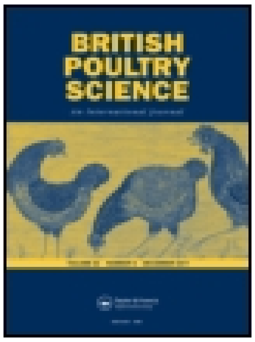

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Accurate prediction of nutritional value of sorghum grain using image analysis

M. R. Ebadi[a], M. Sedghi[b] and R. Akbari Moghaddam Kakhki [c]

[a]Animal Science Research Department, Isfahan Agricultural and Natural Resources Research and Education Centre, AREEO, Isfahan, Iran; [b]Department of Animal Sciences, College of Agriculture, Isfahan University of Technology, Isfahan, Iran; [c]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada

ABSTRACT

1. This study evaluated the application of L (lightness)*a (redness) and *b (blueness) colour analysis and chemical compositions to predict the nutritional value of sorghum grain.
2. A total of 12 varieties of sorghum grain were analysed for L*a*b colours, chemical composition, energy and total and digestible amino acid content. Regression models based on the linear, non-linear and the interaction effects of inputs were applied to predict the nutritional value of sorghum grains either using L*a*b colour or chemical composition, as the model inputs.
3. The results illustrated a significant relationship between a*b and/or chemical compositions with energy content in the samples of sorghum grain. The provided estimation equations presented high goodness of fit in terms of R^2_{adj} ranging from 0.744 to 0.999.
4. Total and digestible amino acids of sorghum grain were estimated based on a*b and chemical compositions data with the goodness of fit ranging from 0.641 to 0.999 (R^2_{adj}).
5. In conclusion, the L*a*b colour analysis may be used for developing equations to predict nutritional value of sorghum grain as an alternative approach to the conventional time-consuming and costly chemical and bioassay methods.

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Amino acids; colour analysis; nutritional value; regression; sorghum

Introduction

A significant portion of poultry rearing expenses are associated with feed cost. Poultry nutritionists have examined feedstuffs continuously and have evaluated their impacts on health and economic performance of birds. Sorghum grain (*Sorghum bicolor*) is a potential alternative grain, possessing approximately 95% of corn grain's nutritional value (Dowling et al. 2002). Considerable drought tolerance of sorghum has made it a unique species to be grown in diverse geographical regions. However, tannins, as the most important anti-nutritional factor in sorghum, can adversely influence the nutritional value of this grain and utilisation of its energy and protein for poultry (Boren and Waniska 1992). Nutrients composition and digestibility of sorghum grain depend on its cultivar and cultivation conditions (Reed et al. 1988; Mabelebele et al. 2015). Khoddami et al. (2015) analysed the concentrations of some non-tannin phenolic compounds in six sorghums and calculated their correlations with energy utilisation in broiler chickens. They concluded that energy utilisation, nitrogen digestibility and chicken performance were adversely affected by the specific non-tannin phenolic compounds.

Today, it is well accepted that the presence of tannin in the seed can be reflected in the colour of sorghum grain (Hahn and Rooney 1986; Khoddami et al. 2015). Thus, colour values have been used by some researchers to determine the nutritional value of sorghum grain (Rooney et al. 1981; Dykes et al. 2005; Sedghi et al. 2012). For instance, Dykes et al. (2005) reported negative correlations between the lightness (L*) and total phenols (r = −0.69) and flavan-4-ol content (r = −0.84), and between the b* value with anthocyanin (r = −0.85) and flavan-4-ol (r = −0.90) contents in sorghum grain. However, no significant correlations were found between the a* value and both flavan-4-ols and total phenols content in their study. Furthermore, Sedghi et al. (2012) stated that a combination of computer image analysis technique with the artificial neural network could be a useful approach to estimate tannin content of sorghum grain based on obtained L*a*b* data.

The correlation between nutrient content with the colour of beans has been reported in several investigations. Moraghan et al. (2002) reported that the iron content of beans can vary according to the colour due to the variation in their tannin content. A higher crude protein and calcium content in coloured beans were reported by Lombardi-Boccia et al. (1998). A similar relationship between colour and mineral and protein contents of beans was reported by Silva et al. (2012).

Currently, there is a lack of information regarding the estimation of chemical composition, energy value and amino acids digestibility of sorghum for poultry based on L*a*b Cielab. Thus, the first objective of this study was to estimate energy content, chemical composition and true digestible amino acids content in samples of sorghum grain based on L*a*b data of colour measurement. The second objective was to re-estimate energy and amino acids contents of sorghum based on available chemical composition data by practical chemical analyses including total phenols (TP), crude protein (CP), crude fibre (CF), ether extract (EE) and ash.

Materials and methods

A total of 12 varieties of sorghum kernels, differing in seed colour and tannin content, were grown in the same year and were scanned by a digital scanner (Hewlett Packard, model 3800). Image processing was performed based on the

CONTACT M. Sedghi mo.sedghi@CC.iut.ac.ir Department of Animal Sciences, College of Agriculture, Isfahan University of Technology, Isfahan, Iran, 84156-83111

described method by Sedghi et al. (2012). Briefly, for colour reproducibility, a 200 × 200 pixel areas were separated from these images and converted into L*a*b* units since the perspective of computer comprehends colour as red, green and blue (RGB) signals. The L*a*b colour space mathematically illustrated all perceived colour in three dimensions including L* for lightness and a* and b* for the colour opponents green–red and yellow–blue, respectively. The L* ranged from 0 (represents the darkest black) to 100 (demonstrates the brightest white). Parameter a* represented the green and red at negative and positive values, respectively. The b* parameter with a negative value displayed yellow colour and with a positive value represented blue colour. The images were analysed using the ImageJ tool-box software version 1.40g as described by Leon et al. (2006).

For chemical composition analysis, six samples from each variety were analysed for ash (method 942.05; AOAC 2005), crude fibre (CF; method 978.10; AOAC 2005), crude protein (CP method 990.03; AOAC 2005) and ether extract (EE; method 920.39; AOAC 2005). Folin–Denis method (method 952.03; AOAC 2005) was used to determine total phenols (TP) content of sorghum grains.

Comprehensive protocols for animal welfare and experimental procedures adhered to comprehensive guides of animal welfare adopted at Ferdowsi University of Mashhad, Mashhad, Iran. Six samples of each variety were used to determine apparent metabolisable energy (AME), apparent metabolisable energy nitrogen-corrected (AMEn), true metabolisable energy (TME) and true metabolisable energy nitrogen-corrected (TMEn) as described by Sibbald (1979b). The gross energy and nitrogen contents of the milled excreta were measured using the bomb calorimeter and Kjeldahl method (Kjeldahl 1883), respectively.

Amino acid digestibility was determined according to the method described by Sibbald (1986). Briefly, 50 Single Comb Leghorn roosters were caecectomised based on Parson's method (Parsons 1985) at 30 weeks of age followed by 10 weeks of recovery. The caecectomised roosters were then weighed, and 24 roosters with a live body weight of 2100 ± 100g were selected for digestibility trials. The digestibility experiments performed during four consecutive periods with 2 weeks' interval for recovery. Each cycle lasted 3 days, and during each period 18 roosters were fed with three sorghum varieties (six replicates per sample). Briefly, six roosters were randomly each given 30 g of one of the six sorghum samples from each sorghum variety via crop intubation followed by returning birds to their cages. A tray was placed under each cage for collecting excreta over a 48-h period. Feathers and offal were removed from the excreta and then put into individual bags in a freezer. Freeze-dried samples were weighed and prepared for amino acid analysis. The remaining six roosters were used for measuring the excreted endogenous amino acids. To do so, the roosters were fed with 30 g of glucose after a 24-h of fasting period (Green et al. 1987; McNab and Blair 1988).

Amino acid contents in the samples of sorghum grains and excreta were determined by ion exchange chromatography following hydrolysis with 6NHCl for 24-h at 110°C in sealed tubes, with four replicates per each sample. Derivation with ninhydrin was accomplished and the quantity of each amino acid was determined using the Bechman Biocrom 20 Amino Acid Analyzer at the University of Manitoba, Canada. Methionine and cystine were measured based on the method described by Moore (1963) in the oxidised samples by performic acid. The method of Hugli and Moore (1972) was applied for the determination of tryptophan. True amino acids digestibility were calculated based on the method described by Sibbald (1979a), in which the amount of dietary amino acids which did not appear in the excreta were referred as digestible amino acids. To determine collinearity between variables, correlation was calculated between L*a*b colour data. Due to the significant correlation ($P < 0.001$) between L* with a* and b* data, the L* component was excluded from the statistical analysis (Robison et al. 2015). All data-lines were subjected to SAS software (SAS 2009) and the linear, quadratic and interaction terms of a × b data were considered as input and regressed through REG procedure. The selection method for choosing the best-fitted model was based on the highest value of R^2_{adj}.

Results

Metabolisable energy, chemical composition and amino acids content in grains of sorghum varieties are shown in Tables 1 and 2, respectively.

Estimation equations to predict energy content, chemical composition and digestibility of sorghum grains based on a*b* colour are shown in Table 3. Analysis of a*b* data, considering linear and quadratic patterns along with their interaction terms, was used to predict the response variables of energy

Table 1. Summary of chemical analysis of different varieties of sorghum kernels.

Items[1]/Sorghum Type	1	2	3	4	5	6	7	8	9	10	11	12
L	44.37	63.22	68.79	36.04	64.45	65.49	42.31	45.02	45.27	49.66	41.67	36.80
a	16.08	8.91	7.15	13.02	7.46	7.89	6.45	17.34	13.30	18.90	17.46	15.47
b	22.73	36.48	30.79	16.86	29.44	31.29	10.29	27.80	26.62	25.50	22.71	18.16
Gross energy	4629	4530	4515	4663	4563	4701	4517	4657	4777	4677	4435	4481
Apparent metabolisable energy	3076	3673	3471	3044	3264	3605	2616	3165	2715	3680	2804	2881
Apparent metabolisable energy $_{N\text{-Corrected}}$	3493	3887	3674	3355	3668	3832	2898	3577	3091	3867	3200	3080
True metabolisable energy	3764	4474	4000	3706	3873	4126	3133	3787	3529	4068	3592	3398
True metabolisable energy $_{N\text{-Corrected}}$	3685	4065	3965	3540	3896	4058	3122	3809	3273	4025	3376	3363
Dry matter	88.30	89.57	91.79	91.75	92.91	91.28	91.76	90.92	87.81	90.77	90.97	94.03
Apparent dry matter digestibility	60.75	73.92	71.72	61.92	61.24	73.38	44.62	45.64	56.59	76.96	56.95	64.24
True dry matter digestibility	82.48	95.04	91.82	82.04	80.86	93.71	64.74	66.13	78.56	97.52	77.84	83.40
Crude protein	12.58	12.73	14.36	11.69	14.97	13.48	10.90	13.84	13.45	13.00	12.28	12.58
Ether extract	2.13	2.89	3.26	2.49	2.65	3.27	3.23	3.53	3.39	3.22	3.09	2.34
Crude fibre	3.44	3.49	1.99	5.94	1.98	2.96	8.99	2.24	3.75	2.95	2.95	1.99
Total phenols	0.34	0.09	0.06	0.56	0.04	0.07	0.37	0.42	0.51	0.19	0.39	0.58
Ash	1.03	1.51	1.74	2.50	1.72	1.75	2.94	1.76	2.07	1.51	2.25	1.46

[1] Energy variables are expressed as kcal per kg and the rest of the response variables are expressed as gram per 100 g.

Table 2. Summary of amino acids analysis of different varieties of sorghum kernels.

Items[1]/Sorghum Type	1	2	3	4	5	6	7	8	9	10	11	12
Total												
Alanine	1.20	1.02	1.38	1.09	1.52	1.42	1.08	1.49	1.37	1.24	1.31	1.17
Arginine	0.35	0.33	0.39	0.39	0.40	0.43	0.27	0.35	0.34	0.33	0.31	0.32
Aspartic acid	0.82	0.82	1.09	0.95	1.04	1.01	0.75	0.94	0.88	0.90	0.87	0.89
Cysteine	0.21	0.20	0.24	0.22	0.26	0.23	0.21	0.23	0.21	0.21	0.22	0.22
Glutamic acid	2.52	2.28	3.19	2.36	3.27	2.96	2.32	3.13	2.78	2.71	2.85	2.50
Glycine	0.33	0.30	0.41	0.36	0.39	0.36	0.29	0.36	0.34	0.31	0.33	0.33
Histidine	0.21	0.22	0.27	0.25	0.28	0.30	0.30	0.26	0.24	0.23	0.24	0.22
Isoleucine	0.44	0.42	0.53	0.48	0.62	0.63	0.47	0.54	0.54	0.44	0.46	0.44
Leucine	1.50	1.31	1.74	1.37	1.88	1.76	1.34	1.80	1.63	1.50	1.59	1.39
Lysine	0.18	0.22	0.23	0.29	0.24	0.29	0.17	0.22	0.24	0.22	0.21	0.23
Methionine	0.17	0.11	0.19	0.19	0.25	0.27	0.18	0.21	0.14	0.18	0.21	0.17
Phenylalanine	0.57	0.52	0.74	0.54	0.74	0.65	0.47	0.67	0.63	0.57	0.59	0.56
Proline	0.98	0.83	1.22	0.89	1.30	1.00	0.78	1.05	1.00	1.09	1.00	0.85
Serine	0.54	0.51	0.63	0.51	0.65	0.60	0.47	0.62	0.60	0.56	0.57	0.53
Threonine	0.36	0.34	0.47	0.38	0.44	0.45	0.35	0.42	0.45	0.39	0.40	0.39
Tryptophan	0.25	0.23	0.26	0.22	0.29	0.22	0.24	0.20	0.30	0.27	0.26	0.25
Tyrosine	0.40	0.40	0.50	0.41	0.53	0.52	0.38	0.53	0.45	0.42	0.45	0.40
Valine	0.60	0.53	0.77	0.63	0.78	0.80	0.59	0.75	0.67	0.63	0.63	0.64
True digestible												
Alanine	0.69	1.00	1.35	0.90	1.49	1.38	0.49	1.18	0.63	1.16	0.86	0.51
Arginine	0.18	0.31	0.38	0.29	0.38	0.40	0.12	0.24	0.12	0.27	0.18	0.14
Aspartic acid	0.49	0.80	1.03	0.78	0.99	0.94	0.40	0.70	0.36	0.84	0.53	0.39
Cysteine	0.09	0.19	0.21	0.11	0.22	0.21	0.11	0.13	0.09	0.18	0.12	0.09
Glutamic acid	1.35	2.25	3.14	1.84	3.20	2.87	1.14	2.37	1.02	2.59	1.74	1.02
Histidine	0.13	0.19	0.24	0.18	0.25	0.24	0.13	0.14	0.06	0.16	0.11	0.08
Isoleucine	0.27	0.40	0.50	0.38	0.60	0.59	0.26	0.42	0.22	0.38	0.29	0.23
Leucine	0.77	1.29	1.69	1.11	1.86	1.70	0.52	1.37	0.46	1.40	0.89	0.33
Lysine	0.12	0.21	0.22	0.24	0.20	0.25	0.11	0.15	0.10	0.17	0.15	0.13
Methionine	0.11	0.12	0.19	0.13	0.24	0.26	0.13	0.14	0.05	0.17	0.15	0.09
Phenylalanine	0.30	0.50	0.72	0.40	0.72	0.62	0.16	0.50	0.18	0.53	0.35	0.21
Proline	0.46	0.76	1.20	0.59	1.25	0.90	0.18	0.68	0.24	0.92	0.51	0.37
Serine	0.31	0.49	0.60	0.38	0.61	0.55	0.21	0.46	0.23	0.50	0.30	0.21
Threonine	0.18	0.33	0.44	0.25	0.39	0.38	0.14	0.29	0.19	0.34	0.21	0.17
Tyrosine	0.14	0.40	0.46	0.31	0.53	0.49	0.09	0.38	0.10	0.38	0.23	0.13
Valine	0.36	0.50	0.74	0.51	0.76	0.74	0.31	0.59	0.28	0.54	0.39	0.34

[1] Energy variables are expressed as kcal per kg and the rest of the response variables are expressed as gram per 100 g.

Table 3. The relation between colour space with chemical composition and some nutrient digestibility of sorghum grain (fitted equations along with coefficients of determination)[1].

Items[2]	Best-fitted equation[3]	R^2_{adj}	*P*-value
Apparent metabolisable energy	$X = -3469.47 + 1750.79\,(a) - 88.24\,(a^2) + 12.28\,(b^2) - 60.29\,(a \times b) - 0.83(a \times b^2) + 4.03\,(a^2 \times b)$	0.744	0.031
Apparent metabolisable energy $_{\text{N-Corrected}}$	$X = -2657.08 + 1573.85\,(a) - 83.31(a^2) + 10.87\,(b^2) - 49.68\,(a \times b) - 0.83\,(a \times b^2) + 3.68\,(a^2 \times b)$	0.760	0.026
True metabolisable energy	$X = -5280.70 + 2683.70\,(a) - 158.64\,(a^2) + 13.01\,(b^2) - 105.16\,(a \times b) - 0.093\,(a^2 \times b^2) + 8.68\,(a^2 \times b)$	0.924	0.002
True metabolisable energy $_{\text{N-Corrected}}$	$X = -2483.13 + 1586.81\,(a) - 84.48\,(a^2) + 11.15\,(b^2) - 49.85\,(a \times b) - 0.87\,(a \times b^2) + 3.75\,(a^2 \times b)$	0.808	0.016
Dry matter	$X = 94.76 - 10.72\,(a) + 1.09\,(a^2) + 5.01\,(b) - 0.15\,(b^2) - 0.0008\,(a^2 \times b^2) + 0.01\,(a \times b^2) - 0.06\,(a^2 \times b)$	0.952	0.002
Crude protein	$X = -12.48 + 0.17(a^2) + 4.63\,(b) - 0.13\,(b^2) - 0.40\,(a \times b) - 0.0001(a^2 \times b^2) + 0.01\,(a \times b^2)$	0.889	0.004
Crude fibre	$X = -103.48 + 28.89\,(a) - 1.58\,(a^2) + 7.75\,(b) - 0.15\,(b^2) - 2.10\,(a \times b) - 0.002\,(a^2 \times b^2) + 0.04\,(a \times b^2) + 0.12\,(a^2 \times b)$	0.957	0.008
Neutral detergent fibre	$X = 49.34 - 2.63\,(a) - 0.06\,(b^2) - 0.23\,(a \times b) - 0.0006\,(a^2 \times b^2) + 0.01\,(a \times b^2) + 0.02\,(a^2 \times b)$	0.979	0.001
Acid detergent fibre	$X = 28.54 - 0.72\,(a) - 0.80\,(b) + 0.001\,(a \times b^2)$	0.793	0.001
Nitrogen free extract	$X = 158.94 - 31.50\,(a) + 2.18\,(a^2) - 2.40\,(b) + 1.63\,(a \times b) + 0.002(a^2 \times b^2) - 0.02\,(a \times b^2) - 0.14\,(a^2 \times b)$	0.905	0.008
Total phenols	$X = -0.004 + 0.009\,(a^2) - 0.001\,(b^2) + 0.009\,(a \times b) + 0.00002\,(a^2 \times b^2) - 0.001\,(a^2 \times b)$	0.919	<0.001

[1] L was excluded from the statistical analysis due to significant collinearity with a and b.
[2] Energy variables are expressed as kcal per kg and the rest of the response variables are expressed as gram per 100 g.
[3] The best-fitted equation was selected based on the highest obtained coefficient of determination (R^2_{adj}).

content (with exception of gross energy, P = 0.140) and chemical composition (with exception of ether extract, P = 0.057 and ash content, P = 0.062). Apparent and true dry matter digestibility were not significantly predicted (P > 0.05) by the regression model.

Fitted prediction equations for total and true digestible amino acids content of sorghum grains are shown in Tables 4 and 5, respectively. Total and true digestible amino acids content of sorghum grains were predicted by the regression model (P < 0.05) based on the linear and quadratic terms along with the interaction between a*b data. However, among the amino acids, methionine, lysine and tryptophan could not be predicted (P > 0.05) by the regression model. No regression model was found to predict true digestible tyrosine content of sorghum grain.

The regression model significantly predicted the metabolisable energy content and nutrient digestibility of sorghum grains (P < 0.05; R^2_{adj}>0.98) based on linear, quadratic and interactions effects between TP, CP, CF and EE data (Table 6). The linear, quadratic and interactive effects between TP, CP, CF and EE data (P < 0.05) created prediction equations for estimation of total and true digestible essential amino acids content of sorghum grains with exception of true digestible phenylalanine (Table 7).

Table 4. The relation between colour space with total amino acids content of sorghum grain (fitted equations along with coefficient of determination)[1].

Amino acids[2]	Best-fitted equation[3]	R^2_{adj}	*P*-value
Alanine	$X = 4.85 - 1.43(a) + 0.08(a^2) - 0.004(b^2) + 0.07(a \times b) + 0.0001(a^2\times b^2) - 0.001(a\times b^2) - 0.005(a^2\times b)$	0.843	0.023
Arginine	$X = -1.06 + 0.27(a) - 0.01(a^2) + 0.06(b) - 0.01(a \times b) - 0.000002(a^2\times b^2) + 0.0006(a^2\times b)$	0.757	0.027
Aspartic acid	$X = -0.607 + 0.124(a) + 0.09(b) + 0.00003(a^2\times b^2) - 0.0003(a\times b^2) - 0.0005(a^2\times b)$	0.947	<0.001
Cysteine	$X = -0.151 + 0.003(a^2) + 0.066(b) - 0.002(b^2) - 0.005(a \times b) + 0.0002(a\times b^2) - 0.00008(a^2\times b)$	0.726	0.036
Glutamic acid	$X = 10.26 - 2.76(a) + 1.16(a^2) - 0.18(b) + 0.16(a \times b) + 0.0002(a^2\times b^2) - 0.003(a\times b^2) - 0.01(a^2\times b)$	0.809	0.034
Glycine	$X = 0.187 - 0.03(b) + 0.002(b^2) + 0.008(a \times b) + 0.00001(a^2\times b^2) - 0.0003(a\times b^2) - 0.0004(a^2\times b)$	0.818	0.014
Histidine	$X = 0.219 + 0.018(b) - 0.0003(b^2) - 0.001(a \times b) - 0.000002(a^2\times b^2)$	0.679	0.015
Isoleucine	$X = -0.0706 + 0.0915(b) - 0.0022(b^2) - 0.0034(a \times b) + 0.09(a\times b^2) + 0.0001(a^2\times b)$	0.710	0.010
Leucine	$X = -0.332 + 0.264(b) - 0.006(b^2) - 0.0088(a \times b) + 0.00029(a\times b^2)$	0.775	0.004
Phenylalanine	$X = 1.131 - 0.342(a) + 0.021(a^2) + 0.023(a \times b) + 0.00003\ (a^2\times b^2) - 0.0004(a\times b^2) - 0.0016(a^2\times b)$	0.885	0.005
Proline	$X = -0.312 + 0.170(b) - 0.003(a^2) - 0.006(a \times b) + 0.000004\ (a^2\times b^2) + 0.0001(a^2\times b)$	0.829	0.005
Serine	$X = 1.340 - 0.355(a) + 0.021(a^2) - 0.0007(b^2) + 0.02(a \times b) + 0.00003\ (a^2\times b^2) - 0.0003(a\times b^2) - 0.0014(a^2\times b)$	0.918	0.007
Threonine	$X = -0.010 + 0.055(b) - 0.001(b^2) - 0.0016(a \times b) + 0.00005(a\times b^2)$	0.705	0.011
Tyrosine	$X = 0.828 - 0.235(a) + 0.016(a^2) + 0.016(a \times b) + 0.000027(a^2\times b^2) - 0.00034(a\times b^2) - 0.0013\ (a^2\times b)$	0.725	0.036
Valine	$X = 1.374 - 0.414(a) + 0.027(a^2) + 0.028(a \times b) + 0.00005(a^2\times b^2) - 0.0006(a\times b^2) - 0.002(a^2\times b)$	0.813	0.015

[1] L was excluded from the statistical analysis due to significant collinearity with a and b.
[2] Amino acids output are expressed as gram per 100 g.
[3] The best fitted equation was selected based on the highest obtained coefficient of determination (R^2_{adj}).

Table 5. The relation between colour space with true digestible amino acids content of sorghum grain (fitted equations along with coefficient of determination)[1].

Amino acids[2]	Best-fitted equation[3]	R^2_{adj}	*P*-value
Alanine	$X = -6.99 + 1.491(a) - 0.066(a^2) + 0.412(b) - 0.075(a \times b) + 0.003(a^2\times b)$	0.887	0.001
Arginine	$X = -2.119 + 0.625(a) - 0.034(a^2) + 0.004(b^2) - 0.018(a \times b) - 0.00038(a\times b^2) + 0.0015(a^2\times b)$	0.871	0.006
Aspartic acid	$X = -18.219 + 3.876(a) - 0.177(a^2) + 1.448(b) - 0.024(b^2) - 0.292(a \times b) - 0.0002(a^2\times b^2) - 0.0049(a\times b^2) + 0.013(a^2\times b)$	0.973	0.004
Cysteine	$X = -3.396 + 0.626(a) - 0.025(a^2) + 0.379(b) - 0.008(b^2) - 0.066(a \times b) - 0.000055(a^2\times b^2) + 0.001(a\times b^2) + 0.003(a^2\times b)$	0.967	0.006
Glutamic acid	$X = -14.08 + 2.98(a) - 0.131(a^2) + 0.905(b) - 0.162(a \times b) + 0.007(a^2\times b)$	0.935	0.003
Histidine	$X = -1.043 + 0.337(a) - 0.019(a^2) + 0.002(b^2) - 0.010(a \times b) - 0.0002(a\times b^2) + 0.0008(a^2\times b)$	0.850	0.009
Isoleucine	$X = -2.433 + 0.539(a) - 0.024(a^2) + 0.148(b) - 0.027(a \times b) + 0.001(a^2\times b)$	0.777	0.010
Leucine	$X = -10.11 + 2.117(a) - 0.095(a^2) + 0.596(b) - 0.109(a \times b) + 0.005(a^2\times b)$	0.840	0.004
Lysine	$X = -1.365 + 0.411(a) - 0.021(a^2) + 0.0025(b^2) - 0.012(a \times b) - 0.0002(a\times b^2) + 0.0009(a^2\times b)$	0.726	0.036
Methionine	$X = 0.135 + 0.00086(b^2) + 0.000005(a^2\times b^2) - 0.00014(a\times b^2)$	0.641	0.010
Phenylalanine	$X = -3.446 + 0.762(a) - 0.033(a^2) + 0.227(b) - 0.040(a \times b) + 0.0017(a^2\times b)$	0.939	<0.001
Proline	$X = -6.349 + 1.238(a) - 0.051(a^2) + 0.394(b) - 0.068(a \times b) + 0.003(a^2\times b)$	0.935	<0.001
Serine	$X = -8.533 + 1.768(a) - 0.079(a^2) + 0.710(b) - 0.012(b^2) - 0.137(a \times b) - 0.0001(a^2\times b^2) + 0.002(a\times b^2) + 0.006(a^2\times b)$	0.949	0.011
Threonine	$X = -3.679 + 0.754(a) - 0.033(a^2) + 0.295(b) - 0.004(b^2) - 0.055(a \times b) - 0.000033(a^2\times b^2) + 0.0008(a\times b^2) + 0.002(a^2\times b)$	0.988	0.001
Valine	$X = -3.932 + 0.848(a) - 0.037(a^2) + 0.229(b) - 0.042(a \times b) - 0.002(a^2\times b)$	0.904	<0.001

[1] L was excluded from the statistical analysis due to significant collinearity with a and b.
[2] Each amino acid output is expressed as gram per 100 g.
[3] The best-fitted equation was selected based on the highest obtained coefficient of determination (R^2_{adj}).

Table 6. The relation between chemical composition with energy content of sorghum grain (fitted equations along with coefficients of determination)[1].

Items[2]	Best Fitted Equation[3]	R^2_{adj}	*P*-value
Apparent metabolisable energy	$X= -2490.21 - 19\,437\ (TP^2) - 252.20\ (CP^2) - 32\,161\ (EE) - 43\,776\ (CF) + 2372.07\ (CF^2) + 22\,227\ (Ash^2) - 4073.53\ (TP\times CF) + 3391.94\ (CP*\ CF) - 18\,765\ (EE\times Ash) - 7664.69\ (CF\times Ash)$	0.999	0.009
Apparent metabolisable energy $_{N\text{-}Corrected}$	$X = -568\,458 - 79\,663\ (CP) - 2619.96\ (CP^2) - 26\,718\ (EE) - 62\,281\ (CF) - 1816.57\ (CF^2) - 389.73\ (Ash^2) + 1128.26\ (CP*EE) - 4863.02\ (CP\times CF)\ -+3463.91\ (EE\times CF) + 1020.10\ (CF\times Ash)$	0.999	0.003
True metabolisable energy	$X = -2\,070\,413 + 5998.06\ (TP) - 56\,479\ (TP2) + 284\,951\ (CP) - 10\,078\ (CP^2) + 63\,768\ (EE) - 14\,306\ (EE2) + 58\,682\ (CF) + 11\,310\ (Ash) - 9275.35\ (TP\times EE) - 4689.68\ (CP\times CF)$	0.999	0.003
True metabolisable energy $_{N\text{-}Corrected}$	$X = -667\,872 + 94\,170\ (TP) + 96\,033\ (CP) - 3501.88\ (CP^2) + 1486.46\ (CF2) + 32\,994\ (Ash) - 10\,729\ (TP*\ CP) + 15\,427\ (TP * EE) - 250.32\ (CP\times Ash) - 4154.39\ (EE * Ash) - 5278.41\ (CF\times Ash)$	0.999	0.002

[1] TP: total phenols, CP: crude protein, CF: crude fibre, EE: ether extract,
[2] Energy variables are expressed as kcal per kg and the rest of the response variables are expressed as gram per 100 g.
[3] The best-fitted equation was selected based on the highest obtained coefficient of determination (R^2_{adj}).

Discussion

Analysis of poultry feedstuffs for digestible values via *in vivo* and/or *in vitro* assays prior to feed formulation is a costly and time-consuming process. Image analysis may provide a rapid and cost-effective approach compared with *in vitro* and *in vivo* measurements, because it resulted in accurate equations for estimating chemical compositions and nutritional values of sorghum grains in the current study. Previously, Sedghi et al. (2012) found a significant correlation between sorghum grain colour and tannin content, which was due to the fact that pigmentation of pericarp and testa in sorghum kernels alter by phenolic compounds like tannin. This pigmentation and colour in sorghum grains are associated with R, Y, B1, B2 and S genes. Domination of each pair of these genes control pigmentation as well as total phenols content. For instance, when both R and Y are dominant, anthocyanidin pigments will enhance and the pericarp become more reddish (Rooney et al. 1981; Hahn and Rooney 1986). Sedghi et al. (2012) regressed L*a*b data with linear regression for predicting tannin content with a goodness of fit of 0.88 (in terms of R^2). However, in the current study, when quadratic and interaction effects of L*a*b colour were included in the model, this resulted in a goodness of fit of 0.919 (in terms of R^2_{adj}).

Table 7. The relation between chemical composition with total and true essential amino acids of sorghum grain (fitted equations along with coefficients of determination)[1].

Items[2]	Best-fitted equation[3]	R^2_{adj}	*P*-value
Total			
Arginine	X = 11.57 −7.27 (TP) +1.06 (EE^2) − 6.26 (CF) + 020 (CF^2) − 1.12 (Ash) − 0.041 (TP ×EE) +0.43 (CP×CF) −0.28 (EE×CF)	0.999	0.006
Cysteine	X = 3.84 −6.85(EE) −2.11(CF) +12.36(Ash) −0.43(TP×CP) +1.87(TP×EE) −0.17(TP×CF) +0.49(CP×EE) +0.16(CP×CF) −1.07(CP×Ash) +0.09(EE×CF)	0.989	0.010
Glycine	X = −0.65 −24.53(TP) +5.21(EE) −3.78(Ash) +2.41(TP×CP) −1.20(TP×EE) +2.22(TP×CF) −4.80(TP×Ash) −0.43(CP×EE) −0.06(CP×CF) +0.49(CP×ASH)	0.982	0.003
Histidine	X = 21.31 −7.84(TP^2) −0.14(CP^2) −17.61(EE) −2.17(CF) +7.88(Ash) −1.04(Ash^2) +1.45(TP×EE) +1.34(CP×EE) +0.86(EE×CF) −1.49(EE×Ash)	0.994	0.017
Leucine	X = −237.61 −5.18(TP^2) +35.36(CP) −1.27(CP^2) +15.16(CF) −0.33(CF^2) +2.55(TP×EE) −3.01(TP×Ash) −0.18(CP×EE) −1.13(CP×CF) +0.40(CF×Ash)	0.969	0.003
Isoleucine	X = −74.76 + 5.64(CP) −7.15(CF) −0.68(CF^2) +61.06(Ash) +2.81(Ash^2) +0.16(TP×CF) +0.86(CP×CF) −4.74(CP×Ash) −0.27(EE×Ash) −4.33(CF×Ash)	0.997	0.012
Lysine	X = 14.81 −51.74(TP) −0.65(CP) −2.38(CF) −0.16(CF^2) +4.41(TP×CP) +3.76(TP×CF) −8.16(TP×Ash) −0.11(CP×EE) +0.21(EE×CF) +0.93(CF×Ash)	0.994	0.019
Methionine	X = 11.54 + 2.63(TP^2) +1.43(EE2) −0.96(CF) +0.06(CF^2) −7.57(Ash) −0.40(TP×CP) +0.58(TP×CF) −0.64(CP×EE) +0.79(CP×Ash) −0.30(EE×Ash)	0.991	0.007
Phenylalanine	X = 76.63 −5.0(CP) −15.83(EE) −0.19(EE2) −12.53(CF) −1.96(TP×CP) +7.36(TP×EE) +1.18(CP×EE) +0.79(CP×CF) −0.37(CP×Ash) +1.57(CF×Ash)	0.986	0.003
Threonine	X = −0.31 + 0.01(CP^2) +1.46(EE) −2.94(Ash) +0.08(TP×CP) −1.10(TP×EE) +1.46(TP×Ash) −0.15(CP×EE) +0.09(CP×Ash) +0.48(EE×Ash) +0.01(CF×Ash)	0.972	0.003
Tryptophan	X = 19.09 −25.48(TP) +13.65(TP^2) +0.22(EE2) −6.65(CF) −3.86(Ash) +6.90(Ash^2) +5.51(TP×EE) +0.48(CP×CF) −1.12(CP×Ash) −1.48(EE×Ash)	0.979	0.013
Valine	X = −4.45 −69.09(TP) −0.12(CF^2) +17.27(Ash) +6.74(TP×CP) +5.70(TP×CF) −18.25(TP×Ash) −0.16(CP×CF) −0.62(CP×Ash) +0.36(EE×CF) −1.35(EE×Ash)	0.981	0.007
True digestible			
Arginine	X = 122.7 −2.67(TP^2) −9.39(CP) −0.61(CF^2) −81.3(Ash) −2.0(Ash^2) +0.06(CP×EE) −0.40(CP×CF) +6.32(CP×Ash) −0.40(EE×Ash) +5.03(CF×Ash)	0.988	0.006
Cysteine	X = 104.76 −35.23(TP^2) −0.69(CP^2) −82.44(EE) −11.47(CF) +29.96(Ash) +6.66(TP×EE) +6.47(CP×EE) +4.70(EE×CF) −9.33(EE×Ash) −0.56(CF×Ash)	0.977	0.036
Histidine	X = 3.25 + 0.02(CP^2) −0.03(CF^2) −8.65(Ash) +2.21(Ash^2) −2.25(TP×EE) −1.11(TP×CF) +6.31(TP×Ash) +0.01(CP×EE) +0.26(EE×CF) −0.10(CF×Ash)	0.948	0.030
Leucine	X = 3286.92 −465.46(CP) +14.97(CP^2) −10.67(EE^2) −330.95(CF) +8.45(CF^2) +44.37(Ash^2) +8.96(CP×EE) +25.59(CP×CF) −23.97(EE×Ash) −18.23(CF×Ash)	0.958	0.002
Isoleucine	X = 219.61 + 44.57(TP^2) −21.27(CP) +74.81(EE) −1.38(EE^2) −270.77(Ash) +38.76(Ash^2) −8.55(TP×CF) −2.61(CP×EE) +18.04(CP×Ash) −19.45(EE×Ash)	0.990	0.012
Lysine	X = 5.20 + 0.03(CP^2) −0.05(CF^2) −13.90(Ash) +3.57(Ash^2) −3.62(TP×EE) −1.87(TP×CF) +10.47(TP×Ash) +0.02(CP×EE) +0.43(EE×CF) −0.20(CF×Ash)	0.989	0.010
Methionine	X = 180.46 −26.42(CP) +1.05(CP^2) −1.66(EE^2) −1.24(CF^2) −20.93(Ash) −15.59(Ash^2) +0.47(CP×Ash) −2.38(EE×CF) +11.04(EE×Ash) +9.34(CF×Ash)	0.990	0.002
Phenylalanine	X = 319.92 −72.67(TP) −1.48(CP^2) −5.20(EE) −2.89(CF^2) −363.63(Ash) +13.40(TP×EE) +14.76(TP×Ash) −1.96(CP×CF) +24.98(CP×Ash) +23.02(CF×Ash)	0.947	0.117
Threonine	X = 226.25 −41.93(TP^2) −19.50(CP) −93.14(EE) −12.58(CF) +37.18(Ash) +2.12(TP×CP) +7.53(CP×EE) +5.88(EE×CF) −11.18(EE×Ash) −1.42(CF×Ash)	0.990	0.008
Valine	X = 57.43 −76.82(TP) +86.49(TP^2) −0.15(CP^2) +4.53(EE^2) −0.64(CF2) −37.56(Ash) +35.24(Ash^2) +31.14(TP×EE) −45.31(TP×Ash) −19.78(EE×Ash)	0.985	0.028

[1] TP: total phenols, CP: crude protein, CF: crude fibre, EE: ether extract.
[2]Amino acids output are expressed as gram per 100 g.
[3]The best fitted equation was selected based on the highest obtained coefficient of determination (R^2_{adj}).

In poultry, sorghum grain is primarily used as an energy source, but the quantity and quality of protein and amino acids are still important. A large number of feeding trials have been conducted to identify factors that influence the energy and amino acids contents of sorghum grains for poultry (Mabelebele et al. 2017; Silveira et al. 2017). Some studies showed that variation in the energy and amino acids digestibility of sorghum grains were associated with differences in phenolic and tannin contents of sorghum grains (Ebadi et al. 2011; Sedghi et al. 2011). Because of the relationship between colour and phenolic contents, this study was conducted to describe the relationship between colour and metabolisable energy in some sorghum varieties. The results indicated that the metabolisable energy contents of sorghum grains could be accurately described by colour image analysis. In addition, Sedghi et al. (2011) reported that the chemical composition of sorghum grains could be used in mathematic modelling to predict energy content of sorghum grains. They used the linear effect of total phenols, ash, CP, CF and EE in the regression model, and predicted the true metabolisable energy nitrogen-corrected (TME_n) of sorghum grains with the R^2 value of 0.71. However, in the current study, beside the linear effect, quadratic and interaction effects of chemical composition were used to develop the regression model to predict $TME_{n,}$ more accurately, which resulted in a goodness of fit of 0.993 in terms of R^2_{adj}.

Moreover, due to the existence of tannins in sorghum grains and its adverse effects on amino acids digestibility, the estimation of amino acids digestibility in this grain has been highlighted in some previous studies (Ebadi et al. 2011). For instance, Mitaru et al. (1985) reported that amino acids digestibility were in the range of 84–93% and 43–73% in high and low-tannin sorghum varieties, respectively. As such, the relationship between colour and amino acids digestibility may be explained by the presence of phenolic compounds and their correlations with the nutrient digestibility in sorghum grains. A number of trials were conducted to compare the nutritional value of sorghum grains with different colour where in some of them the differences between sorghum grains were more associated with variation in endosperm type and texture rather than to the differences in pericarp colour (Liu et al. 2013). Therefore, they concluded that colour of the sorghum grain has little or no correlation with its nutritional value. In contrast, a more recent study showed that white sorghum grain had higher amino acid and starch digestibility than the red variety (Liu et al. 2013). Similar to the prediction for energy content, for amino acid prediction, besides the linear effect, quadratic and interactive effect of colour data and chemical composition (TP, CP, CF and EE) were included in the regression model. Ebadi et al. (2011) created regression models based on the linear effect of CP, CF, EE, TP and ash to estimate true digestible amino acids in sorghum grains with R^2 values ranging from 0.40 to 0.85 for training data and 0.51 to 0.97 for testing data.

The results of previous studies about the correlation between colour and nutritional values were inconsistent. These discrepancies in previous studies might be attributed to the fact that colour of sorghum grains were evaluated visually. Hence, processing methodology in image analysis was quite different from that of in human beings; it seems that the image analysis-based systems show better results than visual assessments.

The current study indicated that digital image analysis is a promising alternative method to the conventional methods for determination of chemical compositions of feedstuffs, which was supported by presenting fitted equations to estimate the nutritional values of sorghum grains with the high goodness of fit in terms of $R^2{}_{adj}$. In addition, energy and amino acids content of sorghum grains can be predicted using the results conventional chemical analysis like CP, CF, EE and TP. It was found that in application of colour analysis and chemical composition to create prediction equations, including the quadratic and interactive effects of input data along with their linear effects, resulted in equations with high goodness of fit. Due to variations among sorghum varieties, further research is warranted to establish more accurate equations for estimating the nutritional values of sorghum grains based on their colour.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

R. Akbari Moghaddam Kakhki http://orcid.org/0000-0003-2621-0623

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