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Correlation between ileal digestibility of amino acids and chemical composition of soybean meals in broilers at 21 days of age

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A B S T R A C T

The correlations between chemical composition and coefficient of standardized ileal digestibility (CSID) of crude protein (CP) and amino acids (AA) were determined in 22 soybean meal (SBM) samples originated from USA ($n=8$), Brazil (BRA; $n=7$) and Argentina (ARG; $n=7$) in 21-day old broilers. Birds were fed a commercial maize-SBM diet from 1 to 17 days of age followed by the experimental diets in which the SBM tested was the only source of protein (205 g CP/kg) for three days. Also, *in vitro* nitrogen (N) digestion study was conducted with these samples using the two-step enzymatic method. The coefficient of apparent ileal digestibility (CAID) of the SBM, independent of the origin, varied from 0.820 to 0.880 for CP, 0.850 to 0.905 for lysine (Lys), 0.859 to 0.907 for methionine (Met) and 0.664 to 0.750 for cysteine (Cys). The corresponding CSID values varied from 0.850 to 0.966 for CP, 0.891 to 0.940 for Lys, 0.931 to 0.970 for Met and 0.786 to 0.855 for Cys. The CSID of CP and Lys of the SBM were positively correlated with CP ($r=0.514$; $P<0.05$ and $r=0.370$; $P=0.09$, respectively), KOH solubility (KOH sol.) ($r=0.696$; $P<0.001$ and $r=0.619$; $P<0.01$, respectively), trypsin inhibitor activity (TIA) ($r=0.541$; $P<0.01$ and $r=0.416$; $P=0.05$, respectively) and reactive Lys ($r=0.563$; $P<0.01$ and $r=0.486$; $P<0.05$) values, but no relation was observed with neutral detergent fiber and oligosaccharide content. No relation between the CSID of CP determined *in vivo* and N digestibility determined *in vitro* was found. The CSID of most key AA were higher for the USA and the BRA meals than for the ARG meals. For Lys, the CSID was 0.921, 0.919 and 0.908 ($P<0.05$) and for Cys 0.828, 0.833 and 0.800 ($P<0.01$) for USA, BRA and ARG meals, respectively. It is concluded that under the conditions of this experiment, the CSID of CP and Lys increased with CP content, KOH sol., TIA and reactive Lys values of the SBM. The CSID of most limiting AA, including Lys and Cys, were higher for USA and BRA meals than for ARG meals.

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1. Introduction

The nutritional value of soybean meal (SBM) for poultry is limited by the presence of several antinutritional factors, including trypsin inhibitors (TI), saponins and oligosaccharides that interfere with feed intake and nutrient utilization.

Abbreviations: AA, amino acid; AIA, acid insoluble ash; AME_N, apparent metabolizable energy corrected for N; ARG, Argentina; BEL, basal endogenous losses; BRA, Brazil; BW, body weight; CAID, coefficient of apparent ileal digestibility; CF, crude fiber; CP, crude protein; CSID, coefficient of standardized ileal digestibility; Cys, cysteine; DM, dry matter; HDI, heat damage index; KOH sol., KOH solubility; Lys, lysine; Met, methionine; N, nitrogen; NDF, neutral detergent fiber; PDI, protein dispersibility index; RSD, residual standard deviation; SBM, soybean meal; Thr, threonine; TI, trypsin inhibitor; TIA, trypsin inhibitor activity; Trp, tryptophan; UA, urease activity.

Heat processing of SBM reduces most of these effects, but an excess of heat increases the incidence of Maillard reactions that inevitable occurs between the amino group of the amino acids (AA) and the reducing sugars present in the meal (Qin et al., 1998). The *in vivo* determination of the AA digestibility of ingredients and diets is expensive and time-consuming. Consequently, alternatives for routine determination of crude protein (CP) and AA digestibility in feeds are needed. Urease activity (UA; American Oil Chemists Society, 2000), protein dispersibility index (PDI; Batal et al., 2000) and KOH solubility (KOH sol.; Araba and Dale, 1990a,b) are laboratory tests widely used to determine the quality of the CP fraction of commercial SBM. However, all of them have some limitations for evaluating the real contribution of SBM to meet AA requirements of non-ruminants (De Coca-Sinova et al., 2008). For example, UA and TI have no negative values and therefore, they do not differentiate well processed SBM from over-processed SBM. Similarly, PDI and KOH sol. analysis lacked sensitivity to indicate accurately if a SBM sample has been over- or under-processed (Anderson-Hafermann et al., 1992; Hsu and Satter, 1995). The correlations between *in vivo* ileal digestibility of CP and AA and the chemical composition of SBM, as well as the use of equations to predict the coefficient of standardized ileal digestibility (CSID) of CP and AA of SBM from chemical data might be of value but have not been studied in detail. An *in vitro* method proposed to simulate the ileal digestion of nitrogen (N) of diets and ingredients is that of Boisen and Fernández (1995). This method has been extensively used in pigs but the information available on its usefulness for poultry is limited.

The AA profile and the quality of the CP fraction of commercial SBM depend on factors such as processing conditions during oil extraction as well as soil, latitude and environmental conditions during growing and harvesting of the beans (Goldflus et al., 2006; Thakur and Hurburgh, 2007). Therefore, the nutritive value of SBM might vary depending on the crushing plant and the country of origin of the beans (Karr-Lilienthal et al., 2004; Mateos et al., 2011). The hypothesis tested in this research was that the CSID of SBM varies among commercial SBM samples and that the variability can be estimated from the chemical and CP quality characteristics of the SBM. The aim of this research was to determine the correlation between the ileal digestibility of AA determined *in vivo* and the chemical laboratory values of SBM samples. A second objective was to study the influence of origin of the bean on ileal digestibility of CP and AA of 22 commercial samples of SBM obtained at random from three countries in 18 to 21-day-old broilers.

2. Material and methods

2.1. Bird management, husbandry and experimental design

All the experimental procedures used in this research were approved by the Animal Ethics Committee of the Universidad Politécnica de Madrid and were in compliance with the Spanish Guidelines for the Care and Use of Animals in Research (Boletín Oficial del Estado, 2007).

In total, 828 one-day-old straight-run broiler chicks (Ross 308) with an initial body weight (BW) of 42.1 ± 3.9 g were obtained from a commercial hatchery (Avicu S.A., Guadalajara, Spain). After arrival at the experimental station, the birds were weighed individually and stratified by BW into six blocks of 138 birds each. Twenty-three uniform groups of chicks (six groups from each BW block) of six birds each were housed in battery cages (1.0 m \times 0.9 m, Avícola Grau S.A., Madrid, Spain). One cage from each of the six blocks differing in BW was randomly assigned to each of the 22 experimental diets based on 22 different SBM of three different origins (USA; Brazil, BRA; Argentina, ARG) and to an extra group that was fed a N-free diet to estimate the basal endogenous losses (BEL) for the determination of the CSID of CP and AA. The cages had wire flooring and were equipped with two drinkers and one open trough feeder. Room temperature was maintained at $33 \pm 2^\circ\text{C}$ during the first three days of life and then was reduced gradually according to age until reaching $24 \pm 2^\circ\text{C}$ at 21st day. During the first week of life, chicks received a 23 h/day light program and then, an 18 h continuous light program until 21 days of age. Chicks had free access to feed and tap water throughout the experiment.

2.2. Soybean meals and diets

Twenty-two batches of SBM, eight from the USA, seven from BRA and seven from ARG, were evaluated in this study. Twenty of the samples (50 kg each) were collected at random at the arrival of the vessels to Hamburg port (Germany) by specialized quality control personnel. The two extra batches of SBM were received in containers directly from two crushers from the East coast of USA (Warsaw, NC and Norfolk, VA). The SBM were ground using a hammer mill fitted with a 2.5-mm screen (Model Z-I, Retsch, Stuttgart, Germany) and included as such in the corresponding experimental diets. From 1 to 17 days of age, all chicks were fed a crumble commercial diet based on maize and SBM with 13.0 MJ apparent metabolizable energy corrected for N (AME_N) and 13.5 g total lysine (Lys)/kg. Then, the birds received their respective experimental diets, based on maize starch and sucrose with the SBM tested as the only source of protein in mash form from 18 to 21 days of age. The SBM were analyzed for CP before diet formulation, and all diets contained 205 g CP (390–433 g SBM/kg depending on CP content of the meal). In addition, a N-free diet based on maize starch and sucrose was fed to an additional group (six cage-replicates) of broilers. Celite, an acid-washed diatomaceous earth (Celite Hispánica S.A., Alicante, Spain), was added at 20 g/kg to all diets to increase the acid insoluble ash (AIA) content.

Table 1Determined chemical composition (g/kg dry matter, unless otherwise indicated) and crude protein (CP) quality of the soybean meals according to origin.^a

| | USA (n=8) | | Brazil (n=7) | | Argentina (n=7) | | S.E.M. | P-value |
|---|-------------------|-------------|--------------------|-------------|-------------------|-------------|--------|---------|
| | Average | Range | Average | Range | Average | Range | | |
| Chemical composition | | | | | | | | |
| Total ash | 77 ^x | 74–81 | 72 ^y | 65–81 | 77 ^x | 75–79 | 1.3 | * |
| CP | 535 ^y | 519–552 | 554 ^x | 526–564 | 523 ^z | 518–530 | 3.8 | *** |
| Ether extract | 23 | 10–29 | 20 | 8–33 | 23 | 19–33 | 2.5 | NS |
| Crude fibre | 48 | 37–61 | 56 | 45–76 | 52 | 45–55 | 3.1 | NS |
| Neutral detergent fibre | 104 | 80–132 | 121 | 98–165 | 112 | 97–120 | 6.6 | NS |
| Stachyose | 59 ^x | 52–67 | 50 ^y | 35–56 | 56 ^x | 52–60 | 2.0 | * |
| Raffinose | 13 | 9–17 | 13 | 12–15 | 13 | 13–14 | 0.6 | NS |
| Sucrose | 75 ^x | 66–89 | 65 ^y | 62–72 | 79 ^x | 74–83 | 2.3 | ** |
| CP quality | | | | | | | | |
| Urease activity (mg N/g) | 0.01 | 0.00–0.01 | 0.00 | 0.00–0.01 | 0.01 | 0.00–0.01 | 0.0019 | NS |
| Protein dispersibility index (%) | 14.3 | 13.0–16.6 | 12.4 | 8.7–14.4 | 13.5 | 11.0–15.9 | 0.64 | NS |
| KOH solubility (%) | 86.5 ^x | 79.6–90.2 | 82.8 ^{xy} | 67.9–88.0 | 80.0 ^y | 77.4–82.1 | 1.633 | * |
| Trypsin inhibitor activity (mg/g DM) | 3.3 | 2.0–3.9 | 3.3 | 2.0–4.2 | 2.5 | 1.8–2.5 | 0.276 | 0.08 |
| Heat damage index ^b | 13.6 | 10–18 | 20 | 11–39 | 15.4 | 8–19 | 2.65 | NS |
| Reactive lysine (proportion total lysine) | 0.858 | 0.852–0.867 | 0.866 | 0.840–0.878 | 0.863 | 0.860–0.868 | 0.003 | NS |
| Non reactive lysine (proportion total lysine) | 0.08 | 0.07–0.09 | 0.08 | 0.07–0.10 | 0.08 | 0.07–0.08 | 0.0022 | NS |

^{xy,z}Within a row, means without a common superscripts differ (P<0.05).

NS, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001.

^a In triplicate.^b Aminored (Evonik, 2010a).

2.3. Laboratory analyses

Soybean meals and diets were analyzed for moisture by oven-drying (method 930.15), total ash with a muffle furnace (method 942.05) and N by combustion (method 990.03) using a Leco equipment (model FP-528, Leco Corporation, St. Joseph, MI) as described by Association of Official Analytical Chemists International (2000). Crude protein content was calculated as N × 6.25. Ether extract of the SBM was analyzed by Soxhlet fat analysis after 3N HCl acid hydrolysis (method 4.b) as described by Boletín Oficial del Estado (1995) and AIA as indicated by De Coca-Sinova et al. (2011). Crude fiber (CF) content was determined by sequential extraction with diluted acid and alkali (method 962.09; Association of Official Analytical Chemists International, 2000) and neutral detergent fiber (NDF) of the SBM as described by Van Soest et al. (1991) and expressed on an ash-free basis. Oligosaccharide (stachyose and raffinose) contents of the SBM were determined by the method of Sánchez-Mata et al. (1998) with modifications. Briefly, the carbohydrate was extracted in water at 50 °C for one hour in agitation and centrifuged at 1780 × g for 15 min, and the extract was analyzed by high-performance liquid chromatography (model 3110, PerkinElmer Inc., Waltham, MA, USA).

The UA of the SBM was determined according to Boletín Oficial del Estado (1995) and the PDI according to method Ba 10–65 of American Oil Chemists Society (2000) using a Hamilton blender (Model G936, VOS Instrument, Zaltbommel, The Netherlands). The KOH sol. was measured as indicated by Araba and Dale (1990a) and the trypsin inhibitor activity (TIA), expressed in mg/g dry matter (DM) of SBM, according to the method of Hamerstrand et al. (1981). Briefly, a sample (1 g) of SBM was ground with a laboratory grinder (ZM-200, Retsch, Stuttgart, Germany) provided with a 0.2-mm screen and extracted with 50 mL of 0.01 mol/L NaOH using a magnetic stirrer for three hours at ambient temperature. The extract was measured for TIA using DL-BAPA (*N*-α-benzoyl-DL-arginine-*ρ*-nitroanilide) as specific substrate and centrifuged at 2500 × g for 10 min. The TIA was calculated from the absorbance read at 410 nm against a blank reagent, using an ultraviolet spectrophotometer (model DU-7, Beckmann Instruments Inc., Fullerton, CA, USA). Heat damage index (HDI) of the SBM was analyzed in the laboratory using the Aminored method as proposed by Evonik-Degussa (Evonik, 2010a). Also, reactive and non-reactive Lys content of the SBM, an indirect measurement of the availability of Lys in non-ruminant animals, were determined according to procedures described by Fontaine et al. (2007).

The AA content of the SBM was determined by ion-exchange chromatography (Hewlett-Packard 1100, Waldbronn, Germany) after acid hydrolysis, following the procedure described by Association of Official Analytical Chemists International (2003). Briefly, samples of SBM were hydrolyzed in 6N HCl for 22 h at 110 °C under reflux conditions. Nitrogen was bubbled through the mixture during the hydrolysis period. A large acid to sample ratio (400 mL/200 mg, volume/weight) was used to reduce AA losses in the presence of carbohydrates. Protein hydrolysates and AA calibration mixture were derivatized with *o*-phthalaldehyde. For determination of methionine (Met) and cysteine (Cys), separate samples were oxidized with performic acid before hydrolysis and measured as Met sulfone and cysteic acid, respectively. Tryptophan (Trp) content was determined after alkaline hydrolysis for 20 h at 110 °C. Data on chemical composition, CP quality traits and AA profile (as proportion of CP) of the SBM tested on individual bases and country of origin are shown in Tables 1 and 2, respectively. The ingredient composition and calculated AA content of the experimental diets are shown in Table 3.

Ileal digesta samples were analyzed for DM, AIA, CP and AA (except Trp), according to the procedures described previously for SBM and these values were used for coefficient of apparent ileal digestibility (CAID) and CSID determinations. All

Table 2Determined amino acid (AA) content (g/kg crude protein) of the soybean meals according to origin.^a

| | USA (n = 8) | | Brazil (n = 7) | | Argentina (n = 7) | | S.E.M. | P-value |
|-------------------------|-------------------|-----------|-------------------|-----------|--------------------|-----------|--------|---------|
| | Average | Range | Average | Range | Average | Range | | |
| Indispensable AA | | | | | | | | |
| Arginine | 74.8 ^x | 73.9–75.8 | 74.5 ^x | 72.9–75.2 | 73.4 ^y | 72.6–74.3 | 0.26 | ** |
| Histidine | 27.3 ^x | 27.0–27.7 | 26.7 ^y | 26.3–27.3 | 27.3 ^x | 27.0–27.9 | 0.11 | *** |
| Isoleucine | 46.0 ^z | 44.7–46.5 | 47.1 ^x | 46.4–47.6 | 46.5 ^y | 46.0–46.8 | 0.18 | *** |
| Leucine | 76.9 ^y | 75.7–77.7 | 78.5 ^x | 77.9–78.9 | 78.0 ^x | 77.4–79.0 | 0.22 | *** |
| Lysine | 62.5 ^x | 62.2–63.4 | 60.7 ^y | 56.5–62.0 | 61.8 ^{xy} | 61.0–62.9 | 0.45 | * |
| Methionine | 14.2 ^x | 14.1–14.3 | 13.0 ^z | 12.5–13.4 | 13.9 ^y | 13.7–14.0 | 0.07 | *** |
| Phenylalanine | 50.7 ^z | 50.2–51.9 | 52.7 ^x | 51.5–53.6 | 51.4 ^y | 50.9–52.7 | 0.24 | *** |
| Threonine | 39.8 ^x | 39.3–40.7 | 39.0 ^y | 38.3–39.5 | 40.0 ^x | 39.6–40.6 | 0.17 | ** |
| Tryptophan | 13.9 ^x | 13.5–14.0 | 13.4 ^y | 13.0–13.7 | 13.7 ^{xy} | 13.5–13.8 | 0.09 | * |
| Valine | 48.2 ^y | 47.2–48.7 | 48.3 ^y | 47.6–48.7 | 49.1 ^x | 48.5–49.5 | 0.16 | ** |
| Dispensable AA | | | | | | | | |
| Alanine | 44.2 ^y | 43.9–45.0 | 44.0 ^y | 43.7–44.3 | 44.9 ^x | 44.6–45.6 | 0.13 | *** |
| Asparagine | 116 ^y | 115–118 | 117 ^x | 117–119 | 115 ^z | 115–117 | 0.28 | *** |
| Cysteine | 14.9 ^x | 14.7–15.2 | 13.2 ^z | 12.6–13.7 | 14.4 ^y | 13.8–15.0 | 0.13 | *** |
| Glutamine | 183 ^x | 181–186 | 185 ^x | 183–186 | 182 ^y | 180–186 | 0.53 | ** |
| Glycine | 43.2 | 42.5–43.9 | 42.6 | 42.0–43.5 | 42.9 | 42.5–43.4 | 0.18 | 0,05 |
| Proline | 51.7 | 51.1–52.8 | 51.3 | 51.0–51.9 | 51.6 | 51.1–52.6 | 0.19 | NS |
| Serine | 50.6 | 49.9–51.7 | 50.8 | 50.1–51.7 | 50.1 | 49.0–51.6 | 0.26 | NS |
| Tyrosine | 35.0 ^y | 34.3–35.8 | 36.0 ^x | 35.3–37.1 | 35.5 ^{xy} | 35.0–36.3 | 0.21 | ** |

^{x,y,z} Within a row, means without a common superscripts differ (P < 0.05).

NS, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

^a In duplicate.

laboratory analyses were conducted in triplicate except for AA of SBM and diets and for all ileal digesta measurements that were determined in duplicate.

2.4. *In vitro* digestibility assay

The *in vitro* digestibility of N of the 22 SBM samples was conducted in two successive steps, simulating gastric and intestinal digestion, respectively, as proposed by Boisen and Fernández (1995). Briefly, the samples (1.0 g) were ground (Model Z-I, Retsch, Stuttgart, Germany) using a 0.5-mm screen and placed in a 100 mL flask. Each sample was incubated in duplicate for the gastric and the ileal digestibility determinations. For gastric digestion (one step), the samples were incubated for 6 h at 39 °C with 1 mL of a pepsin solution (10 mg/mL of porcine pepsin, 2000 FIP-U/g, Merck no. 7190, Whitehouse Station, NJ, USA) at pH 2.0. For ileal digestion (two steps), gastric simulation was followed by hydrolysis of the remaining of the samples with a mixture of protease, amylase and lipase (porcine, pancreatin solution 25 mg/mL, Sigma P-1790, St. Louis, MO, USA) at pH 6.8 and 39 °C for 18 h. After incubation, N was solubilised adding 5 mL of 20% (volume/volume) sulphosalicylic acid solution (Sigma P-7626, Steinheim, Germany) to the samples. The undigested residues (non solubilised and precipitated protein) were collected in a filtration unit (Fibertec System M, Tecator, Höganäs, Sweden), washed with ethanol and acetone and dried at 80 °C overnight, and the N content was measured. A blank sample (with enzymes) and a control sample (using one of the SBM samples chosen at random) were included in each of the two digestion simulation processes. The coefficients of *in vitro* digestibility of N were calculated by difference from data in samples and in the undigested residues after correction for the N content of the enzymes used for the blank.

2.5. Growth performance and coefficients of apparent and standardized ileal digestibility determinations

At 18 days of age, birds were fed their respective experimental diets *ad libitum* for three days and BW of the chicks and feed consumption corrected for feed wastage were measured by cage. Then, all birds were euthanized by CO₂ asphyxiation and the contents of the ileal portion, defined as the small intestine section extending from the vitelline Meckel's diverticulum to 4 cm anterior to the ileocecal junction, were immediately removed (Ravindran et al., 1999). The ileal digesta of each of the six birds of each cage were collected by gently flushing the contents with distilled water into plastic containers, pooled, homogenized, frozen at –20 °C and freeze-dried. Dried ileal digesta samples were ground using a pestle and mortar to pass through a 0.5-mm screen and stored in airtight containers at room temperature until chemical analyses. The CAID of DM, CP and AA of the diets were calculated as follows:

$$\text{CAID} = 1 - [(\text{Nutrient}_d / \text{Nutrient}_f) \times (\text{AIA}_f / \text{AIA}_d)],$$

Table 3
Ingredient composition and calculated amino acid content of the experimental diets (g/kg, as fed basis).^a

| SBM origin ^d | Ingredient composition | | | | Amino acid content ^{b,c} | | | | | | | |
|--------------------------|------------------------|--------------|---------|--------------------------|-----------------------------------|------|------|------|-----|-----|-----|-----|
| | SBM ^e | Maize starch | Sucrose | Common part ^f | Arg | Ile | Lys | Met | Thr | Val | Cys | |
| USA | 401.8 | 246.1 | 246.1 | 106 | 14.7 | 9.2 | 12.2 | 2.8 | 7.7 | 9.6 | 2.9 | |
| | 406.8 | 243.6 | 243.6 | 106 | 14.4 | 9.0 | 12.0 | 2.7 | 7.6 | 9.4 | 2.9 | |
| | 426.0 | 234.0 | 234.0 | 106 | 14.5 | 9.0 | 12.3 | 2.8 | 7.7 | 9.5 | 3.0 | |
| | 409.3 | 242.3 | 242.3 | 106 | 14.5 | 9.0 | 12.1 | 2.7 | 7.8 | 9.4 | 2.9 | |
| | 407.6 | 243.2 | 243.2 | 106 | 14.5 | 9.1 | 12.1 | 2.7 | 7.6 | 9.5 | 2.9 | |
| | 407.1 | 243.5 | 243.5 | 106 | 14.6 | 8.8 | 12.2 | 2.7 | 7.6 | 9.3 | 2.9 | |
| | 415.8 | 239.1 | 239.1 | 106 | 14.8 | 8.9 | 12.6 | 2.9 | 8.0 | 9.4 | 3.0 | |
| | 432.9 | 230.6 | 230.6 | 106 | 15.2 | 9.2 | 12.7 | 2.9 | 8.1 | 9.7 | 3.0 | |
| | Brazil | 422.2 | 235.9 | 235.9 | 106 | 14.5 | 9.2 | 12.0 | 2.6 | 7.5 | 9.5 | 2.6 |
| | | 393.5 | 250.3 | 250.3 | 106 | 14.4 | 9.3 | 12.1 | 2.5 | 7.6 | 9.5 | 2.6 |
| 392.2 | | 250.9 | 250.9 | 106 | 14.5 | 9.3 | 11.7 | 2.4 | 7.3 | 9.6 | 2.4 | |
| 390.2 | | 251.9 | 251.9 | 106 | 14.4 | 9.0 | 11.8 | 2.5 | 7.6 | 9.2 | 2.6 | |
| 390.3 | | 251.8 | 251.8 | 106 | 14.5 | 9.5 | 11.8 | 2.5 | 7.3 | 9.7 | 2.5 | |
| 392.0 | | 251.0 | 251.0 | 106 | 14.6 | 9.4 | 12.1 | 2.6 | 7.4 | 9.5 | 2.7 | |
| 420.2 | | 236.9 | 236.9 | 106 | 14.6 | 9.4 | 11.3 | 2.6 | 7.9 | 9.6 | 2.6 | |
| Argentina | | 414.0 | 240.0 | 240.0 | 106 | 14.2 | 9.3 | 12.1 | 2.7 | 7.7 | 9.8 | 2.8 |
| | | 418.9 | 237.6 | 237.5 | 106 | 14.3 | 9.1 | 12.1 | 2.7 | 7.7 | 9.6 | 2.9 |
| | | 415.5 | 239.2 | 239.2 | 106 | 14.3 | 9.2 | 12.1 | 2.7 | 7.7 | 9.8 | 2.9 |
| | 420.3 | 236.9 | 236.9 | 106 | 14.2 | 9.1 | 12.0 | 2.7 | 7.7 | 9.6 | 2.7 | |
| | 420.1 | 237.0 | 237.0 | 106 | 14.3 | 9.3 | 11.9 | 2.7 | 7.6 | 9.8 | 2.8 | |
| | 410.8 | 241.6 | 241.6 | 106 | 14.3 | 9.0 | 12.1 | 2.7 | 7.8 | 9.5 | 2.9 | |
| 432.0 | 231.0 | 231.0 | 106 | 14.9 | 9.3 | 12.6 | 2.8 | 8.1 | 9.8 | 2.8 | | |
| N free diet ^g | – | 427.5 | 427.5 | 145 | – | – | – | – | – | – | – | |

^a All diets contained 205 g crude protein/kg and the soybean meal tested was the only source of crude protein.

^b Values calculated from determined amino acid content of the soybean meal and the proportion of soybean meal in the diet.

^c Arg: Arginine, Ile: Isoleucine, Met: Methionine, Thr: Threonine, Val: Valine, Cys: Cysteine.

^d Soybean meal.

^e g of SBM included per kg of diet.

^f The common part (g/kg) of the experimental diets consisted of 50 soybean oil, 20 celite (Celite Hispánica S.A., Alicante, Spain), 19 dicalcium phosphate, 10 calcium carbonate, 5 vitamin and mineral premix and 2 sodium chloride. The vitamin and mineral premix contained the following (per kg of diet): vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D₃ (cholecalciferol), 2,000 IU; vitamin E (all-ractocopherol acetate), 20 IU; vitamin K (bisulfate menadiolone complex), 3 mg; riboflavin, 5 mg; pantothenic acid (d-calcium pantothenate), 10 mg; nicotinic acid, 30 mg; pyridoxine (pyridoxine-HCl), 3 mg; thiamin (thiamin mononitrate), 1 mg; vitamin B₁₂ (cyanocobalamin), 12 µg; biotin, 0.15 mg; choline (choline chloride), 300 mg; folic acid, 0.5 mg; Se (Na₂SeO₃), 0.1 mg; I (KI), 2.0 mg; Cu (CuSO₄·5H₂O), 10 mg; Fe (FeSO₄·7H₂O), 30 mg; Mn (MnSO₄·H₂O), 100 mg; Zn (ZnO), 100 mg; ethoxyquin, 110 mg.

^g The common part of the nitrogen-free diet included also 30 g of cellulose and 9 g of potassium carbonate/kg.

where, Nutrient_d and AIA_d are the concentrations of dietary components (DM, CP and AA) and of AIA in the ileal digesta and Nutrient_f and AIA_f are the concentrations of the same dietary components in the feed, all of them expressed in mg/kg DM. The BEL of CP and AA were determined in birds fed the N-free diet as indicated by Adedokun et al. (2008):

$$\text{BEL (mg/kg of DM intake)} = \text{Nutrient}_d \times (\text{AIA}_f / \text{AIA}_d).$$

The flow of endogenous CP and AA was calculated by multiplying the BEL of CP and AA by the daily DM intake. The CSID was calculated after correcting the CAID for the endogenous losses estimates of CP and each particular AA:

$$\text{CSID} = \text{CAID} + (\text{BEL} / \text{Nutrient}_f).$$

2.6. Statistical analyses

Data on chemical composition of SBM were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Institute Inc., 1990) with origin of the SBM as main effect of the model. Digestibility data were analyzed as a nested design using the MIXED procedure of SAS (SAS Institute Inc., 1990). The main effect of the model was the country of origin of the SBM and the nested effects were the SBM sample within origin and the cage within the SBM. When the model was significant, the Tukey test was used to make pairwise comparisons between treatment means. Data on *in vitro* N digestibility of the 22 diets was plotted against their respective *in vivo* values and the REG procedure (SAS Institute Inc., 1990) was used for the comparisons. Also, Pearson correlation analyses were conducted to study the relation between the CSID of CP and AA and laboratory determinations of the SBM. Prediction equations of CSID of CP and Lys of the SBM samples from chemical analysis were developed by multiple stepwise regression analysis, using the REG procedure of SAS Institute Inc. (1990). Only linear models were tested and it was assumed that there were no interactions among variables. In addition, only those variables that contributed to a significant improvement in the estimation of the dependent variable were introduced in the models. A probability value of 0.05 was considered significant, whereas values between 0.05 and 0.10 were considered a tendency.

Table 4Influence of origin of the soybean meal (SBM) on *in vitro* digestibility coefficients of nitrogen.

| SBM origin | One step ^a | Two steps ^b | Difference |
|---------------------|-----------------------|------------------------|--------------------|
| USA | 0.658 ^y | 0.882 ^x | 0.225 ^x |
| Brazil | 0.677 ^x | 0.864 ^y | 0.187 ^y |
| Argentina | 0.649 ^y | 0.875 ^{xy} | 0.226 ^x |
| S.E.M. ^c | 0.00553 | 0.00467 | 0.00768 |
| P-value | ** | * | ** |

^{xy} Within a column, means without a common superscripts differ ($P < 0.05$).

*, $P < 0.05$; **, $P < 0.01$.

^a Simulating stomach digestion.

^b Simulating stomach and small intestine digestion.

^c Eight samples from USA meals and seven samples from Brazil and Argentina meals.

3. Results

3.1. Laboratory analyses

The CP and NDF contents of the individual SBM samples ranged from 518 to 564 g/kg DM and from 80 to 165 g/kg DM, respectively (Table 1). The PDI, KOH sol. and TIA values varied from 8.7 to 16.6%, 67.9 to 90.2% and 1.8 to 4.2 mg/g DM, respectively and the HDI varied from 8 to 39. The AA profile differed among samples with Lys and Met contents ranging from 56.5 to 63.4 and from 12.5 to 14.3 g/kg, respectively (Table 2).

3.2. Influence of origin of the beans on chemical composition of the soybean meals

The SBM from ARG had less CP content than the SBM from USA and both less than those from BRA ($P < 0.001$; Table 1). No differences among origins were observed for NDF content but stachyose ($P < 0.05$) and sucrose ($P < 0.01$) concentrations were higher for USA and ARG meals than for BRA meals. Origin of the SBM did not affect UA or PDI values but KOH sol. was higher ($P < 0.05$) for the USA meals than for the ARG meals with the BRA meals being intermediate. The SBM from USA and BRA tended to have ($P = 0.08$) higher TIA than the SBM from ARG. Heat damage index varied from 8 to 39 with average values of 13.6, 20.0 and 15.4 for USA, BRA and ARG meal, respectively ($P > 0.10$). Reactive Lys (as a proportion of total Lys) of the individual SBM varied between 0.840 and 0.878 and was not affected by origin of the meal. The AA profile (g/kg CP) of the SBM varied with the origin of the beans, with Lys ($P < 0.05$), Met ($P < 0.001$), threonine (Thr; $P < 0.01$), Trp ($P < 0.05$) and Cys ($P < 0.001$) contents being higher for USA than for BRA meals with ARG meals being intermediate.

3.3. *In vitro* digestibility assay

Origin of the SBM affected the *in vitro* N digestibility in different ways (Table 4). When the one step (gastric digestion) method was used, N digestibility was higher ($P < 0.01$) for BRA meals than for USA or ARG meals. However, when the two steps method (gastric and small intestine digestion) was used, N digestibility was higher ($P < 0.05$) for the USA than for the BRA meals, with the ARG meals being intermediate. The differences obtained with the two methods were higher ($P < 0.01$) for SBM from USA and ARG than for SBM from BRA.

3.4. Growth performance and coefficients of apparent and standardized ileal digestibility

From 18 to 21 days of age, BW gain of the experimental birds was of 38.4 g/d as an average. In this short period of time, broilers fed USA SBM had higher average daily feed intake and better feed conversion ratio than broilers fed the BRA or ARG meals ($P < 0.05$; data not shown).

The CAID of CP and most key indispensable AA were higher for USA and BRA SBM than for the ARG SBM (Table 5). In fact, the CAID of arginine ($P < 0.01$), Lys ($P < 0.05$), Met ($P < 0.01$) and Thr ($P < 0.01$) were 0.911, 0.882, 0.880 and 0.797 for SBM from USA, 0.913, 0.881, 0.891 and 0.811 for SBM from BRA and 0.898, 0.867, 0.866 and 0.785 for SBM from ARG, respectively. Also, the CAID for Cys was higher for USA and BRA meals than for ARG meals ($P < 0.01$). The CSID of most limiting indispensable AA were higher for the USA and BRA meals than for the ARG meal (Table 6). The CSID were 0.943 and 0.944 vs. 0.933 ($P < 0.05$) for arginine and 0.921 and 0.919 vs. 0.908 ($P < 0.05$) for Lys for USA, BRA and ARG meals, respectively. Also, the CSID of Cys was higher for USA and BRA meals than for the ARG meals (0.828, 0.833 and 0.800; $P < 0.01$).

3.5. Correlations between the coefficient of standardized ileal digestibility of crude protein and amino acids and the laboratory analyses of soybean meals

The KOH sol. values of the SBM were positively correlated with PDI ($r = 0.508$; $P < 0.05$), TIA ($r = 0.732$; $P < 0.001$) and reactive Lys ($r = 0.398$; $P = 0.07$) (Table 7). Heat damage index was negatively correlated ($P < 0.01$) with PDI ($r = -0.614$), KOH

Table 5Influence of origin of the soybean meal (SBM) on the coefficient of apparent ileal digestibility of dry matter (DM)^a, crude protein (CP) and amino acids (AA).

| | USA | | Brazil | | Argentina | | S.E.M. ^b | P-value |
|------------------|---------------------|-------------|---------------------|-------------|---------------------|-------------|---------------------|---------|
| | Average | Range | Average | Range | Average | Range | | |
| DM | 0.795 ^{xv} | 0.776–0.814 | 0.808 ^x | 0.781–0.823 | 0.784 ^y | 0.776–0.792 | 0.0044 | ** |
| CP | 0.847 ^{xy} | 0.837–0.880 | 0.854 ^x | 0.820–0.869 | 0.835 ^y | 0.829–0.841 | 0.0047 | * |
| Indispensable AA | | | | | | | | |
| Arginine | 0.911 ^x | 0.901–0.931 | 0.913 ^x | 0.893–0.921 | 0.898 ^y | 0.892–0.909 | 0.0031 | ** |
| Histidine | 0.870 | 0.856–0.892 | 0.877 | 0.845–0.887 | 0.864 | 0.853–0.873 | 0.0043 | NS |
| Isoleucine | 0.863 ^{xy} | 0.851–0.891 | 0.876 ^x | 0.852–0.886 | 0.854 ^y | 0.846–0.864 | 0.0042 | ** |
| Leucine | 0.863 ^{xy} | 0.850–0.888 | 0.874 ^x | 0.855–0.883 | 0.852 ^y | 0.846–0.863 | 0.0039 | ** |
| Lysine | 0.882 ^x | 0.871–0.905 | 0.881 ^{xy} | 0.850–0.891 | 0.867 ^y | 0.860–0.880 | 0.0041 | * |
| Methionine | 0.880 ^{xy} | 0.863–0.907 | 0.891 ^x | 0.870–0.904 | 0.866 ^y | 0.859–0.880 | 0.0045 | ** |
| Phenylalanine | 0.857 ^y | 0.844–0.880 | 0.875 ^x | 0.852–0.883 | 0.854 ^y | 0.836–0.864 | 0.0043 | ** |
| Threonine | 0.797 ^{xy} | 0.778–0.827 | 0.811 ^x | 0.795–0.824 | 0.785 ^y | 0.772–0.801 | 0.0051 | ** |
| Valine | 0.849 ^{xy} | 0.834–0.878 | 0.860 ^x | 0.836–0.870 | 0.838 ^{by} | 0.829–0.846 | 0.0044 | * |
| Dispensable AA | | | | | | | | |
| Alanine | 0.846 ^{xy} | 0.828–0.872 | 0.858 ^x | 0.835–0.867 | 0.836 ^y | 0.829–0.845 | 0.0043 | ** |
| Asparagine | 0.845 | 0.834–0.875 | 0.845 | 0.817–0.858 | 0.831 | 0.823–0.849 | 0.0045 | NS |
| Cysteine | 0.714 ^x | 0.690–0.750 | 0.712 ^x | 0.673–0.741 | 0.675 ^y | 0.664–0.693 | 0.0072 | ** |
| Glutamine | 0.893 | 0.881–0.916 | 0.893 | 0.863–0.903 | 0.881 | 0.873–0.895 | 0.0040 | NS |
| Glycine | 0.815 ^{xy} | 0.797–0.846 | 0.824 ^x | 0.798–0.841 | 0.802 ^y | 0.792–0.816 | 0.0049 | * |
| Proline | 0.854 ^{xy} | 0.841–0.881 | 0.860 ^x | 0.831–0.870 | 0.841 ^y | 0.834–0.850 | 0.0041 | * |
| Serine | 0.843 ^{xy} | 0.825–0.865 | 0.852 ^x | 0.827–0.868 | 0.832 ^y | 0.818–0.848 | 0.0044 | * |

^{xv} Within a row, means without a common superscripts differ (P < 0.05).

NS, P > 0.05; *, P < 0.05; **, P < 0.01.

^a Data for ileal digestibility of DM corresponded to that of the diets (different levels of SBM and maize starch depending on CP content of the SBM).^b Six replicates of six birds each per each SBM sample (eight samples from USA meals and seven samples from Brazil and Argentina meals).

sol. ($r = -0.559$) and reactive Lys ($r = -0.542$). The CSID of CP, Lys and Cys of the SBM were positively correlated with CP content (P < 0.05, P < 0.10 and P < 0.05, respectively), KOH sol. (P < 0.001, P < 0.01 and P < 0.01, respectively) and TIA (P < 0.01, P < 0.10 and P < 0.05, respectively). However, no correlations between CSID of CP and AA and NDF content were found. The CSID of CP and Lys were positively correlated with reactive Lys ($r = 0.563$; P < 0.01 and $r = 0.486$; P < 0.05, respectively) and negatively with HDI ($r = -0.378$; P < 0.10 and $r = -0.535$; P < 0.01, respectively). The CSID of CP, Lys and Cys were better correlated with KOH sol. ($r = 0.696$, P < 0.001; $r = 0.619$, P < 0.01; $r = 0.589$; P < 0.01) than with PDI ($r = 0.257$, P > 0.10; $r = 0.385$, P < 0.10; $r = 0.0952$; P > 0.10). The CAID and CSID of CP obtained *in vivo* were positively correlated with the one step N *in vitro* digestibility ($r = 0.45$ and 0.43 , respectively; P < 0.05; data not shown) but not with the two steps procedure.

Table 6

Influence of origin of the soybean meal (SBM) on the coefficient of standardized ileal digestibility of crude protein (CP) and amino acids (AA).

| | USA | | Brazil | | Argentina | | S.E.M. ^a | P-value |
|------------------|---------------------|-------------|---------------------|-------------|--------------------|-------------|---------------------|---------|
| | Average | Range | Average | Range | Average | Range | | |
| CP | 0.925 | 0.897–0.962 | 0.926 | 0.878–0.951 | 0.916 | 0.850–0.966 | 0.0083 | † |
| Indispensable AA | | | | | | | | |
| Arginine | 0.943 ^x | 0.934–0.960 | 0.944 ^x | 0.925–0.950 | 0.933 ^y | 0.928–0.941 | 0.0028 | * |
| Histidine | 0.913 | 0.904–0.931 | 0.919 | 0.888–0.932 | 0.910 | 0.900–0.917 | 0.0039 | NS |
| Isoleucine | 0.921 ^{xy} | 0.913–0.942 | 0.928 ^x | 0.905–0.934 | 0.913 ^y | 0.907–0.920 | 0.0033 | * |
| Leucine | 0.909 ^{xy} | 0.899–0.930 | 0.916 ^x | 0.898–0.923 | 0.900 ^y | 0.895–0.907 | 0.0033 | ** |
| Lysine | 0.921 ^x | 0.912–0.940 | 0.919 ^{xy} | 0.891–0.927 | 0.908 ^y | 0.901–0.918 | 0.0035 | * |
| Methionine | 0.946 ^{xy} | 0.935–0.968 | 0.959 ^x | 0.940–0.970 | 0.938 ^y | 0.931–0.953 | 0.0042 | ** |
| Phenylalanine | 0.923 | 0.910–0.941 | 0.935 | 0.912–0.951 | 0.923 | 0.907–0.932 | 0.0038 | NS |
| Threonine | 0.916 ^{xy} | 0.906–0.936 | 0.925 ^x | 0.909–0.934 | 0.909 ^y | 0.900–0.916 | 0.0037 | * |
| Valine | 0.914 ^{xy} | 0.905–0.937 | 0.921 ^x | 0.899–0.928 | 0.905 ^y | 0.899–0.914 | 0.0034 | * |
| Dispensable AA | | | | | | | | |
| Alanine | 0.908 ^{xy} | 0.895–0.929 | 0.916 ^x | 0.894–0.923 | 0.901 ^y | 0.894–0.906 | 0.0036 | * |
| Asparagine | 0.891 | 0.881–0.916 | 0.887 | 0.860–0.899 | 0.880 | 0.872–0.893 | 0.0041 | NS |
| Cysteine | 0.828 ^x | 0.812–0.855 | 0.833 ^x | 0.797–0.852 | 0.800 ^y | 0.786–0.820 | 0.0058 | ** |
| Glutamine | 0.929 | 0.918–0.949 | 0.927 | 0.897–0.936 | 0.919 | 0.913–0.931 | 0.0037 | NS |
| Glycine | 0.895 | 0.883–0.919 | 0.900 | 0.874–0.911 | 0.886 | 0.877–0.894 | 0.0039 | NS |
| Proline | 0.905 ^x | 0.892–0.926 | 0.907 ^x | 0.881–0.916 | 0.894 ^y | 0.887–0.901 | 0.0035 | * |
| Serine | 0.917 | 0.906–0.934 | 0.922 | 0.897–0.933 | 0.911 | 0.901–0.920 | 0.0034 | NS |

^{xv} Within a row, means without a common superscripts differ (P < 0.05).

NS, P > 0.05; †, P < 0.10; *, P < 0.05; **, P < 0.01.

^a Six replicates of six birds each per each SBM sample (eight samples from USA meals and seven samples from Brazil and Argentina meals).

Table 7

Pearson coefficient of correlation (r) between nutrient content of the soybean meal (n=22) and the coefficient of standardized ileal digestibility (CSID) of crude protein (CP) and selected amino acids.

| | CP | NDFom ^a | Oligos. ^b | PDI ^c | KOH sol. ^d | TIA ^e | Reactive Lys ^f | HDI ^g | CSID CP | CSID Lys | CSID Met ^h | CSID Thr ⁱ | CSID Cys ^j |
|--------------|----------------------|-----------------------|-----------------------|-----------------------|-----------------------|----------------------|---------------------------|-----------------------|----------------------|----------------------|-----------------------|-----------------------|-----------------------|
| CP | 1 | | | | | | | | | | | | |
| NDF | 0.0873 ^{NS} | 1 | | | | | | | | | | | |
| Oligos. | 0.250 ^{NS} | -0.671 ^{***} | 1 | | | | | | | | | | |
| PDI | -0.108 ^{NS} | -0.578 ^{**} | 0.693 ^{***} | 1 | | | | | | | | | |
| KOH sol. | 0.156 ^{NS} | -0.342 ^{NS} | 0.564 ^{**} | 0.508 [*] | 1 | | | | | | | | |
| TIA | 0.294 ^{NS} | -0.172 ^{NS} | 0.258 ^{NS} | 0.419 [*] | 0.732 ^{***} | 1 | | | | | | | |
| Reactive Lys | 0.222 ^{NS} | -0.238 ^{NS} | 0.289 ^{NS} | 0.310 ^{NS} | 0.398 [†] | 0.233 ^{NS} | 1 | | | | | | |
| HDI | 0.282 ^{NS} | 0.576 ^{**} | -0.733 ^{***} | -0.614 ^{**} | -0.559 ^{**} | -0.179 ^{NS} | -0.542 ^{**} | 1 | | | | | |
| CSID CP | 0.514 [†] | -0.198 ^{NS} | 0.238 ^{NS} | 0.257 ^{NS} | 0.696 ^{***} | 0.541 ^{**} | 0.563 ^{**} | -0.378 [†] | 1 | | | | |
| CSID Lys | 0.370 [†] | -0.354 ^{NS} | 0.302 ^{NS} | 0.385 [†] | 0.619 ^{**} | 0.416 [†] | 0.486 [†] | -0.535 ^{**} | 0.868 ^{***} | 1 | | | |
| CSID Met | 0.080 ^{NS} | 0.0805 ^{NS} | -0.157 ^{NS} | 0.0748 ^{NS} | 0.308 ^{NS} | 0.259 ^{NS} | 0.521 [*] | -0.0596 ^{NS} | 0.776 ^{***} | 0.789 ^{***} | 1 | | |
| CSID Thr | 0.410 [†] | 0.0333 ^{NS} | -0.131 ^{NS} | 0.00540 ^{NS} | 0.303 ^{NS} | 0.260 ^{NS} | 0.472 [†] | -0.127 ^{NS} | 0.750 ^{***} | 0.807 ^{***} | 0.929 ^{***} | 1 | |
| CSID Cys | 0.423 [†] | -0.0952 ^{NS} | 0.0929 ^{NS} | 0.0952 ^{NS} | 0.589 ^{**} | 0.449 [†] | 0.388 [†] | -0.232 ^{NS} | 0.760 ^{***} | 0.783 ^{***} | 0.766 ^{***} | 0.827 ^{***} | 1 |

NS, P > 0.10; †, P < 0.10; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

^a Neutral detergent fibre.^b Oligosaccharides (stachyose and raffinose).^c Protein dispersibility index.^d KOH solubility.^e Trypsin inhibitor activity.^f Lysine.^g Heat damage index (Aminored; Evonik, 2010a).^h Methionine.ⁱ Threonine.^j Cysteine.

Table 8

Prediction equations of the coefficient of standardized ileal digestibility (CSID) of crude protein (CP) and lysine (Lys) of soybean meal ($n=22$) based on chemical analyses (g/100g dry matter).

| CSID | Regression equation ^a | R^2 | RSD ^b |
|------|---|-------|------------------|
| CP | 75.5 (± 3.10) + 0.161 (± 0.0372) KOH solubility | 0.485 | 0.882 |
| | 59.7 (± 5.82) + 0.146 (± 0.0317) KOH solubility + 0.318 (± 0.105) CP | 0.654 | 0.742 |
| | 29.3 (± 16.7) + 0.123 (± 0.032) KOH solubility + 0.284 (± 0.099) CP + 0.395 (± 0.206) Reactive lysine | 0.713 | 0.695 |
| Lys | 81.0 (± 3.00) + 0.127 (± 0.036) KOH solubility | 0.383 | 0.855 |
| | 71.6 (± 6.43) + 0.118 (± 0.035) KOH solubility + 0.190 (± 0.116) CP | 0.459 | 0.821 |

^a Values in parentheses are standard errors. KOH solubility values are given in percentage (g/100g) and reactive lysine values are given in g/100g of total lysine.

^b Residual standard deviation.

3.6. Prediction equations of the coefficient of standardized ileal digestibility of crude protein and lysine

The KOH sol. value was the best predictor of the CSID of CP of the SBM [$R^2=0.485$, residual standard deviation (RSD)=0.882; $P<0.001$; Table 8]. Also, a strong positive effect of CP content ($P<0.01$) and reactive Lys ($P<0.05$) on CSID of CP was observed. When these two variables were included as independent variables in the model, the R^2 increased from 0.485 to 0.713, and the RSD decreased from 0.882 to 0.695 ($P<0.001$).

The CSID of Lys of the SBM was best predicted by the KOH sol. value ($R^2=0.383$, RSD=0.855; $P<0.01$). In a second step, a positive effect of CP content ($P<0.01$) of the SBM was detected. When the CP content was included as independent variable in the model, the regression equation was improved ($R^2=0.459$, RSD=0.821; $P<0.01$).

4. Discussion

4.1. Influence of origin of the beans on chemical composition of the soybean meals

The BRA meals had higher CP content than the USA meals and both higher than the ARG meals, in agreement with data of Thakur and Hurburgh (2007). Differences in CP content of the meals depend on the proportion of hulls removed during the process as well as on bean genotype (Wilcox and Shibles, 2001) and environmental and agronomic conditions during harvesting (Grieshop and Fahey, 2001). The Lys, Met and Cys content per unit of CP was higher for the USA meals than for the BRA meals, with the ARG meals being intermediate, results that agree with data of Evonik (2010b) and Mateos et al. (2011).

The concentration of stachyose and sucrose was lower for BRA than for USA and ARG meals, consistent with data of Mateos et al. (2011) and in agreement with data of Kumar et al. (2010) who showed higher sucrose content for beans grown at cooler locations. Solubility in KOH was higher for USA meals than for ARG meals, with BRA meals being intermediate, results that agree with data of Thakur and Hurburgh (2007) and Mateos et al. (2011). A lower KOH sol. value might be indicative of a more aggressive processing for the ARG than for the USA beans. Although the difference was not significant, the TIA varied with the origin of the beans with lowest values for the ARG meals, in agreement with data reported by Mateos et al. (2011) and consistent with the lower PDI and KOH sol. values observed for ARG meals.

4.2. In vitro digestibility assay

Stomach digestion (one step *in vitro* digestion) contributed as an average to 66.1% of the total N of the SBM digested with higher digestibility values for the BRA than for the USA and ARG meals. The stomach is the main site of solubilisation of dietary protein and the difference observed among origins might result from the higher CP content of the BRA meals compared to the USA and ARG meals. However, when the ileal digestibility (two steps) was determined, the higher N digestion values were observed for the USA meals. Consequently, the contribution of the small intestine to total digestion of N was lower for the BRA meals than for the USA and ARG meals, a reduction that might be related to the higher N content of the non-starch polysaccharides of the BRA meals. Probably, these compounds were not fully hydrolysed by the amylase included at the second step of the *in vitro* method.

The CAID of N determined *in vivo* was correlated with the *in vitro* digestibility of N obtained by using the one step method, but not with the two steps procedure. In contrast, De Coca-Sinova et al. (2008) reported a high correlation ($r=0.82$; $P<0.05$) between *in vivo* and *in vitro* ileal digestibility of N of six SBM samples in broilers. The reasons for these discrepancies are unclear. In the current study, the *in vivo* N digestibility values of the SBM ($n=22$) ranged from 0.820 to 0.880 whereas in the study of De Coca-Sinova et al. (2008) ($n=6$) varied from 0.773 to 0.855. Probably, the *in vitro* procedure of Boisen and Fernández (1995) might not predict accurately small differences in *in vivo* N digestibility of commercial SBM samples for poultry.

4.3. Coefficients of apparent and standardized ileal digestibility of crude protein and amino acids of diets and correlation with chemical composition of soybean meals

The CAID of CP and AA of the SBM were within the range reported by Pérez et al. (1993), Huang et al. (2005), Valencia et al. (2009a) and Bandegan et al. (2010), although lower than values reported by Ravindran et al. (1999, 2005) and higher than those reported by De Coca-Sinova et al. (2008). The reasons for the differences in CAID of the AA among researchers are not known but might be related to the conditions applied during the processing of the beans as well as the methodology used, including type of marker, age of the birds, slaughter technique and segment of the ileum used to collect the samples (Rynsburger, 2009). For example, Ravindran et al. (1999, 2005) used 42-day-old broilers whereas in the current research birds were 21-day-old and it has been reported that CP digestibility increased as the chick become older (Gracia et al., 2003; Adedokun et al., 2008).

The CAID of DM of the diet was higher for the BRA than for the ARG meals, with USA meals being intermediate. The level of inclusion of SBM, maize starch and sucrose varied slightly among experimental diets because the diets were formulated to have similar CP content. Therefore, the effects of SBM origin and inclusion level of SBM in the diet on DM digestibility were confounded, and no conclusions can be reached in respect to the AMEn content of the different SBM.

The CAID of CP and of most AA were variable and higher for the USA and BRA meals than for the ARG meals. The differences in digestibility observed might depend on the processing conditions applied to reduce the moisture or the antinutritional factors content of the meals (Opapeju et al., 2006). Also, the physical and chemical composition of the original beans vary depending on agronomic conditions and geographical location (Yaklich et al., 2002; Karr-Lilienthal et al., 2004; Thakur and Hurburgh, 2007) which in turn might affect AA digestibility. De Coca-Sinova et al. (2008) reported that the CAID of CP and Lys of SBM were positively correlated with the CP content and were higher for USA meals than for BRA and ARG meals. Burgos et al. (1973) studied the coefficient of total tract apparent retention of 13 different varieties of SBM of USA origin that were grown in the same location under similar agronomic conditions in 21-day-old broilers, and reported values that varied from 0.667 to 0.861 for CP, 0.708 to 0.876 for Lys and 0.636 to 0.856 for Met. Also, Douglas and Parsons (2000) reported differences in ileal digestibility of energy from 11.83 to 13.38 MJ/kg DM in chicks fed diets based on SBM collected at seven different locations within the USA. This information together with that of the current experiment, support the suggestion of Dudley-Cash (1997) and Mateos et al. (2011) that the nutritive value of commercial SBM is more variable than accepted in practice.

The quality and digestibility of the AA of the SBM depend on a series of interrelated factors difficult to separate and characterize. Kakade et al. (1973) indicated that only 40% of the growth depressing effect observed in rat fed unheated soybean diets was due to the TI content and attributed the remaining of the growth depression observed to poor digestibility of the undenaturalized protein of the raw soybeans. The ileal digestibility of most AA was lower for the ARG than for the USA and BRA meals but no significant differences on TIA content of the meals were observed among origins. Moreover in the current experiment, the TIA of the SBM was positively correlated with the CSID of CP, Lys and Cys, an observation which contrasts with most published reports which correlate the TIA content of the meal with reduced AA digestibility both in pigs (Qin et al., 1996; Valencia et al., 2008a,b) and poultry (Leeson and Atteh, 1996; Wiseman et al., 2003; Valencia et al., 2009b). Temperature, pressure and time applied during the processing of the beans denature the structure of the protein fraction and inactivate most of the heat-labile antinutritional factors presents in the meal (Marsman et al., 1997; Batal et al., 2000). However, under severe processing conditions, the incidence of Maillard reactions increases, counteracting the benefits of heating on protein denaturation and reduction of TIA. In this respect, Parsons et al. (1992) reported that BW gain and feed efficiency of broilers were depressed by 28 and 18%, respectively, and Lys digestibility decreased by more than 20% as autoclaving time (121 °C and 105 kPa) increased from 0 to 40 min. The data suggest that the ARG meals were processed under more severe conditions than the USA and BRA meals, resulting in lower TI content but also in higher incidence of Maillard reactions. This suggestion is consistent with the lower KOH sol. values observed for the ARG meals as compared with the USA meals, although HDI and reactive Lys values were not significant different among origins. In fact, Wang and Johnson (2001) correlated the TIA with PDI and KOH sol. in 27 samples of SBM from USA and found values of 80 and 96% for PDI and KOH sol., respectively. It has to be considered that the ARG meals had lower CP content than the USA and the BRA SBM and De Coca-Sinova et al. (2008) have shown that the ileal AA digestibility for SBM increased as the CP content increased. Therefore, the effects of origin (USA and BRA vs. ARG) and CP content of the meals on AA digestibility were confounded.

The positive correlation observed between the CSID of CP, Lys and Cys and the CP content of the SBM was consistent with data on ingredient composition from Institut National Recherche Agronomique (2002) and Fundación Española Desarrollo Nutrición Animal (2010) which give higher AA digestibility for the high-protein SBM than for the regular SBM. In the current experiment, no significant correlation between CSID of CP and Lys and NDF content of the SBM was detected. In contrast, De Coca-Sinova et al. (2008) reported a Pearson correlation coefficient of -0.745 ($P < 0.001$) between NDF and CAID for Lys in a study with six SBM samples. Similar results have been reported by Fan and Sauer (1999) in peas ($n = 6$). Mateos et al. (2011) documented that SBM from BRA contained more NDF but also more CP than SBM from USA and ARG. Consequently, the positive effects of higher CP content on AA digestibility of the meals, as reported by De Coca-Sinova et al. (2008) and the negative effects of higher NDF on the same variables, as reported by Dilger et al. (2004), may counteract each other resulting in a neutral final effect.

The correlation between the CSID of CP and key indispensable AA and oligosaccharides content was not significant, in disagreement with data of Baker and Stein (2009) who observed poorer digestibility of most AA in a diet that contained SBM

with 56.5 g of oligosaccharides (raffinose and stachyose) than in a diet based on a SBM with only 17.3 g/kg. Oligosaccharides cannot be digested in the small intestine of poultry because of the absence of endogenous α -(1, 6)-galactosidase enzyme (Choct et al., 2010). In addition, raffinose and stachyose may increase digesta passage rate and decrease digestion and absorption of nutrients (Parsons et al., 2000), increasing the incidence of diarrhoea (Wiggins, 1984). Probably, the differences in the level of oligosaccharides of the diets (24.6–31.2 g/kg) were not high enough to produce any significant effect on digesta passage rate in broilers (Parsons et al., 2000).

The first independent variable included in the model to predict CSID of CP and Lys from the determined chemical and quality characteristics of the SBM was KOH sol. This relation confirms the strong negative effect of aggressive processing conditions on N utilization of SBM in poultry (Parsons et al., 1992). For CSID of CP, the stepwise procedure showed that the inclusion of CP content and of reactive Lys of the SBM improved the accuracy of the equation. Reactive Lys has been recommended as a sound trait to evaluate the incidence of Maillard reactions caused by aggressive heat processing of the meal. For CSID of Lys, CP content was also included in the second step, which further explained the variation of available Lys content among meals. The equations reported herein might facilitate the accurate evaluation of the nutritional value of commercial SBM, improving prediction of CSID of CP or Lys for commercial SBM and the assessment of the economical value of SBM in poultry diets.

5. Conclusions

The commercial SBM tested differed widely in nutrient content, including CP quality and ileal digestibility of most AA. The coefficients of standardized ileal digestibility of CP and lysine of the SBM were positively correlated with CP content, KOH sol., TIA and reactive lysine but not with NDF and oligosaccharide contents. The CSID of lysine and other AA were higher for the USA and Brazil meals than for the Argentina meals, suggesting that the meals from Argentina were processed under more severe conditions. KOH sol. was the best predictor of the ileal digestibility of both CP and lysine. The use of correlations between ileal digestibility and chemical analyses may allow to improve the evaluation of the nutritive value of commercial SBM. Feed compound managers and nutritionists should be aware of differences in nutritive value among commercial sources of SBM.

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