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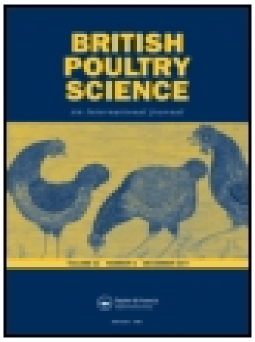
Abdulla, J.M., Rose, S.P., Mackenzie, A.M. and Pirgozliev, V. 2021. Variability of amino acid digestibility in different field bean cultivars for broilers. *British Poultry Science*.

19 February 2021



British Poultry Science

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/cbps20

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Publisher: Taylor & Francis & British Poultry Science Ltd

Journal: *British Poultry Science*

DOI: 10.1080/00071668.2021.1891525

Variability of amino acid digestibility in different field bean cultivars for broilers

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ABSTRACT

1. The amino acid composition of 10 different UK-grown field bean cultivar samples from the same harvest year were determined.

2. Diets included each bean sample at a level of 200 g/kg feed compared against a control diet formulated with only soyabean sources, whereby the beans replaced the soyabean meal, and were used to compare ileal amino acid digestibility. The amino acid digestibility coefficients for the bean samples were obtained via substitution method.

3. The data showed that one field bean sample, cultivar Sultan, had a lower amino acid digestibility ($P<0.05$) than that of the other nine . This sample had a higher tannin content that may have affected amino acid digestibility.

Keywords: field beans, amino acid digestibility, broilers

INTRODUCTION

Pulses are edible dry mature seeds of leguminous crops, and, in the UK, pulses are almost exclusively peas (*Pisum sativum*) and field beans (*Vicia faba*). The main agronomic advantage of pulses over other combinable crops is that pulses require no nitrogen fertiliser

Accepted for publication 24 January 2021

and provide residual nitrogen for the following crop which can help to reduce greenhouse gas emissions (Canfield *et al.,* 2010). The mean crude protein (CP) concentration of beans is higher than peas (Jezierny *et al.,* 2011) but lower than soybean meal (Ravindran *et al.,* 2014). There is a similarity between the amino acid profiles of beans and soybean meal (Gatta *et al.,* 2013), thus beans can be used as alternative protein sources for poultry. However, the relatively high concentration of antinutrients, primarily non-starch polysaccharides (NSP), some oligosaccharides, tannins and pyrimidine in the field beans, has led to a low uptake of feed industry use of this grain in commercial poultry diets (Longstaff and McNab 1991a, 1991b; Nalle *et al.,* 2010b).

Consideration of amino acid (AA) digestibility in feed formulation for broiler chickens is critical to increase the efficacy of protein utilisation. However, this approach to feed formulation requires knowledge of AA digestibility of feed ingredients (Zuber *et al.,* 2016). One additional marked advantage of diet formulations based on digestible AA is the ability to utilise protein sources with variable digestibility coefficients (Bryden and Li 2010). Although a number of studies on AA digestibility of field beans in broilers have been conducted, there were differences in the methods employed (O'Neill *et al.,* 2012), geographical location (Nalle *et al.,* 2010; Woyengo and Nyachoti 2012), and the year of investigation (Longstaff and McNab 1991a, 1991b). The chemical composition of crops varies due to a number of major factors including husbandry, location and seasonal as well as genetic factors (Pirgozliev *et al.,* 2002; Abdulla *et al.,* 2016 a, b; Mphande *et al.,* 2020).

The aim of the current experiment was to investigate the variation in AA digestibility of ten UK-grown field bean cultivar samples produced during the same harvest year when fed to broilers. The hypotheses tested were that variability due to agronomic differences may affect amino acid digestibility in bean cultivar samples. Understanding this variability would allow greater confidence in their use.

Accepted for publication 24 January 2021

MATERIAL AND METHODS

Field bean cultivar samples

Ten different flower colour, UK-grown field beans, including three spring (Fuego, Fury and Maris Bead) and seven winter grown cultivars (Arthur, Buzz, Clipper, Divine, Honey, Sultan and Wizard) from the 2013 harvest year were obtained from Askew & Barrett (Pulses) Ltd, Wisbech, UK. The beans were grown at different locations although there was no information on agronomy or soil type available. All harvested field bean samples were kept at ambient air temperatures in a dry store and were used in the broiler feeding experiment after approximately 6 months of storage. Before the feeding trial, the bean samples were hammer-milled using a 4 mm screen and then mixed in a horizontal mixer with the other feed ingredients. Freshly milled field beans were used for analyses and in the feeding study, to avoid spoilage. Proximate analysis, gross energy, carbohydrate and mineral contents, phenolic compounds, grain quality and viscosity of the bean samples were measured and presented in an earlier paper (Abdulla *et al.,* 2020).

Amino acid analysis

Analysis of AA in experimental diets and ileal digesta samples was performed (ISO 13903:2005). The samples were oxidised with hydrogen peroxide/formic acid/phenol solution, which converted any methionine to methionine sulphone and cystine to cysteic acid, as some of the cystine and methionine would otherwise be lost upon hydrolysis. Excess oxidation reagent was decomposed with sodium metabisulphite. The oxidised sample was hydrolysed with 6 M hydrochloric acid for 24 hours at 110°C. The hydrolysate was adjusted to pH 2.20 and the amino acids separated by ion exchange chromatography and determined by post column reaction with Ninhydrin using photometric detection at 570nm (440nm for Proline). Titanium dioxide (TiO_2) content in digesta was quantified following the method by Short *et al.,* (1999).

Accepted for publication 24 January 2021

Diet formulation

The balancer diet contained higher metabolisable energy (ME) than breeder's recommendation (Aviagen Ltd., Edinburgh, UK), to allow the ME of the field bean diets to be close to requirements. The balancer diet contained 5 g/kg of TiO_2 as an indigestible marker. Ten test diets were produced, which included 200 g/kg of each of the 10 different field bean cultivars, and 800 g/kg of the balancer feed. Note: The same dietary formulation was published previously (Abdullah et al 2020) - see Table 1.

Table 1 here

Husbandry and sample collection

The experiment was conducted at the National Institute of Poultry Husbandry and was approved by the Research Ethics Committee of Harper Adams University, Newport, UK. Approximately 500, one-day-old male Ross 308 broiler chicks were obtained from a commercial hatchery (Cyril Bason, Shropshire, UK). Chicks were placed in pens and were reared on wood shavings litter in an environmentally controlled room, and fed a proprietary broiler starter feed *ad libitum* for the first seven days.

On d 1 of the experiment (8 d of age) all chicks were individually weighed, and small and malformed birds were discarded, leaving 440 birds with average weight of 151 g. These birds were then randomly allocated into 88 raised-floor pens (0.36 m^2 floor area; five birds in each pen). The pens were arranged as a one tier level within a controlled environment room, and each pen was equipped with a plastic feeder and drinker and bedded with wood shavings. Each of the 11 experimental diets (control plus ten bean samples) was fed to eight randomised pen replicates. Feed and water were provided *ad libitum* throughout the experimental period.

The trial feeing period continued for 13 d (until 21 d old). The rearing temperature and lighting programme followed industry standards (Aviagen Ltd., Edinburgh, UK).

On the final day of the experiment birds, aged 21 d, were slaughtered and the ileum, situated between Meckel's diverticulum and the ileocaecal junction, was removed. The ileal contents from five birds per pen were then pooled and freeze dried in preparation for future analyses and TiO marker determination.

Determination of ileal amino acid digestibility coefficients

The dried digesta and balancer diet samples were ground to pass through a 0.8 mm screen. The amino acids of each dried pooled digesta sample and the balancer diet samples were determined in duplicate as described for the field bean samples earlier. The coefficients of ileal amino acid digestibility were determined *via* the indigestible marker technique (Whiting *et al.,* 2019), using the following equation:

$$Amino\ acid\ digestibility\ coefficients = 1 - \frac{digAA/digti}{dietAA/dietti}$$

where digAA was the concentration of the respective amino acid in the digesta, dig_{ti} the concentration of titanium dioxide in the digesta, $diet_{AA}$ the concentration of the respective amino acid in the diet and $diet_{ti}$ the concentration of titanium in the diet.

Statistical analysis

The observational unit was the raised-floor pen containing five birds. Statistical analyses were performed using the Genstat 18th statistical software package (Genstat 17 release 3.22 for Windows; IACR, Rothamstead, Hertfordshire, UK). The amino acid digestibility coefficients of the bean samples were statistically compared using a randomised block ANOVA. The position of pens within the room was used as the blocking factor. Tukey's range test was used to determine significant differences between treatment groups. Confidence limits were set at 95%, with $P<0.05$ being significant. The amino acid digestibility coefficients the diets (excluding the balancer diet) were obtained using the substitution method (Watts *et al.,* 2020).

Accepted for publication 24 January 2021

RESULTS

The chemical composition and physical characteristics, on a dry matter (DM) basis, of the experimental field bean cultivar samples were presented in previous report (Abdulla *et al.,* 2020). In summary, there was a range of crude protein (CP) contents with 244 g/kg the lowest (*cv.* Sultan) and 304 g/kg the highest (*cv.* Maris Beads) (CV=6.5%). The gross energy (GE) contents were similar between different cultivars, which ranged from 18.27 (*cv.* Bazz and Sultan) to 18.60 MJ/kg (*cv.* Divine), with a difference of 0.33 MJ (CV=0.7%). The range of lightness scores varied from 88 (*cv.* Sultan) to 95 (*cv.* Fury) (CV=2.4%). The cotyledon portion varied between 843 (*cv.* Clipper) and 890 g/kg whole bean (*cv.* Honey) (CV=1.6%). The range of total phenols varied from 4.5 (*cv.* Arthur) to 10.9 mg/g (*cv.* Sultan) (CV=25.7%). The mean total tannin concentration was 5.11 mg/g (CV=34.3%), and *cv.* Buzz had the lowest (2.2 mg/g) and *cv.* Sultan the highest (8.3 mg/kg) total tannin concentrations. Condensed tannins concentrations varied between 2.8 mg/g (*cv.* Arthur) and 7.3 mg/g (*cv.* Sultan) (CV=30.9%). Trypsin inhibitor levels were relatively low, and ranged between 2.3 mg/g (*cv.* Sultan) and 4.4 mg/g (*cv.* Fuego) (CV=19.2%).

The amino acid contents of the field bean cultivars are summarised in Table 2. The amino acid composition of the field bean samples agreed with previous research (O'Neill *et al.,* 2012; Woyengo and Nyachoti 2012). However, *cv.* Sultan had lower content for the majority of the amino acids when compared to the rest of the beans used in the study.

Table 2 here

Apparent ileal amino acid digestibility of the field bean

Apparent ileal digestibility coefficients (AID) of amino acids of the field bean cultivars are summarised in Table 3. The mean ileal digestibility coefficient of the field bean cultivar

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samples was 0.817 for indispensable AA (CV=5.9%), 0.807 for dispensable AA (CV=5.4%), and 0.811 for total AA (CV=5.4%). The lowest mean digestibility was observed for proline (0.623; CV=12.0%) and the highest for lysine (0.861; CV=4.2%). The mean digestibility coefficients for methionine and threonine were 0.821 (CV=12.6%), and 0.810 (CV=7.4%), respectively. The highest variation was found for methionine (CV=12.6%), proline (CV=12.0%), tyrosine (CV=9.3%) and histidine (CV=8.6%), and the lowest was for lysine (CV=4.2%) followed by arginine and glutamic acid (CV=4.3%).

There were differences in AA digestibility between the field bean cultivars. Except for methionine, the digestibility of all of the studied amino acids was significantly different ($P<0.05$) among the bean samples. The lowest AA digestibility was observed for proline in *cv.* Sultan (0.466) followed by *cv.* Clipper (0.585) and then *cv.* Arthur (0.593) and the highest digestibility were for lysine in *cv.* Wizard (0.883) followed by *cv.* Honey (0.882). The lowest and the highest lysine, threonine and cystine digestibility coefficients were for *cv.* Buzz (0.810) and *cv.* Wizard (0.883), *cv.* Sultan (0.703) and *cv.* Fury (0.853), *cv.* Sultan (0.680) and *cv.* Honey (0.840), respectively. The variation in lysine, threonine and cystine between bean samples was 8.3, 17.6 and 19.0 percentage points, respectively.

As with the exception of lysine (*cv.* Buzz had the lowest), *cv.* Sultan had the lowest AA digestibility of all amino acids and the lowest dispensable, indispensable and total AA digestibility coefficients. The highest indispensable and total AA was seen for *cv.*Wizard, and *cv.* Honey had the highest dispensable AA digestibility.

On examination of the data, *cv.* Sultan had significantly lower amino acid digestibility than most of the other samples. There were no significant differences ($P>0.05$) between any of the other bean samples.

DISCUSSION

The nutritional value of a raw material may be expressed in two ways with reference to amino acids (Wiseman *et al.,* 2003). The first is total content, and the AA profiles of the beans used in this study were similar to previous reports (Nalle *et al.,* 2010a, 2010b; O'Neill *et al.,* 2012). Faba beans are moderately good sources of lysine, but low in sulphur-containing amino acids, which are characteristics of grain legumes in general (Gatel 1994).

The other assessment is the digestibility of amino acids, which is more meaningful compared to the total content, as it is usually related to bird performance. The overall average digestibility coefficients for the bean samples were similar to previous research (Szczurek 2009; Nalle *et al.,* 2010a, 2010b; O'Neill *et al.,* 2012).

Of the 10 bean cultivars used in the study only one (*cv*. Sultan) had significantly lower overall amino acid digestibility compared to the others. This may be explained by its high tannin content, as it has been shown that tannins have a high affinity to interact with dietary cysteine, histidine, methionine and proline and reduce their bioavailability (Longstaff and McNab 1991a; Jansman 1993). This was in accord with the relatively low digestibility coefficients of cysteine, histidine, methionine and proline in *cv.* Sultan compared to the rest of the bean samples. The colour score of the bean is correlated to its antinutrient content including tannins (Beninger *et al.,* 2005). Duc *et al.,* (1995) observed that tannin activity in field beans was strongly associated with darkness of their hulls. It is generally agreed that pale legume seeds (high colour score) have higher nutritive value than dark seeded cultivars (Marquardt *et al.,* 1978). It was observed that *cv.* Sultan had a relatively low colour score, as observed in a previous study (Abdulla *et al.,* 2020). Trypsin inhibitor activity is known to affect amino acid digestibility. However, the trypsin inhibitor activity in all the samples was relatively low and the activity in *cv.* Sultan was numerically the lowest of all samples.

The results of the current experiment indicated that all 10 field bean cultivar samples had different amino acid compositions and digestibility. The commercial poultry industry requires high digestible amino acid contents in broiler diets. Nutritionists can only incorporate significant amounts of field beans in poultry diets if they have a high available energy and amino acids, and there is a need to be able to identify suitable cultivars. The results of the present experiment showed expected variability in the determined amino acid digestibility of nine different bean samples, except for one sample which had much lower total protein and amino acid digestibility. This sample was characterised by high tannin content, dark hull colour and relatively small seed size. It is possible that these criteria could be used to rapidly assess the feeding value of individual batches of field beans for poultry by feed mills. This information can be used to provide a guide to plant breeders to incorporate the best feeding characteristics in the development of new field bean cultivars.

Acknowledgments

The authors would like to thank Richard James and Ros Crocker for their technical support. Thanks to Askew & Barrett (Pulses) Ltd., who donated the field bean samples for this study.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This experiment is a part of a PhD project funded by the Ministry of Higher Education and Scientific Research – Kurdistan Regional Government – Iraq and Harper Adams University, UK.

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Accepted for publication 24 January 2021

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Accepted for publication 24 January 2021

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Table 1. Approximate chemical composition and ingredients (g/kg, as-fed) of the experimental diets and as-hatched nutrition specification for Ross 308 broilers

Ingredient	Balancer diet (g/kg)
Wheat	533.2
SBM (CP=48%)	150
Full fat soy meal	175
Maize gluten meal	37.4
Soy oil	50
L - Lysine HCl	1.9
DL - Methionine	6.3
L - Threonine	1.9
Monocalcium phosphate	20
Limestone	15.5
Sodium chloride	3.8
Vitamin/mineral premix*	5

Accepted for publication 24 January 2021

Total	1000
Calculated composition	
ME (MJ/kg)	13.71
Crude protein (g/kg)	231
Lysine (g/kg)	12.4
Methionine + cysteine (g/kg)	11.1
Calcium (g/kg)	11.1
Phosphorus av (g/kg)	8.5
Sodium (g/kg)	2.0

*The vitamin and mineral premix contained vitamins and trace elements to meet breeder's recommendation (Aviagen Ltd., Edinburgh, UK). The vitamin and mineral premix provided per kg diet: 50 mg nicotinic acid, 34 mg α-tocopherol, 15 mg pantothenic acid, 7 mg riboflavin, 5 mg pyridoxine, 3.6 mg retinol, 3 mg menadione, 2 mg thiamine, 1 mg folic acid, 200 μg biotin, 125 μg cholecalciferol, 15 μg cobalamin, 100 mg manganese, 80 mg iron, 80 mg zinc, 10 mg copper, 1 mg iodine, 0.5 mg cobalt, 0.5 mg molybdenum and 0.2 mg selenium.

Note: The same dietary formulation was published previously (Abdullah et al 2020) - see Table 1.

Table 1. Crude protein, amino acid, and antinutritive factors concentrations of ten UK grown studied field bean cultivars

	Bean cultivar										CV%	Balancer Diet
	Arthur	Buzz	Clipper	Divine	Fuego	Fury	Honey	Maris Bead	Sultan	Wizard		
Crude protein[a] (g/kg DM)	270.6	276.0	284.8	299.6	269.8	281.0	293.8	304.5	244.6	299.7	6.5	231
Indispensable amino acids[a] (g/kg DM)												
Arginine	24.0	26.3	22.8	30.3	23.0	27.6	28.1	30.4	19.5	29.5	14.0	8.1
Histidine	7.2	8.4	7.1	8.9	6.6	8.4	8.9	8.4	6.4	8.5	11.9	3.1
Isoleucine	11.9	13.5	11.1	14.0	11.1	13.0	13.9	12.8	10.2	13.1	10.5	4.5
Leucine	21.0	23.6	19.4	24.7	19.5	22.9	24.3	23.2	17.8	23.2	10.8	9.1
Lysine	18.9	20.7	17.6	21.0	17.4	20.0	20.7	19.2	16.2	20.0	8.5	6.7
Methionine	2.3	2.2	2.1	2.4	2.0	2.3	2.3	2.3	1.8	2.3	9.3	4.3
Phenylalanine	14.1	14.4	12.1	14.8	11.5	13.8	14.8	13.4	10.7	14.0	10.8	5.8
Threonine	10.2	10.8	9.5	11.8	9.4	11.1	11.4	11.1	8.5	11.0	9.9	4.9
Tryptophan	2.4	2.4	2.1	2.9	2.1	2.5	2.4	2.2	1.9	2.3	16.9	-
Valine	13.3	14.7	12.5	15.4	12.5	14.5	15.1	14.2	11.2	14.3	9.7	5.0
Dispensable amino acids[a] (g/kg DM)												
Alanine	12.2	12.7	10.9	13.9	11.0	13.1	13.3	12.8	9.9	12.7	10.1	5.0
Aspartic acid	32.2	34.9	28.9	36.3	29.6	34.8	35.9	34.0	26.3	35.0	10.4	10.1
Cysteine	3.4	3.3	3.0	4.4	3.4	3.9	4.1	3.7	2.6	3.9	14.9	1.3
Glutamic acid	47.8	52.2	44.2	55.7	44.8	52.4	53.8	52.3	40.3	51.7	10.1	22.2
Glycine	12.5	13.1	11.6	14.6	11.6	13.4	13.9	13.4	10.4	13.4	9.9	4.4
Proline	8.4	11.7	10.1	13.6	10.9	12.1	12.7	12.8	9.2	12.3	14.9	8.0

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Serine	13.7	15.0	12.9	16.1	13.1	14.9	15.7	15.3	11.4	15.4	10.4	5.4
Tyrosine	5.8	6.7	6.4	8.5	4.4	7.4	7.4	7.0	5.7	6.5	16.9	3.9
Antinutritive factors (mg/g DM)[b]												
Total phenols[c]	4.5	4.7	7.1	7.1	8.3	6.3	7.3	6.9	10.9	8.1	25.7	-
Condensed tannins[d]	2.8	2.9	5.3	6.2	6.8	4.7	3.9	4.5	7.3	6.0	30.9	-
Trypsin inhibitor	3.1	2.6	3.3	4.2	4.4	3.7	3.4	3.8	2.3	3.8	19.2	-

[a]Each value represents mean of duplicate; [b]Each value represents the mean of triplicate analysis. [c]Tannic acid equivalents; [d]Leukocyanidin equivalents

Table 3. Apparent ileal digestibility coefficient of amino acids of ten UK-grown field bean cultivar samples fed to 21 day old broiler chickens

Amino acid digestibility coefficient	Bean Cultivar										Mean	CV %	SEM (df=63)	P value
	Arthur	Buzz	Clipper	Divine	Fuego	Fury	Honey	Maris Bead	Sultan	Wizard				
Indispensable amino acids														
Arginine	0.848^{bc}	0.798^{ab}	0.842^{bc}	0.864^{c}	0.846^{bc}	0.854^{bc}	0.867^{c}	0.876^{c}	0.775^{a}	0.878^{c}	0.845	4.3	0.0127	< 0.001
Histidine	0.779^{ab}	0.763^{ab}	0.765^{ab}	0.818^{ab}	0.774^{ab}	0.822^{b}	0.834^{b}	0.839^{b}	0.710^{a}	0.829^{b}	0.793	8.6	0.0241	0.005
Isoleucine	0.825^{b}	0.784^{ab}	0.791^{ab}	0.829^{b}	0.807^{ab}	0.832^{b}	0.844^{b}	0.825^{b}	0.745^{a}	0.835^{b}	0.812	5.5	0.0156	< 0.001
Leucine	0.811^{b}	0.773^{ab}	0.769^{ab}	0.822^{b}	0.808^{b}	0.819^{b}	0.830^{b}	0.824^{b}	0.704^{a}	0.825^{b}	0.798	5.8	0.0164	< 0.001
Lysine	0.878^{b}	0.810^{a}	0.844^{ab}	0.870^{b}	0.871^{b}	0.864^{ab}	0.882^{b}	0.878^{b}	0.826^{ab}	0.883^{b}	0.861	4.2	0.0126	< 0.001
Methionine	0.881	0.756	0.836	0.816	0.825	0.808	0.86	0.802	0.756	0.865	0.821	12.6	0.036	0.215

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							5						7	
Phenylalanine	0.820^{b}	0.767^{ab}	0.760^{ab}	0.806^{b}	0.775^{ab}	0.798^{b}	0.820^{b}	0.800^{b}	0.706^{a}	0.808^{b}	0.786	5.8	0.0160	< 0.001
Threonine	0.810^{b}	0.768^{ab}	0.796^{ab}	0.840^{b}	0.810^{b}	0.853^{b}	0.836^{b}	0.849^{b}	0.703^{a}	0.839^{b}	0.810	7.4	0.0213	< 0.001
Valine	0.821^{b}	0.778^{ab}	0.798^{ab}	0.829^{b}	0.804^{ab}	0.840^{b}	0.845^{b}	0.834^{b}	0.725^{a}	0.835^{b}	0.811	6.1	0.0174	< 0.001
Dispensable amino acids														
Alanine	0.840^{b}	0.780^{ab}	0.803^{ab}	0.841^{b}	0.826^{b}	0.846^{b}	0.854^{b}	0.844^{b}	0.746^{a}	0.849^{b}	0.823	5.9	0.0172	< 0.001
Aspartic acid	0.841^{b}	0.805^{ab}	0.829^{ab}	0.857^{b}	0.844^{b}	0.867^{b}	0.858^{b}	0.862^{b}	0.764^{a}	0.860^{b}	0.839	5.0	0.0149	< 0.001
Cysteine	0.795^{b}	0.765^{ab}	0.750^{ab}	0.814^{b}	0.812^{b}	0.792^{b}	0.840^{b}	0.801^{b}	0.680^{a}	0.776^{b}	0.782	7.4	0.0205	< 0.001
Glutamic acid	0.861^{b}	0.809^{ab}	0.826^{ab}	0.850^{b}	0.833^{ab}	0.848^{b}	0.860^{b}	0.853^{b}	0.776^{a}	0.863^{b}	0.838	4.3	0.0128	< 0.001
Glycine	0.803^{b}	0.766^{ab}	0.779^{ab}	0.831^{b}	0.801^{b}	0.840^{b}	0.841^{b}	0.842^{b}	0.708^{a}	0.831^{b}	0.804	6.6	0.0187	< 0.001
Proline	0.593^{b}	0.634^{b}	0.585^{ab}	0.674^{b}	0.612^{b}	0.641^{b}	0.691^{b}	0.649^{b}	0.466^{a}	0.685^{b}	0.623	12.0	0.0264	< 0.001
Serine	0.812^{b}	0.770^{ab}	0.788^{ab}	0.835^{b}	0.826^{b}	0.831^{b}	0.836^{b}	0.839^{b}	0.712^{a}	0.832^{b}	0.808	5.9	0.0169	< 0.001
Tyrosine	0.706^{abc}	0.688^{abc}	0.714^{abc}	0.784^{c}	0.644^{ab}	0.753^{bc}	0.766^{c}	0.752^{bc}	0.642^{a}	0.734^{abc}	0.718	9.3	0.0237	< 0.001
Indispensable amino acids	0.832^{b}	0.783^{ab}	0.802^{ab}	0.839^{b}	0.812^{b}	0.837^{b}	0.826^{b}	0.845^{b}	0.745^{a}	0.847^{b}	0.817	5.9	0.0171	0.001
Dispensable amino	0.819^{b}	0.778^{a}	0.785^{a}	0.829	0.806	0.827	0.83	0.829^{b}	0.725	0.833^{b}	0.807	5.4	0.015	<

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acids		^{b}	^{b}	^{b}	^{b}	^{b}	6^{b}		^{a}				4	0.001
Total amino acids	0.825^{b}	0.780^{ab}	0.793^{ab}	0.833^{b}	0.808^{b}	0.832^{b}	0.832^{b}	0.836^{b}	0.734^{a}	0.840^{b}	0.811	5.4	0.0156	< 0.001

Each value represents mean of eight replicate pens of five birds each; CV, coefficient of variation; SEM, standard error of the mean; ^{a,b,c}Values within a column with different superscripts differ significantly at P≤0.05.

Accepted for publication 24 January 2021