs/ml/g for anthocyanins and 3.81 abs/ml/g for flavan-4-ols in six sorghum varieties harvested on the Liverpool Plains in 2009. Thus the PGLP sorghums contained lesser anthocyanins and flavan-4-ols concentrations than these two reports. However, concentrations of total phenolic compounds anthocyanins and flavan-4-ols were not correlated with AME, ( $P > 0.25$ ), ME:GE ratios ( $P > 0.20$ ) or ADE ( $P > 0.30$ ) across the PGLP sorghums. Unsurprisingly, all sorghums were negative to the Clorox bleach test, which means that they did not possess a pigmented testa nor did they contain condensed tannin.

The concentrations of soluble (free and conjugated) and insoluble (bound) phenolic acids in retained sorghum samples are shown in Tables 9 and 10. Collectively, mean total concentrations were 26  $\mu\text{g/g}$  for p-hydroxybenzoic acid, 7.2  $\mu\text{g/g}$  for syringic acid, 98  $\mu\text{g/g}$  for p-coumaric acid and 471  $\mu\text{g/g}$  for ferulic acid. Again, by way of comparison Khoddami et al. (2015) reported mean values 33  $\mu\text{g/g}$  for p-hydroxybenzoic acid, 21  $\mu\text{g/g}$  for syringic acid, 50  $\mu\text{g/g}$  for p-coumaric acid and 415  $\mu\text{g/g}$  for ferulic acid. Also, Truong et al. (2016) found average values of 25  $\mu\text{g/g}$  for p-hydroxybenzoic acid, 17  $\mu\text{g/g}$  for syringic acid, 46  $\mu\text{g/g}$  for p-coumaric acid and 319  $\mu\text{g/g}$  for ferulic acid. Thus the values reported are in broad agreement with ferulic acid clearly being dominant. However, concentrations of soluble phenolic acids were not correlated with AME, ( $P > 0.13$ ), ME:GE ratios ( $P > 0.25$ ) or ADE ( $P > 0.10$ ) across the PGLP sorghums. There was, however, a significant negative correlation ( $r = -0.616$ ;  $P < 0.01$ ) between insoluble p-hydroxybenzoic acid and AME in poultry. There was also a significant negative correlation ( $r = -0.507$ ;  $P < 0.05$ ) between total p-coumaric acid and ME:GE ratios in poultry.

The concentrations of phytate or phytate-P and proportions of phytate-P relative to total P in retained sorghums are shown in Table 11. On average, sorghum contained 1.84 g/kg phytate-P or 6.53 g/kg IP6 phytate and phytate-P represented 69.2% of total P. These average values are noticeably lower than those recorded in the earlier Selle et al. (2003) survey of 15 sorghums where the mean phytate-P level was 2.41 g/kg, which represented 82.7% of total P in sorghum. However, concentrations of soluble phytate were not correlated with AME, ( $P > 0.80$ ), ME:GE ratios ( $P > 0.45$ ) or ADE ( $P > 0.45$ ) across the PGLP sorghums.

Concentrations of ten minerals in sorghum samples analysed by ICP-OES are shown in Table 12. The variation in Na levels is noteworthy as Na concentrations ranged from 2.7 to 13.5 mg/kg around a mean value of 6.9 mg/kg. The coefficient of variation was 53% but we have found even higher variations in a previous survey. Curiously, copper concentrations, which averaged 3.00 mg/kg, were



negatively correlated with AME ( $r = -0.499$ ;  $P < 0.04$ ) and ME:GE ratios ( $r = -0.5651$ ;  $P < 0.03$ ) in poultry and AME was positively correlated with manganese ( $r = 0.515$ ;  $P < 0.03$ ), which averaged 15.0 mg/kg across the PGLP sorghums. On the other hand, zinc, with an average concentration of 17.8 mg/kg, was negatively correlated ( $r = -0.859$ ;  $P < 0.02$ ) with ADE in pigs.

The rapid visco-analysis (RVA) starch pasting profiles of 19 retained sorghum samples are shown in Table 13. The mean peak, holding and final RVA viscosities were 2,166, 1,802 and 4,126 cP, respectively. These values are noticeably less than the corresponding mean viscosities of 4,280, 2,969 and 5,958 cP recorded in 13 grain sorghum varieties by Truong et al. (2017). In poultry, peak RVA viscosity was positively correlated ( $r = 0.522$ ;  $P < 0.04$ ) with ME:GE ratios. Alternatively, in pigs, peak RVA viscosity ( $r = -0.503$ ;  $P < 0.04$ ) and breakdown RVA viscosity ( $r = -0.472$ ;  $P < 0.05$ ) were negatively correlated with ADE.

Quantification of grain sorghum colour for the parameters  $L^*$ ,  $a^*$  and  $b^*$  are shown in Table 14 where the overall mean  $L^*$  value for lightness was 39.2 (where 100 = white and 0 = black). However, the three white (Liberty) and one yellow (Karper) sorghum had an average  $L^*$  value of 52.8 as opposed to the average of 35.6 for fifteen red varieties. This  $L^*$  value ranged from 29.6 to 40.2 for red sorghums; whereas, one white sorghum (Liberty 56) had an  $L^*$  value of 58.7. There were not any significant correlations ( $P < 0.25$ ) between sorghum colour scores and ADE in pigs. However,  $L^*$  values were positively correlated ( $r = 0.585$ ;  $P < 0.02$ ) with AME in poultry and the  $b^*$  value approached significance ( $r = 0.446$ ;  $P = 0.064$ ). Alternatively, the  $a^*$  value was negatively correlated ( $r = -0.655$ ;  $P < 0.005$ ) with AME in poultry.

### **5.1 Results of multiple regressions**

Grain colour was a strong predictor of AME. The equation involving all sorghums was highly significant ( $R^2 = 0.61$ ;  $P = 0.0054$ ) with all independent variables being significant ( $P < 0.05$ ):

$$\text{AME(MJ/kg dry matter)} = 18.15 - 0.091L^* - 0.143a^* + 0.213b^*$$

The equation involving only red sorghums was also highly significant ( $R^2 = 0.70$ ;  $P = 0.0031$ ;) with all independent variables being significant ( $P < 0.05$ ):

$$\text{AME(MJ/kg dry matter)} = 21.41 - 0.182L^* - 0.307a^* + 0.382b^*$$

Grain was not a significant predictor of ADE when all sorghums were included ( $P = 0.31$ ), nor when only red sorghums were considered ( $P = 0.13$ ).

RVA was not a significant predictor of AME when all sorghums were included ( $P = 0.98$ ), nor when only red sorghums were considered ( $P = 0.13$ ). In contrast, RVA was a strong predictor of ADE. The 7-variable equation involving all sorghums was highly significant ( $P = 0.0023$ ;  $R^2 = 0.87$ ). The equation involving only red sorghums was also highly significant ( $P = 0.00911$ ;  $R^2 = 0.88$ ). The coefficients and significance of individual variables are shown in the Table 15.

Multiple regression involving all phenolic compounds resulted in significant 5-variable ( $P = 0.0002$ ;  $R^2 = 0.86$ ) and 10-variable ( $P = 0.03$ ;  $R^2 = 0.96$ ) equations for AME and ADE, respectively, in which all independent variables were significant ( $P < 0.05$ ). The coefficients and significance of individual variables are shown in the Tables 16 and 17.

The 1-variable equation involving insoluble benzoic acid weakly predicted AME ( $P = 0.02$ ;  $R^2 = 0.32$ ), but higher order equations were not significant ( $P > 0.05$ ). Insoluble phenolic acids had no significant effects on ADE ( $P > 0.05$ ). Further analysis of soluble phenolic acids resulted in no significant equations for either AME or ADE. Similarly, anthocyanins and flavan-4-ols had no predictive power for AME or ADE.

## 6. Discussion

The relationship between AME in poultry and ADE in pigs across the retained PGLP sorghum samples was not significant ( $P = 0.741$ ). The relative availability of energy in sorghum is approximately 14% higher in chickens compared with pigs. Given the very real differences in the anatomy and physiology of the digestive tracts of these two animal species this is perhaps not surprising. As explained by Black (2016), pigs masticate their feed poorly, and unless the grain is processed before ingestion, large quantities of starch, protein, and fiber in grain particles can be fermented in the hind gut. In contrast, poultry have a gizzard within their digestive tract, and the intense muscular contractions of this organ are extremely effective for physically breaking the grain into small particles and disrupting the integrity of the endosperm cell walls and the protein matrix.

### 6.1 RVA starch pasting profiles

The lack of an AME-ADE relationship contributes to the differences observed between pigs and poultry in some of the parameters considered. For example, peak RVA viscosity of the PGLP sorghums was negatively correlated with ADE in pigs ( $r = -0.503$ ;  $P = 0.033$ ) as was breakdown RVA viscosity ( $r = -0.472$ ;  $P = 0.048$ ). RVA viscosities were not correlated with AME in poultry; however, peak RVA viscosity was positively correlated with AME in poultry ( $r = 0.522$ ;  $P = 0.038$ ) and the relationship with breakdown RVA viscosity ( $r = 0.473$ ;  $P = 0.064$ ) approached significance. The likelihood is that ME:GE ratios are more indicative of energy utilisation in poultry than AME outcomes. Truong et al. (2017) advanced the case for RVA starch pasting profiles to gauge the quality of sorghum as a feed grain for chicken-meat production. ME:GE ratios were positively correlated to peak ( $P = 0.001$ ), holding ( $P = 0.001$ ), breakdown ( $P = 0.003$ ) and final ( $P = 0.002$ ) RVA viscosities to significant extents in a series of five broiler feeding studies involving nine sorghum varieties. Thus a sorghum with especially a high peak RVA viscosity is likely to be a good feed grain for poultry but a poor feedstuff for pigs, which is a salient dichotomy.

### 6.2 Cielab colour values

Interestingly, this dichotomy extends to Cielab colour values for sorghum. As shown in Table 18, both  $L^*$  and  $b^*$  colour values were significantly correlated with AME in poultry but this was not the case with ADE in pigs. In turn,  $L^*$  values were negatively correlated with concentrations of both anthocyanins ( $r = -0.540$ ;  $P < 0.02$ ) and flavan-4-ols ( $r = -0.498$ ;  $P < 0.04$ ); whereas,  $a^*$  values were positively correlated with anthocyanins ( $r = 0.459$ ;  $P < 0.05$ ) and flavan-4-ols ( $r = 0.458$ ;  $P < 0.05$ ) in the PGLP sorghums, as shown in Table 19. Therefore, it is relevant Dykes et al. (2005) found significant correlations between Cielab colour values and phenolic compounds across 13 sorghum genotypes. For example, the  $L^*$  value was negatively correlated to total phenols ( $r = -0.69$ ;  $P < 0.01$ ), flavan-4-ols ( $r = -0.84$ ;  $P < 0.001$ ), luteolinidin ( $r = -0.6i$ ;  $P < 0.05$ ) and apigeninidin ( $r = -0.62$ ;  $P < 0.05$ ). As discussed further in the next section, the implication is that phenolic compounds in sorghum are influencing both grain colour and energy utilisation in birds offered sorghum-based diets. It is also relevant that Khoddami et al. (2015) assessed Cielab colour values of six red sorghum varieties harvested on the Liverpool Plains of NSW in 2009. These researchers found that the  $L^*$  colour value of these six sorghums tended to be positively related to protein (N) digestibility coefficients in the distal jejunum and distal ileum in a linear manner in broiler chickens offered sorghum-casein diets. The corresponding quadratic relationships are displayed in Figure 3.

Clearly, white sorghums will have different Cielab colour values than red varieties. Indeed the 3 white PGLP sorghums had significantly higher L\* scores (54.4 versus 35.6;  $P < 0.001$ ) and lower a\* scores (3.5 versus 14.4;  $P < 0.001$ ) than the 15 red sorghums, as shown in Table 23. However, when only the red sorghum varieties are considered the relationships between the Cielab colour values for sorghum and AME in poultry remain significant. Indeed, all three colour values are significantly related including L\* ( $r = 0.589$ ;  $P < 0.02$ ), a\* ( $r = -0.585$ ;  $P < 0.02$ ) and b\* ( $r = 0.523$ ;  $P < 0.04$ ). Moreover, there is a significant multiple linear regression ( $r = 0.785$ ;  $P < 0.01$ ) between AME and Cielab colour values where the relevant equation is as follows:  
$$\text{AME(MJ/kg dry matter)} = 19.132 + 0.268*b^* - 0.128*L^* - 0.168*a^*$$

The vast majority of sorghums grown in Australia, perhaps more than 95%, are red. Nevertheless, it appears that Cielab colour values could be used to predict the value of a red sorghum as a feed grain for poultry on the basis of energy utilisation.

### **6.3 Phenolic compounds**

Phenolic compounds are a diverse group of phytochemicals ranging from highly-polymerised inert lignins to simple phenolic acids (Mangan, 1988) and sorghum contains higher concentrations of phenolic compounds than other feed grains (Bravo, 1998). Condensed tannin is a polyphenolic compound with potent anti-nutritive properties but it is highly improbable that it is present in contemporary Australian sorghum crops as confirmed by the negative Clorox bleach tests for all the PGLP sorghums. However, our contention is that other, 'non-tannin' phenolic compounds in sorghum possess anti-nutritive properties. Instructively, Taylor (2005) concluded that grain sorghum cultivars contain higher levels of phenolic compounds than other cereals and red (non-tannin) sorghums are highly pigmented with polyphenols, including anthocyanins, and these phenols bind strongly to starch. Phenolic compounds are more likely to form starch-phenolic complexes with amylose than amylopectin (Tomasik and Schilling, 1998). The interactions between phenolic compounds and starch have been investigated in some detail by Yu et al. (2001), Kandil et al. (2012) and Zhu (2015).

Given the above it is noteworthy that anthocyanins and flavan-4-ols in the PGLP sorghums were negatively correlated to L\* colour scores and positively correlated to a\* colour scores as discussed above. This is entirely consistent with the facts that anthocyanins are red phenolic pigments and flavan-4-ols are precursors of phenolic pigments. It is also interesting that total phenolic compounds are negatively correlated with peak, holding, final and setback RVA paste viscosities in the PGLP sorghums as shown in Table 20. That is total phenolic compounds depressed these RVA parameters which probably indicates that interactions between phenolic compounds and starch were taking place during the starch pasting process and depressed RVA starch pasting profiles hold negative implications for the quality of sorghum as a feed grain for poultry.

### **6.4 Kafirin**

Kafirin concentrations in PGLP sorghums were not correlated with AME ( $P > 0.20$ ) or ME:GE ratios ( $P > 0.40$ ) in poultry or ADE ( $P > 0.20$ ) in pigs, which was not the anticipated outcome. Compelling direct evidence that kafirin compromises energy utilisation in broiler chickens was generated by Truong et al. (2015). Two red sorghums were compared as the basis of conventional broiler diets;

kafirin concentrations in sorghums per se were 50.7 and 61.5 g/kg but this translated to dietary kafirin concentrations of 29.0 and 39.6 g/kg. The sorghum with the lesser kafirin concentration supported superior AME by 1.06 MJ (13.31 versus 12.55 MJ/kg), ME:GE ratios by 4.81% (0.800 versus 0.769) and AMEn by 1.03 MJ (12.38 versus 11.35 MJ/kg) and these differences were attributed to the kafirin differential. It is largely accepted that biophysical and biochemical starch-protein interactions involving kafirin protein bodies and starch granules in sorghum endosperm compromise starch digestion and energy utilisation in animals offered sorghum-based diets.

The amino acid profiles of kafirin, expressed as a percentage of a total of 15 amino acids are shown in Table 21. The kafirin profiles reported by Xiao et al. (2015) and Truong et al. (2017) are compared with the mean values of the nineteen PGLP sorghums and the three data-sets are in close agreement. Then the amino acid profiles of kafirin per se are compared with the amino acid profiles of total protein in the PGLP sorghums on the same basis. It has been suggested that kafirin, as a proportion of total protein, has been increasing in local sorghum crops (Selle, 2011). This is probably an inadvertent outcome of breeding programs as selection has targeted red sorghums with relatively dense or corneous endosperms in a quest to enhance grain weathering resistance (Henzell, 1992). Importantly, it is almost axiomatic that selecting sorghums with hard, corneous endosperms will lead to higher kafirin concentrations. Instructively, the texture or 'hardness' of Australian sorghums are relatively high by international standards.

As shown in Table 22, Ravindran et al. (1998) and Bryden et al. (2009) completed amino acid analyses of 17 sorghum varieties in projects funded by RIRDC Chicken-meat. When the two amino acid profiles are compared statistically there are differences in the relative quantities that were either significant or approached significance for four amino acids. The 2009 sorghums contained less arginine by 11.9% (4.00 versus 4.54%;  $P = 0.001$ ), more leucine by 11.9% (15.52 versus 15.02%;  $P = 0.018$ ), less methionine 12.4% (1.69 versus 1.90%;  $P = 0.041$ ) and more glutamic acid by 4.27% (4.00 versus 4.54%;  $P = 0.084$ ) than the 1998 sorghums. This is in agreement with differences in the amino acid profiles of kafirin or total protein across the PGLP sorghums where kafirin contained 17.6% less arginine, 9.29% more leucine, 45.4% less methionine and 4.97% more glutamic acid than total sorghum protein. Thus these differences between 1998 and 2009 sorghums are entirely consistent with the proposition that kafirin concentrations in local sorghums are increasing. Given that kafirin is an important limitation to starch/energy utilisation in broilers offered sorghum-based diets, breeding programs should develop new directions to reverse this trend as a priority (Selle et al., 2018).

### **6.5 Phytate**

Phytate concentrations in PGLP sorghums were not correlated with AME or ME:GE ratios in poultry or ADE in pigs to significant extents. Sorghum contains at least as much phytate, or phytate-P, as other feed grains on the basis of a survey of local feedstuffs (Selle et al., 2003). A total of 15 sorghum varieties contained 2.92 g/kg total P and 2.41 g/kg phytate-P in this survey which are higher than the corresponding values of 2.67 g/kg total P and 1.84 g/kg phytate-P found in the PGLP sorghums.

Not surprisingly, phytate concentrations were correlated with P ( $r = 0.891$ ;  $P < 0.001$ ) but they were also correlated with Mg ( $r = 0.902$ ;  $P < 0.001$ ) and K ( $r = 0.786$ ;  $P < 0.001$ ) to highly significant extents and Fe ( $r = 0.601$ ;  $P < 0.01$ ). This lends weight to the assertion of Lott et al. (2000) that IP6 phytate is predominantly present in feedstuffs as a mineral-phytate complex linking three  $Mg^{2+}$  and six  $K^{+}$  ions with the polyanionic IP6 phytate molecule. It is also noteworthy that phytate was

correlated with total phenolic compounds ( $r = 0.621$ ;  $P < 0.005$ ) and total ferulic acid ( $r = 0.523$ ;  $P < 0.025$ ). That this is the case may stem from the fact that both phytate and phenolic compounds are located in the periphery of sorghum grain. Interestingly, it has been proposed that phytate and phenolic compounds share analogous anti-nutritive properties (Selle et al., 2010). One example is that both phytate and phenolic compounds have been found to reduce blood glycaemic indices in humans (Thompson et al., 1984, 1987). While speculative, this outcome could be attributed to phytate and phenolic compounds impeding intestinal uptakes of glucose along the small intestine. Glucose and Na are co-absorbed via the Na<sup>+</sup>-dependent transporter, SGLT-1, which is driven by the activity of the sodium pump (Na<sup>+</sup>/K<sup>+</sup>-ATPase) located in the baso-lateral membrane of enterocytes. Therefore, it is relevant that both phenolic compounds (Welsch et al., 1989) and phytate (Dilworth et al., 2005) have been reported to retard sodium pump activity. Finally, phytate was negatively correlated with final RVA viscosity ( $r = -0.533$ ;  $P < 0.025$ ) and setback RVA viscosity ( $r = -0.532$ ;  $P < 0.025$ ), which implies that phytate is interacting with starch and this has negative ramifications for poultry.

### ***6.6 Red versus white sorghums***

Local anecdotal field evidence suggests that white sorghums (Liberty) are better feed grains for pigs and poultry than red sorghum varieties. This appears to be valid for poultry as two white sorghums generated higher AME values than fifteen red sorghums by 0.67 MJ (16.53 versus 15.86 MJ/kg) and one white sorghum generated higher ME:GE ratios than fourteen red sorghums by 4.44% (0.988 versus 0.946) in this project. Alternatively, there was not any difference in ADE values for pigs (14.58 versus 14.56 MJ/kg) between two white and fifteen red varieties.

Given the above, a statistical comparison of selected parameters between red ( $n = 15$ ) and white ( $n = 3$ ) PGLP sorghums was completed as shown in Table 23. Anthocyanin is a red polyphenolic pigment and predictably its concentration in white sorghums was significantly 65% lower than in red sorghums. Also, white sorghums tended ( $P < 0.10$ ) to have lower concentrations of total phenolic compounds by 28% and flavan-4-ols by 63%; the latter is not surprising in that flavan-4-ols are precursors of polyphenolic pigments. While unrelated to colour, it is noteworthy that white sorghums contained significantly lower concentrations of insoluble or bound ferulic acid.

Both crude protein and kafirin contents of red and white sorghums were similar. Interestingly, kafirin represented 54.8% of sorghum protein in white sorghums as opposed to 46.9% in red sorghums and this difference was significant ( $P < 0.01$ ). In contrast, the phytate-P proportions of total P in red and white sorghums were nearly identical.

The RVA starch viscosities of white sorghums were invariably of numerically greater magnitudes than red sorghums. As an example the peak RVA viscosity of white sorghums was 24% (2563 versus 2059 cP) than red sorghums and this difference approached significance ( $P < 0.08$ ). Across the PGLP sorghums there was no relationship ( $P > 0.50$ ) between peak RVA viscosity and AME in poultry but there was a negative relationship between peak RVA viscosity and ADE in pigs ( $r = -0.503$ ,  $P = 0.033$ ) which was significant. However, there was a significant, positive relationship ( $r = 0.522$ ;  $P = 0.038$ ) peak RVA viscosity and ME:GE ratios in poultry as illustrated in Figure 4. Truong et al. (2017) reported that peak, holding, breakdown and final RVA viscosities of sorghums were positively correlated with ME:GE ratios across five studies. In this meta-analysis, peak RVA viscosity was

positively correlated with ME:GE ratio ( $r = 0.810$ ;  $P = 0.001$ ), AMEn ( $r = 0.588$ ;  $P = 0.035$ ), N retention ( $r = 0.587$ ;  $P = 0.035$ ) and AME ( $r = 0.475$ ;  $P = 0.101$ ).

## 7. Implications & Recommendations

Our recommendation would be that the investigation of three indicators as predictors of sorghum quality as a feed grain for chicken-meat production should be pursued. The three indicative systems comprising (i) Cielab colour values, (ii) RVA starch pasting profiles and (iii) Promatest protein solubilities. Such an evaluation could be completed for pigs but, given the differences between the two species, the two projects would be best kept separate.

We feel that this project was entirely satisfactory in respect of determining sorghum characteristics in the laboratory but the evaluation of sorghums in broiler chickens could be improved substantially. Our contention is that the sorghums being evaluated should be included in complete, nutritionally equivalent diets rather than atypical 'sorghum-only' diets. Also the parameters determined in poultry should be extended from just AME to include growth performance (weight gain, feed intake, FCR), nutrient utilisation (AME, ME:GE ratios, N retention, AMEn) and, ideally, apparent digestibility coefficients and disappearance rates of starch and protein/amino acids.

From our experience, the likelihood is that there will be more variation in performance parameters when birds are offered typical diets than was the case with the PGLP data for AME where there was very little variation. In this project the mean AME was 15.98 MJ/kg with a 2.82% coefficient of variation and the mean ME:GE ratio was 0.949 with a 2.13% coefficient of variation. By way of contrast, the Poultry Research Foundations has completed six studies involving 10 sorghum varieties and 19 observations in which birds were offered typical sorghum-based diets. Across these feeding studies, the mean AME was 12.45 MJ (range: 11.50 to 13.61) with a 5.00% coefficient of variation and the mean ME:GE ratio was 0.750 (range: 0.690 to 0.827) with a 5.60% coefficient of variation. Very clearly, the energy values stemming from birds offered typical diets were of a lower order with greater variation. The limited variation in the PGLP data for AME and ADE was a fundamental obstacle in the current project.



## **8. Intellectual Property**

We do not believe any issues in respect of intellectual property have arisen from the research conducted.

## 9. Technical Summary

The major outcome of this project in the sense of a 'discovery' was the finding that Cielab colour values of sorghums were indicative of the quality of sorghum as a feed grain for broiler chickens in terms of energy utilisation. Moreover, Cielab colour values remained indicative when only red sorghum varieties were considered. That sorghum Cielab colour values are indicative of energy utilisation in poultry appears to be linked to concentrations of phenolic compounds in the feed grain. Even in the absence of condensed tannin, it is our contention that phenolic compounds, including flavan-4-ols and ferulic acid, have deleterious effects in broiler chickens which may stem mainly from compromised starch digestion and glucose absorption. Also, it is our understanding that an overseas university has been evaluating Cielab colour values for grain sorghum in poultry with promising outcomes. The likelihood is that Cielab colour values could be used in practice as a rapid and inexpensive means to predict the value of sorghum as a feed grain for poultry.

Our group quite recently completed two AgriFutures sorghum-related projects (PRJ-007639 and PRJ-008695) in which a number of sorghum varieties were extensively evaluated. The Cielab colour values for ten of these sorghums were determined and our intention is to complete a retrospective investigation of the six relevant feeding studies to examine the relationships between sorghum Cielab colour values and growth performance, nutrient utilisation, starch and protein (N) digestibility coefficients in birds offered conventional, nutritionally equivalent sorghum-based diets.

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## **11. Publications Arising**

It certainly is our intention to submit one, and probably two, research articles for publication in an appropriate peer-reviewed journal such as *Animal Feed Science and Technology* from this project. Provisionally, one paper will focus on Cielab colour scores for grain sorghum and the other will focus on kafirin and the amino acid profiles of kafirin versus sorghum protein. The likely co-authors will include Dr Robert Hughes, Dr Sonia Yun Liu, Ms Amy Moss, Dr Ali Khoddami, Mr Peter Chrystal and Dr Peter Selle. We are more than prepared to forward the manuscripts to APL and the Feed Grain Partnership for approval prior to their submission to the selected journal.

## 12. Appendix I – Tables

Table 1 Apparent metabolisable energy (AME), ME:GE ratios in poultry and apparent digestible energy (ADE) in pigs of 19 retained PGLP sorghum samples

Sorghum ID	Description	Broiler chickens AME (MJ/kg DM)	Broiler chickens ME:GE ratios	Pigs ADE (MJ/kg DM)
7712	Buster	16.25	0.947	14.33
7811	Boomer	15.48	0.939	14.23
7814	Goldrush	15.62	0.935	15.03
7815	MR 31 B	15.40	0.931	14.96
7816	MR 31 T	15.53	0.925	14.87
7817	MR 31 W	15.58	na	14.11
7818	Pac 2391	15.48	0.922	14.57
7819	Success 42	15.72	0.940	14.95
7820	Thunder	15.65	0.960	14.08
7828	W-isoline	15.99	0.974	14.40
7830	Mr Maxi B	16.06	0.987	14.90
7855	Mr Maxi	15.97	0.942	14.22
7856	Liberty 56	16.15	na	14.59
7859	Buster T	16.38	0.947	14.84
7864	Red 2	16.24	0.932	14.48
7869	Karper	16.63	0.955	14.53
7872	Liberty 72	na	na	14.56
7876	Ridley	16.54	0.956	14.43
7885	Liberty 85	16.91	0.988	na
	Mean	15.98	0.949	14.56
	Standard deviation	± 0.450	± 0.0202	± 0.305
	Coefficient of Variation (%)	2.82	2.13	2.09

Table 2 Gross energy densities, starch and protein (N) concentrations, amylose proportions and Symes PSI textures of 19 retained PGLP sorghum samples

Sorghum	Gross energy (MJ/kg DM)	Starch <sup>1</sup> (g/kg)	Amylose <sup>2</sup> (%)	Crude protein (g/kg)	PSI texture (%)
Buster	16.35	774	30.0	99.2	12
Boomer	15.97	781	35.4	80.6	16
Goldrush	16.21	739	35.4	108.8	16
MR 31 B	16.36	786	36.3	94.8	6
MR 31 T	16.48	742	34.4	119.4	10
MR 31 W	16.35	738	34.5	98.7	12
Pac 2391	16.33	735	35.8	85.6	13
Success 42	16.04	733	34.5	91.0	11
Thunder	16.10	731	33.7	74.9	14
W-isoline	16.49	699	5.3	118.2	6
Mr Maxi B	16.04	757	35.7	98.4	12
Mr Maxi	16.09	677	19.5	98.3	14
Liberty 56	16.21	651	20.3	97.5	18
Buster T	16.39	682	20.8	95.5	10
Red 2	16.57	646	11.5	120.9	8
Karper	16.13	na	na	106.4	12
Liberty 72	16.08	na	na	86.3	9
Ridley	16.54	na	na	93.0	6
Liberty 85	16.04	na	na	81.6	6
Mean	16.25	725	28.2	97.3	11.1
St deviation	± 0.192	± 44.5	± 10.1	± 13.0	± 3.76
C of V (%)	1.2	6.1	35.8	13.4	33.9

<sup>1</sup>The first 9 sorghums have a mean starch concentration of 751 g/kg ± 22.4 (3.0%)

<sup>2</sup>The first 9 sorghums have a mean amylose content of 34.4% ± 1.85 (5.4%)

Table 3 kaffirin concentrations, protein (N) concentrations, kaffirin proportions of sorghum protein and kaffirin index in 19 retained PGLP sorghum samples

Sorghum	Crude protein (g/kg)	Kaffirin (g/kg)	Kaffirin proportion of protein (%)	Kaffirin index <sup>1</sup>
Buster	99.2	44.6	44.9	6.7
Boomer	80.6	42.7	52.9	4.9
Goldrush	108.8	52.3	48.1	6.7
MR 31 B	94.8	48.9	51.5	5.1
MR 31 T	119.4	53.1	44.5	7.9
MR 31 W	98.7	44.5	45.1	6.6
Pac 2391	85.6	37.9	44.3	3.7
Success 42	91.0	39.5	43.4	4.9
Thunder	74.9	33.0	44.0	3.2
W-isoline	118.2	56.7	48.0	7.5
Mr Maxi B	98.4	49.7	50.5	7.3
Mr Maxi	98.3	47.6	48.4	6.4
Liberty 56	97.5	49.8	51.0	6.0
Buster T	95.5	42.5	44.5	5.3
Red 2	120.9	48.4	40.0	8.4
Karper	106.4	52.0	48.9	8.1
Liberty 72	86.3	46.6	54.0	5.2
Ridley	93.0	49.8	53.5	5.5
Liberty 85	81.6	48.5	59.4	5.1
Mean	97.3	46.7	48.3	6.03
St deviation	± 13.0	± 5.78	± 4.74	± 1.448
C of V (%)	13.4	12.4	9.8	24.0

<sup>1</sup>Kaffirin index is the concentration (g/kg) of leucine minus the sum of the basic amino acids (arginine, histidine, lysine)

Table 4 Amino acid concentrations in kafirin (g/kg) of essential amino acids in 19 retained PGLP sorghum samples

Sorghum	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Valine
Buster	1.4	1.1	2.2	8.3	0.5	0.3	3.0	1.1	2.7
Boomer	1.4	1.0	2.2	7.8	0.6	0.3	2.9	1.2	2.5
Goldrush	1.7	1.3	2.6	9.3	0.7	0.6	3.5	1.7	3.1
MR 31 B	1.7	1.2	2.4	8.7	0.7	0.6	3.2	1.6	2.9
MR 31 T	1.6	1.2	2.7	9.9	0.4	0.5	3.6	1.5	3.2
MR 31 W	1.3	1.0	2.3	8.5	0.4	0.3	3.1	1.2	2.5
Pac 2391	1.4	0.9	1.9	6.8	0.5	0.4	2.6	1.1	2.2
Success 42	1.3	1.0	2.0	7.2	0.4	0.4	2.7	1.2	2.3
Thunder	1.1	0.7	1.7	6.0	0.3	0.2	2.3	0.9	2.0
W-isoline	1.9	1.2	2.9	10.6	0.6	0.4	3.8	1.6	3.3
Mr Maxi B	1.6	1.2	2.6	9.1	0.6	0.5	3.4	1.5	2.9
Mr Maxi	1.5	1.2	2.4	8.9	0.6	0.4	3.2	1.4	2.8
Liberty 56	1.6	1.1	2.4	8.5	0.7	0.5	3.2	1.5	2.7
Buster T	1.4	1.0	2.1	7.7	0.5	0.4	2.9	1.2	2.5
Red 2	1.4	1.2	2.4	9.1	0.3	0.3	3.2	1.4	2.9
Karper	1.4	1.2	2.7	9.8	0.4	0.5	3.6	1.5	3.1
Liberty 72	1.8	1.2	2.4	8.2	0.9	0.4	3.2	1.5	2.8
Ridley	1.7	1.2	2.5	9.1	0.7	0.4	3.3	1.5	2.9
Liberty 85	1.9	1.2	2.5	8.6	0.9	0.4	3.3	1.5	2.9
Mean	1.53	1.11	2.36	8.53	0.56	0.41	3.16	1.37	2.75
St deviation	± 0.216	± 0.145	± 0.297	± 1.107	± 0.117	± 0.105	± 0.369	± 0.213	± 0.344
C of V (%)	14.1	13.1	12.6	13.0	20.9	25.6	11.7	15.5	12.5

Table 5 Amino acid concentrations in kafirin (g/kg) of non-essential amino acids in 19 retained PGLP sorghum samples

Sorghum	Alanine	Aspartic acid	Glutamic acid	Glycine	Proline	Serine	Tyrosine	Total amino acids
Buster	5.2	3.4	12.4	1.4	4.8	1.6	2.4	51.8
Boomer	4.9	3.3	11.6	1.3	4.7	1.6	2.5	49.8
Goldrush	5.9	4.2	13.9	1.6	5.6	2.5	2.9	61.1
MR 31 B	5.6	3.8	12.8	1.6	5.2	2.2	2.8	57.0
MR 31 T	6.2	4.1	14.8	1.6	5.8	2.0	2.9	62.0
MR 31 W	5.3	3.5	12.3	1.2	4.7	1.8	2.5	51.9
Pac 2391	4.3	2.9	10.0	1.3	4.1	1.5	2.2	44.1
Success 42	4.5	3.0	10.7	1.3	4.5	1.5	2.3	46.3
Thunder	3.8	2.5	9.1	1.1	3.5	1.2	1.8	38.2
W-isoline	6.6	4.4	15.7	1.5	6.3	2.2	3.2	66.2
Mr Maxi B	5.7	3.6	13.5	1.5	5.4	2.0	2.9	58.0
Mr Maxi	5.5	3.5	13.0	1.4	5.2	1.9	2.7	55.6
Liberty 56	5.4	3.7	12.6	1.5	5.1	1.9	2.7	55.1
Buster T	4.9	3.2	11.4	1.6	4.6	1.7	2.4	49.5
Red 2	5.7	3.5	13.5	1.4	5.4	2.0	2.5	56.2
Karper	6.1	4.0	14.5	1.5	5.8	2.1	2.7	60.9
Liberty 72	5.3	3.7	12.0	1.6	4.8	2.0	2.6	54.4
Ridley	5.8	4.0	13.2	1.6	5.3	2.0	2.8	58.0
Liberty 85	5.5	3.8	12.5	1.7	5.1	2.0	2.7	56.5
Mean	5.38	3.58	12.61	1.46	5.05	1.88	2.61	54.35
St deviation	± 0.681	± 0.474	± 1.624	± 0.161	± 0.647	± 0.305	± 0.312	± 6.744
C of V (%)	12.7	13.2	12.9	11.0	12.8	16.2	12.0	12.4

Table 6 Concentrations (g/kg) of essential amino acids in 19 retained PGLP sorghum samples

Sorghum	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Valine
Buster	3.4	2.5	4.3	14.7	2.1	1.3	5.7	3.3	5.3
Boomer	2.5	2.0	3.3	11.0	1.6	1.2	4.2	2.5	4.0
Goldrush	3.6	2.6	4.6	15.3	2.4	1.4	6.0	3.5	5.6
MR 31 B	3.4	2.4	3.9	13.0	2.1	1.4	5.1	3.2	4.9
MR 31 T	4.0	2.8	5.1	17.1	2.4	1.7	6.5	3.8	6.2
MR 31 W	3.2	2.3	4.2	14.2	2.1	1.2	5.5	3.2	5.1
Pac 2391	3.3	2.2	3.5	11.3	2.1	1.2	4.5	2.9	4.4
Success 42	3.2	2.3	3.7	12.4	2.0	1.4	4.9	3.0	4.6
Thunder	2.7	1.8	3.1	9.6	1.9	1.0	4.0	2.5	3.8
W-isoline	4.0	2.7	4.8	16.6	2.4	1.6	6.3	3.7	5.9
Mr Maxi B	2.8	2.4	4.2	14.3	1.8	1.4	5.4	3.1	4.9
Mr Maxi	2.9	2.3	4.0	13.5	1.9	1.3	5.1	3.0	4.9
Liberty 56	3.3	2.4	4.2	13.9	2.2	1.3	5.5	3.3	5.1
Buster T	3.3	2.4	3.9	13.1	2.1	1.2	5.2	3.1	4.9
Red 2	4.0	3.0	5.1	17.8	2.4	1.4	6.8	3.9	6.3
Karper	3.4	2.6	4.8	16.3	2.2	1.5	6.3	3.6	5.7
Liberty 72	2.9	2.2	3.6	12.2	1.9	1.2	4.8	2.9	4.4
Ridley	3.4	2.4	4.1	13.6	2.3	1.2	5.4	3.2	5.1
Liberty 85	2.9	2.1	3.6	12.0	1.9	1.1	4.8	2.8	4.4
Mean	3.27	2.39	4.11	13.78	2.10	1.32	5.37	3.18	5.03
St deviation	± 0.431	± 0.281	± 0.580	± 2.176	± 0.227	± 0.171	± 0.769	± 0.393	± 0.692
C of V (%)	13.2	11.7	14.1	15.8	10.8	13.0	14.3	12.4	13.8



Table 7 Concentrations (g/kg) of non-essential and total amino acids in 19 retained PGLP sorghum samples

Sorghum	Alanine	Aspartic acid	Glutamic acid	Glycine	Proline	Serine	Tyrosine	Total amino acids
Buster	9.4	6.8	22.5	3.2	8.7	4.8	1.8	99.7
Boomer	7.1	5.4	16.8	2.5	6.6	3.7	1.7	76.3
Goldrush	10.0	7.6	23.8	3.4	9.3	5.1	2.0	106.4
MR 31 B	8.4	6.2	19.6	3.2	7.9	4.5	1.7	91.2
MR 31 T	11.2	8.2	26.6	3.6	10.1	5.5	2.6	117.3
MR 31 W	9.2	7.0	21.7	3.0	8.1	4.6	1.8	96.4
Pac 2391	7.4	5.9	17.6	3.0	6.9	4.1	1.6	82.0
Success 42	8.0	6.0	19.2	3.0	7.7	4.2	2.2	87.9
Thunder	6.4	5.0	14.9	2.6	5.8	3.6	1.6	70.1
W-isoline	10.7	8.1	25.6	3.5	9.8	5.3	2.7	113.8
Mr Maxi B	9.1	6.5	21.6	2.9	8.5	4.5	2.1	95.5
Mr Maxi	8.7	6.5	20.6	3.0	8.0	4.4	2.3	92.5
Liberty 56	9.1	6.8	21.5	3.3	8.4	4.7	1.9	96.9
Buster T	8.5	6.3	20.0	3.2	7.9	4.5	1.7	91.3
Red 2	11.5	8.0	27.3	3.6	10.4	5.7	2.5	119.9
Karper	10.6	7.7	25.1	3.4	9.6	5.3	2.2	110.2
Liberty 72	7.9	5.8	18.7	2.8	7.3	4.1	1.8	84.5
Ridley	8.9	7.0	21.4	3.2	8.1	4.6	1.9	95.6
Liberty 85	7.8	5.7	18.7	2.8	7.4	4.0	1.5	83.5
Mean	8.94	6.66	21.22	3.12	8.24	4.59	1.98	95.32
St deviation	± 1.394	± 0.940	± 3.357	± 0.315	± 1.213	± 0.587	± 0.354	± 13.589
C of V (%)	15.6	14.1	15.8	10.1	14.7	12.8	17.9	14.3

Table 8 Concentrations of total phenolic compounds, anthocyanins and flavan-4-ols and Clorox bleach test in 19 retained PGLP sorghum samples

Sorghum	Total phenolic compounds (mg GAE/g DM)	Anthocyanins (abs/ml/g DM)	Flavan-4-ols (abs/ml/g DM)	Clorox bleach test <sup>1</sup>
Buster	3.558	5.685	1.543	negative
Boomer	2.641	4.641	1.968	negative
Goldrush	2.609	4.443	0.909	negative
MR 31 B	3.223	3.746	1.604	negative
MR 31 T	4.467	5.203	3.808	negative
MR 31 W	3.776	4.392	0.980	negative
Pac 2391	3.345	2.968	0.788	negative
Success 42	3.624	2.829	1.727	negative
Thunder	4.120	5.860	2.642	negative
W-isoline	4.496	4.307	3.588	negative
Mr Maxi B	1.748	11.102	1.625	negative
Mr Maxi	2.842	8.893	2.342	negative
Liberty 56	2.905	1.844	0.504	negative
Buster T	3.180	4.672	1.869	negative
Red 2	5.153	3.853	3.728	negative
Karper	2.952	4.154	0.745	negative
Liberty 72	1.865	1.374	1.234	negative
Ridley	3.454	3.770	0.871	negative
Liberty 85	2.786	2.143	0.507	negative
Mean	3.302	4.520	1.736	-
St deviation	± 0.865	± 2.308	± 1.059	-
C of V (%)	26.2	51.1	61.0	-

Table 9 Concentrations ( $\mu\text{g/g DM}$ ) of soluble (free and conjugated) phenolic acids in 19 retained PGLP sorghum samples

Sorghum	<i>p</i> -hydroxybenzoic acid	Syringic acid	<i>p</i> -coumaric acid	Ferulic acid
Buster	15.3	5.54	22.9	40.3
Boomer	13.0	4.88	22.0	33.4
Goldrush	18.9	3.66	35.0	29.4
MR 31 B	16.5	3.89	17.5	33.9
MR 31 T	29.1	3.90	32.5	36.1
MR 31 W	19.0	5.62	24.2	28.2
Pac 2391	9.77	7.72	23.5	37.4
Success 42	7.64	2.07	11.0	18.3
Thunder	19.7	3.84	50.3	37.6
W-isoline	25.8	3.81	81.0	57.1
Mr Maxi B	18.3	4.28	19.6	33.7
Mr Maxi	15.7	3.83	22.5	43.8
Liberty 56	38.0	3.83	27.5	35.4
Buster T	11.3	6.05	19.9	33.0
Red 2	11.9	4.51	18.1	31.0
Karper	3.1	4.51	34.5	40.5
Liberty 72	3.86	3.97	26.2	28.0
Ridley	9.97	7.37	26.3	38.7
Liberty 85	4.86	3.88	26.3	31.2
Mean	15.3	4.59	28.5	35.1
St deviation	$\pm 8.88$	$\pm 1.36$	$\pm 15.24$	$\pm 7.81$
C of V (%)	58.0	29.6	53.5	22.3

Table 10 Concentrations ( $\mu$  g/g DM) of insoluble (bound) phenolic acids in 19 retained PGLP sorghum samples

Sorghum	<i>p</i> -hydroxybenzoic acid	Syringic acid	<i>p</i> -coumaric acid	Ferulic acid
Buster	9.65	1.88	71.2	493
Boomer	9.29	1.99	53.3	515
Goldrush	18.9	1.38	106	444
MR 31 B	18.5	1.81	59.1	480
MR 31 T	14.7	1.35	60.3	476
MR 31 W	11.6	8.40	62.3	642
Pac 2391	10.5	2.88	49.1	553
Success 42	7.35	0.00	38.2	225
Thunder	13.2	1.59	70.2	417
W-isoline	13.3	0.00	38.3	204
Mr Maxi B	18.7	11.4	86.1	488
Mr Maxi	15.8	1.96	49.0	510
Liberty 56	12.8	2.16	83.8	332
Buster T	8.60	2.21	59.6	474
Red 2	5.95	1.90	47.2	544
Karper	3.14	1.98	14.7	352
Liberty 72	4.87	2.41	110	317
Ridley	7.33	2.65	68.4	569
Liberty 85	4.42	2.07	58.8	249
Mean	11.0	2.63	69.4	436
St deviation	$\pm$ 4.91	$\pm$ 2.71	$\pm$ 27.36	$\pm$ 123.4
C of V (%)	44.6	97.0	39.4	28.3

Table 11 Concentrations of phytate, total phosphorus (P), phytate-P and proportion of phytate-P of total P in 19 retained PGLP sorghum samples

Sorghum	Phytate (g/kg)	Total P (g/kg)	Phytate-P (g/kg)	Phytate-P proportion of total P (%)
Buster	8.15	3.11	2.30	74.0
Boomer	4.65	2.40	1.31	54.6
Goldrush	6.25	2.66	1.76	66.3
MR 31 B	5.30	2.19	1.49	68.2
MR 31 T	6.90	2.92	1.95	66.6
MR 31 W	6.60	3.13	1.86	59.5
Pac 2391	4.70	1.93	1.33	68.7
Success 42	6.10	2.67	1.71	64.0
Thunder	7.05	2.86	1.99	69.5
W-isoline	9.65	4.09	2.72	66.5
Mr Maxi B	5.25	2.14	1.48	69.2
Mr Maxi	5.30	1.98	1.49	75.5
Liberty 56	5.55	2.90	1.57	54.0
Buster T	6.60	2.42	1.86	76.9
Red 2	9.00	3.43	2.54	74.0
Karper	9.60	3.49	2.71	77.6
Liberty 72	4.90	1.86	1.38	74.3
Ridley	6.15	2.24	1.73	77.4
Liberty 85	6.40	2.30	1.80	78.5
Mean	6.53	2.67	1.84	69.2
St deviation	± 1.56	± 0.60	± 0.44	± 7.39
C of V (%)	23.9	22.5	23.9	10.7

Table 12 Concentrations (mg/kg) of ten minerals in sorghum samples analysed by inductively coupled plasma optical emission spectrometry (ICP-OES) in 19 retained PGLP sorghum samples

Sorghum	Ca	Cu	Fe	K	Mg	Mn	Na	P	Sr	Zn
Buster	104	3.09	44.6	2773	1359	14.4	5.41	3110	0.72	15.4
Boomer	110	2.20	25.1	2694	1100	9.14	5.58	2395	0.78	18.2
Goldrush	196	4.49	33.0	2654	1260	18.9	5.64	2657	3.94	20.0
MR 31 B	194	4.18	27.3	2475	1116	14.2	3.19	2185	1.41	18.1
MR 31 T	124	3.88	39.2	2615	1365	12.6	11.2	2916	1.65	19.9
MR 31 W	120	3.49	34.5	2830	1344	8.5	5.93	3128	1.33	19.7
Pac 2391	179	2.95	26.0	2459	1032	13.2	2.66	1931	1.24	17.2
Success 42	143	3.00	30.3	2810	1232	15.0	6.86	2668	1.88	17.8
Thunder	210	3.08	25.8	2899	1199	15.2	7.18	2863	1.60	19.7
WV-isoline	95.9	3.37	39.4	4225	1600	16.0	11.2	4086	0.66	16.4
Mr Maxi B	139	2.11	24.7	2278	1116	12.1	3.67	2140	1.88	13.9
Mr Maxi	145	2.44	24.6	2331	1036	14.6	2.80	1978	2.61	15.2
Liberty 56	161	2.18	31.4	3164	1224	21.0	2.52	2900	3.23	20.8
Buster T	144	3.14	27.8	2828	1205	12.9	6.17	2419	2.88	19.1
Red 2	125	3.33	33.6	3378	1452	19.7	9.92	3429	2.41	23.0
Karper	193	2.26	28.9	3255	1469	18.6	10.2	3486	1.65	17.5
Liberty 72	91	2.59	24.8	2481	1079	15.0	13.1	1859	2.81	12.7
Ridley	145	3.18	31.1	2628	1165	16.9	3.68	2244	1.52	18.7
Liberty 85	102	1.99	23.5	2614	1051	17.7	13.5	2301	1.46	14.7
Mean	143	3.00	30.3	2810	1232	15.0	6.86	2669	1.88	17.8
St deviation	± 36.9	± 0.71	± 5.90	± 451	± 162	± 3.31	± 3.61	± 599	± 0.89	± 2.59
C of V (%)	25.8	23.7	19.5	16.0	13.1	22.1	52.6	22.4	47.3	14.6

Table 13 Rapid visco-analysis (RVA) starch pasting profiles of 19 retained PGLP sorghum samples

Sorghum	RVA viscosity (cP)					Pasting time (min)	Peak temp. (°C)
	Peak	Holding	Breakdown	Final	Setback		
Buster	1700	1597	104	3320	1725	5.84	81.1
Boomer	2837	2418	419	5565	3147	5.60	83.3
Goldrush	1573	1529	44	3729	2200	6.27	84.4
MR 31 B	1880	1650	230	3568	1918	5.57	79.5
MR 31 T	1487	1452	35	3835	2383	6.67	82.6
MR 31 W	2329	1815	514	4216	2402	5.30	78.7
Pac 2391	2177	1734	443	3658	1924	5.30	80.8
Success 42	2097	1752	345	3789	2037	5.40	79.9
Thunder	2777	1931	846	4078	2147	5.00	77.5
W-isoline	1971	1693	279	2818	1125	5.27	75.2
Mr Maxi B	2364	2136	228	5625	3489	5.97	85.2
Mr Maxi	2327	2050	227	5079	3029	5.77	77.4
Liberty 56	2150	1868	283	4322	2455	5.63	81.1
Buster T	1807	1554	254	3593	2040	5.54	82.0
Red 2	1459	1425	34	3087	1662	6.44	84.0
Karper	2582	1912	670	4679	2767	5.37	83.2
Liberty 72	2670	1955	716	4763	2809	5.30	80.6
Ridley	2098	1708	390	4008	2300	5.50	74.4
Liberty 85	2868	2062	806	4674	2613	5.20	78.3
Mean	2166	1802	364	4126	2324	5.63	80.5
St. dev.	± 444.0	± 252.6	± 248.2	± 769.4	± 562.7	± 0.439	± 3.23
C of V (%)	20.5	14.0	68.2	18.6	24.2	7.8	4.0

Table 14 Quantification of grain sorghum colour using the Hunterlab system giving L\*, a\* and b\* measurements of 19 retained PGLP sorghum samples

<b>Sorghum</b>	<b>L*</b>	<b>a*</b>	<b>b*</b>
Buster	36.0	13.0	14.5
Boomer	39.2	13.2	14.4
Goldrush	34.3	14.9	13.1
MR 31 B	32.6	17.2	13.7
MR 31 T	35.4	15.7	13.7
MR 31 W	38.7	14.8	15.2
Pac 2391	33.3	16.2	14.4
Success 42	36.5	16.8	16.4
Thunder	29.6	14.9	10.5
W-isoline	34.6	16.8	15.6
Mr Maxi B	36.5	16.8	16.4
Mr Maxi	32.2	12.7	11.1
Liberty 56	58.7	3.4	17.6
Buster T	36.5	15.5	15.8
Red 2	38.5	12.7	15.0
Karper	47.9	4.5	17.7
Liberty 72	53.3	3.2	15.5
Ridley	40.2	13.4	16.4
Liberty 85	51.1	3.9	14.6
Mean	39.2	12.6	14.8
Standard deviation	± 7.85	± 4.88	± 1.90
C of V (%)	20.0	38.7	12.8



Table 15 Coefficients and significance of individual rapid visco-analysis (RVA) of starch pasting variables in equations to predict pig digestible energy (DE) values for sorghum

Variable	All sorghums		Red sorghums	
	Coefficient	Probability	Coefficient	Probability
Intercept	18.92	< 0.001	18.92	< 0.001
Peak	-0.0085	0.069	-0.0082	0.123
Holding	-0.2932	0.003	-0.3103	0.007
Breakdown	0.0066	0.137	0.0062	0.210
Final	0.3004	0.003	0.3171	0.007
Setback	-0.2998	0.003	-0.3165	0.007
Peak time	-1.0704	0.002	-1.0670	0.005
Pasting temp.	0.0444	0.046	0.0447	0.090

Table 16 Coefficients and significance of phenolic compounds in equations to predict AME values for broiler chickens offered PGLP sorghums

<b>Variable</b>	<b>Coefficient</b>	<b>Probability</b>
Intercept	13.85	< 0.001
Total phenolic compounds	-0.258	0.008
Soluble <i>p</i> -hydroxybenzoic acid	0.040	0.001
Insoluble <i>p</i> -hydroxybenzoic acid	-0.083	< 0.001
Insoluble <i>p</i> -coumaric acid	-0.047	< 0.001
Total syringic acid	0.047	< 0.001

Table 17 Coefficients and significance of phenolic compounds in equations to predict ADE values for pigs offered PGLP sorghums

<b>Variable</b>	<b>Coefficient</b>	<b>Probability</b>
Intercept	14.01	< 0.001
Anthocyanin	-0.036	0.003
Flavan-4-ols	0.244	< 0.001
Soluble <i>p</i> -hydroxybenzoic acid	-0.011	< 0.001
Soluble syringic acid	0.288	< 0.001
Soluble <i>p</i> -coumaric acid	-0.016	< 0.001
Soluble ferulic acid	-0.027	< 0.001
Insoluble <i>p</i> -hydroxybenzoic acid	0.080	< 0.001
Insoluble <i>p</i> -coumaric acid	0.006	< 0.001
Insoluble ferulic acid	-0.004	< 0.001
Total ferulic acid	0.018	< 0.001

Table 18 Pearson correlations between AME in poultry and ADE in pigs with Cielab colour values for PGP sorghums

Item	AME (MJ/kg)	ADE (MJ/kg)	$L^*$	$a^*$	$b^*$
AME	1.000				
ADE	r = -0.086 P = 0.741	1.000			
$L^*$	r = 0.585 P = 0.011	r = 0.001 P = 0.998	1.000		
$a^*$	r = -0.655 P = 0.003	r = 0.192 P = 0.445	r = -0.913 P < 0.001	1.000	
$b^*$	r = 0.446 P = 0.064	r = 0.283 P = 0.255	r = 0.614 P = 0.005	r = -0.339 P = 0.156	1.000

Table 19 Pearson correlations between polyphenolic compounds and Cielab colour values of PGLP sorghums

Item	Polyphenolic compounds			Cielab colour values		
	Totals	Anthocyanins	Flavan-4-ols	<i>L</i> *	<i>a</i> *	<i>B</i> *
Totals	1.000					
Anthos.	r = -0.149 P = 0.541	1.000				
Flavan.	r = 0.665 P = 0.002	r = 0.300 P = 0.212	1.000			
<i>L</i> *	r = -0.381 P = 0.107	r = -0.540 P = 0.017	r = -0.498 P = 0.030	1.000		
<i>a</i> *	r = 0.374 P = 0.115	r = 0.459 P = 0.048	r = 0.458 P = 0.048	r = -0.913 P < 0.001	1.000	
<i>b</i> *	r = -0.171 P = 0.484	r = -0.323 P = 0.177	r = -0.356 P = 0.135	r = 0.614 P = 0.005	r = -0.339 P = 0.156	1.000

Table 20 Pearson correlations between polyphenolic compounds and RVA starch pasting viscosities of PGLP sorghums

Item	Polyphenolic compounds			Peak	RVA starch pasting viscosities			
	Totals	Anthocyanins	Flavan-4-ols		Holding	Breakdown	Final	Setback
Totals	1.000							
Anthocyanins	r = -0.149 P = 0.541	1.000						
Flavan-4-ols	r = 0.665 P = 0.002	r = 0.300 P = 0.212	1.000					
Peak	r = -0.498 P = 0.030	r = -0.033 P = 0.895	r = -0.398 P = 0.091	1.000				
Holding	r = -0.614 P = 0.005	r = 0.224 P = 0.357	r = -0.309 P = 0.198	r = 0.888 P < 0.001	1.000			
Breakdown	r = -0.260 P = 0.283	r = -0.308 P = 0.204	r = -0.402 P = 0.088	r = 0.876 P < 0.001	r = 0.558 P = 0.013	1.000		
Final	r = -0.740 P < 0.001	r = 0.362 P = 0.128	r = -0.351 P = 0.141	r = 0.717 P = 0.001	r = 0.873 P < 0.001	r = 0.379 P = 0.109	1.000	
Setback	r = -0.737 P < 0.001	r = 0.394 P = 0.095	r = -0.342 P = 0.152	r = 0.582 P = 0.009	r = 0.745 P < 0.001	r = 0.269 P = 0.266	r = 0.976 P < 0.001	1.000

Table 21 Amino acid profiles of kafirin in sorghum (percentage of 15 amino acids) as reported by Xiao et al. (2015), Truong et al. (2017) and kafirin and total protein in PGLP sorghums

Amino acid	Xiao et al (2015)	Truong et al (2017)	Kafirin PGLP sorghums	Total protein PGLP sorghums
Arginine	2.31	2.43	3.10	3.76
Histidine	1.32	2.10	2.25	2.75
Isoleucine	4.07	4.54	4.79	4.72
Leucine	18.59	17.48	17.30	15.83
Lysine	0.22	0.55	1.14	2.41
Methionine	1.43	1.33	0.83	1.52
Phenylalanine	5.94	6.31	6.41	6.17
Threonine	2.75	2.99	2.78	3.65
Valine	4.62	5.31	5.58	5.78
Alanine	12.32	11.17	10.91	10.27
Aspartic acid	6.60	6.75	7.26	7.65
Glutamic acid	29.70	26.88	25.58	24.37
Glycine	1.21	2.32	2.96	3.58
Serine	4.07	4.65	3.81	5.27
Tyrosine	4.84	5.20	5.29	2.27

Table 22 Statistical comparison of amino acid profiles (percentage of 15 amino acids) in 1998 (n=6) and 2009 (n=11) grain sorghums

<b>Amino acid</b>	<b>1998 sorghums (% 15 amino acids)</b>	<b>2009 sorghums (% 15 amino acids)</b>	<b>SEM</b>	<b>Significance (P =)</b>
Arginine	4.55	4.00	0.0952	0.001
Histidine	2.53	2.64	0.0782	0.387
Isoleucine	5.30	4.59	0.2904	0.120
Leucine	15.02	15.52	0.1279	0.018
Lysine	2.45	2.28	0.0797	0.175
Methionine	1.90	1.69	0.0632	0.041
Phenylalanine	5.93	5.86	0.0840	0.536
Threonine	3.47	3.67	0.0881	0.136
Valine	5.65	5.69	0.1127	0.810
Alanine	10.22	10.26	0.1964	0.898
Aspartic acid	7.22	7.37	0.1158	0.377
Glutamic acid	22.97	23.95	0.3570	0.084
Glycine	3.45	3.48	0.0711	0.768
Serine	4.98	5.26	0.2650	0.597
Tyrosine	4.33	3.68	0.2541	0.104



Table 23 Statistical comparison of selected parameters between red and white varieties of PGLP sorghums

Parameter	Red sorghums (n = 15)	White sorghums (n = 3)	SEM	Significance (P =)
<u>Polyphenolics</u>				
Total phenolic compounds	3.48	2.52	0.277	0.085
Anthocyanins	5.09	1.79	0.690	0.022
Flavan-4-ols	2.00	0.75	0.325	0.060
<u>Insoluble phenolic acids</u>				
p-hydroxybenzoic acid	12.2	7.4	1.468	0.101
syringic acid	2.76	2.21	0.954	0.766
p-coumaric acid	61.2	84.2	6.339	0.074
ferulic acid	469	299	36.76	0.027
<u>Crude protein</u>				
Crude protein	98.5	88.5	4.346	0.242
Kafirin	46.1	48.3	1.972	0.563
Kafirin % of protein	46.9	54.8	1.314	0.006
<u>Phytate-P</u>				
Phytate-P	1.83	1.58	0.133	0.334
Total P	2.68	2.35	0.195	0.394
Phytate-P % of total P	68.7	68.9	2.513	0.965
<u>RVA pasting properties</u>				
Peak viscosity	2059	2563	139.6	0.075
Holding viscosity	1763	1962	85.8	0.240
Breakdown viscosity	293	602	74.9	0.045
Final viscosity	3998	4586	259.8	0.250
Setback viscosity	2235	2626	191.0	0.297
Pasting time	5.70	5.38	0.158	0.275
Peak temperature	80.4	80.0	1.045	0.843
<u>Cielab colour scores</u>				
L*	35.6	54.4	1.020	< 0.001
a*	15.0	3.5	0.510	< 0.001
b*	14.4	15.9	0.589	0.202

### 13. Appendix 2 – Figures

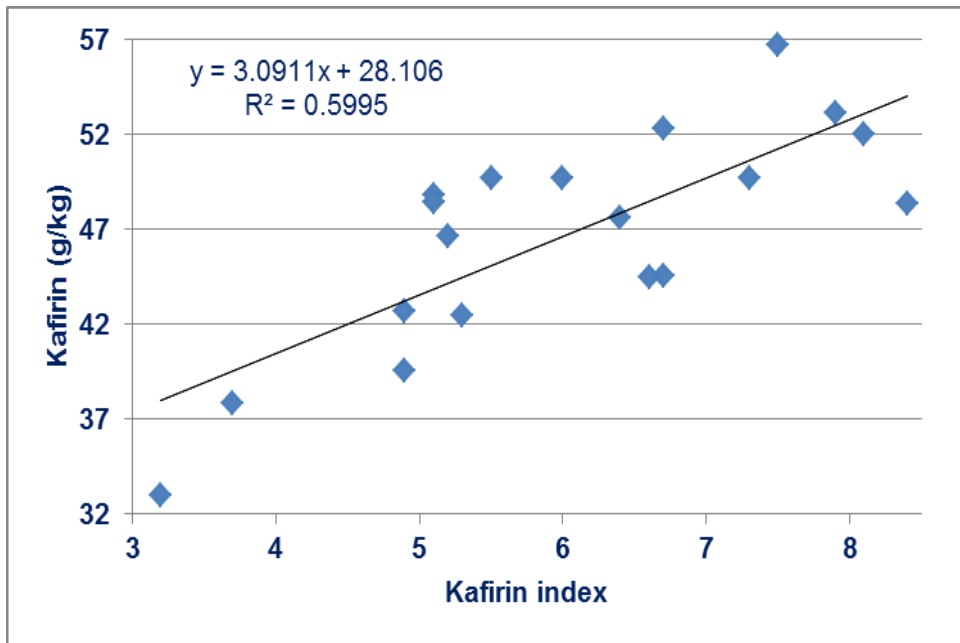


Figure 1 Linear relationship ( $r=0.774$ ;  $P<0.001$ ) between kafirin index and kafirin concentrations in 19 retained PGLP sorghum samples

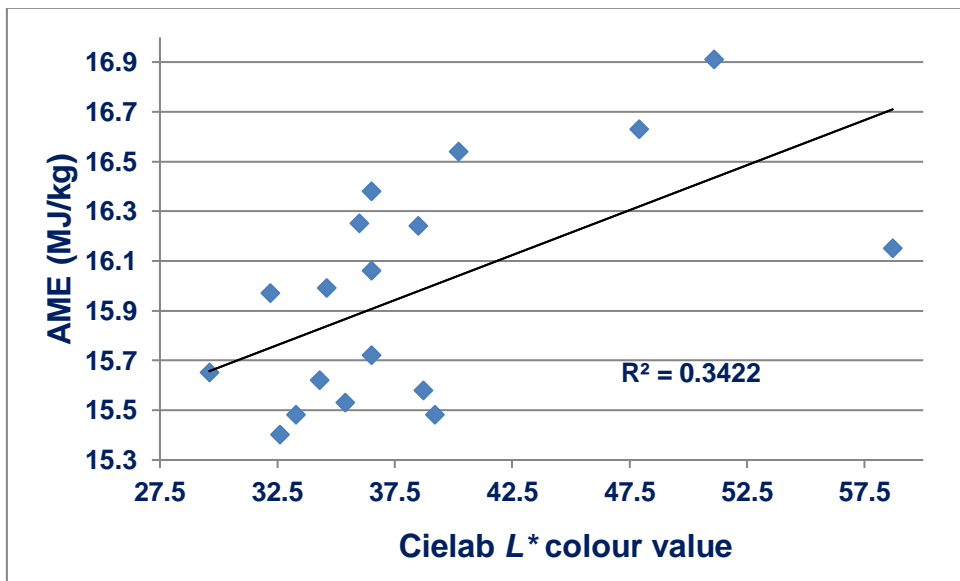
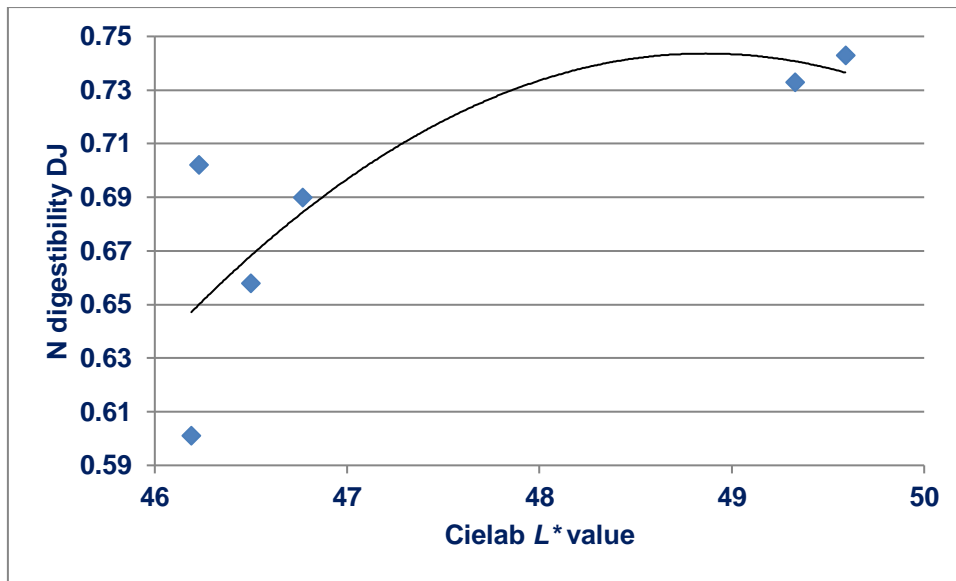
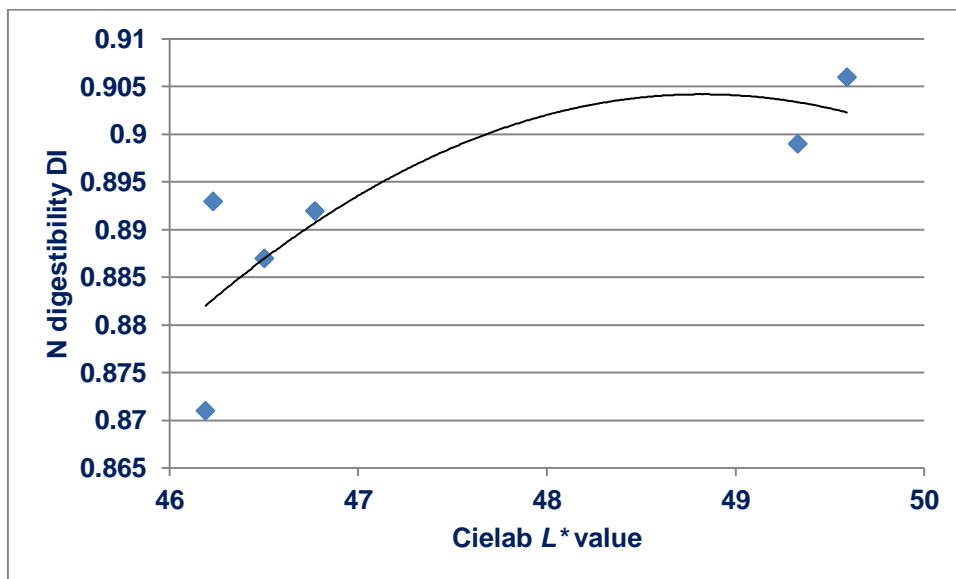


Figure 2 Linear relationship ( $r=0.585$ ;  $P<0.015$ ) between Cielab L\* colour value and AME in poultry



A



B

Figure 3 Quadratic relationships between Cielab L\* values of six red sorghums and crude protein (N) digestibility coefficients in (A) distal jejunum ( $r=0.776$ ;  $P=0.070$ ) and (B) distal ileum ( $r=0.774$ ;  $P=0.071$ ) in broiler chickens. Adapted from Khoddami et al. (2015)

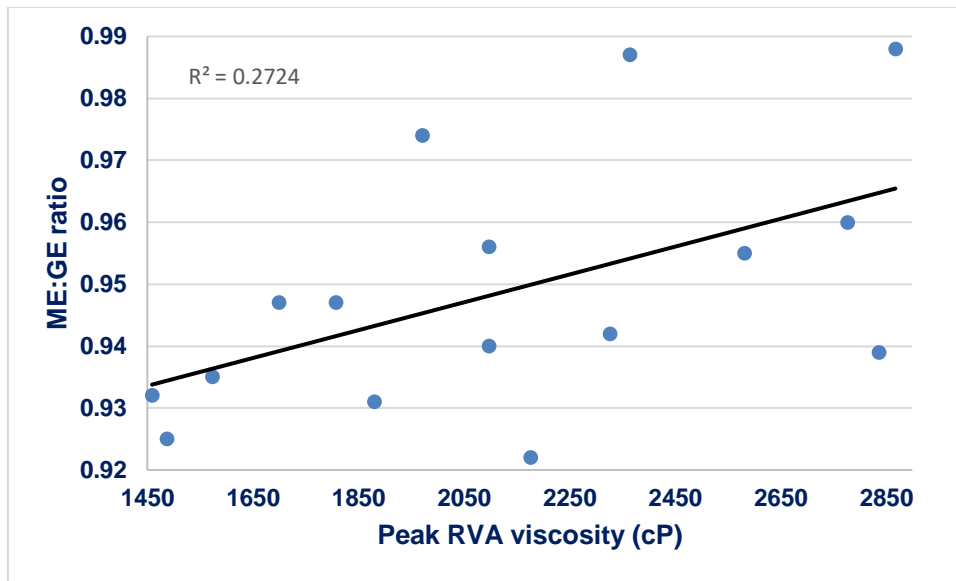


Figure 4 Linear relationship ( $r=0.552$ ;  $P=0.038$ ) between Peak RVA viscosity of sorghums and ME:GE ratios in poultry  
 $y_{(ME:GE\ ratio)} = 0.901 + 0.00002 * peak\ viscosity$