

UNIVERSIDAD POLITÉCNICA DE MADRID
ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA AGRONÓMICA,
ALIMENTARIA Y DE BIOSISTEMAS



**Influence of the origin of the soybeans on
the chemical composition, protein
quality, and apparent metabolizable
energy of the soybean meals in broilers**

DOCTORAL THESIS

Submitted for the degree of Doctor by:

Lewis Alfonso Aguirre Toribio

M.Sc. Animal Production and Health

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Dr. Gonzalo González Mateos
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SUMMARY

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In the present Doctoral Thesis, 2 experiments were conducted to evaluate the influence of the origin on chemical composition, protein fraction quality, energy content, and nutritional value of commercial soybean meals (SBM) for poultry diets. Also, to study correlations between the laboratory data (proximal analyses and protein fraction) and the *in vivo* energy content and digestibility of crude protein (CP) and amino acids (AA) of commercial SBM.

Experiment 1. An experiment was conducted to study the influence of the origin of the soybeans on the chemical composition, protein quality, and nutritive value of the SBM. A total of 160 commercial samples of SBM from South Africa (SAF), Argentina (ARG), Brazil (BRA), and USA, in equal numbers, were collected by specialized quality control personnel. The samples were analyzed for main components [CP, AA, crude fiber (CF), neutral detergent fiber, sugars, and minerals] and for protein quality indicators [urease activity (UA), protein dispersibility index (PDI), protein solubility in KOH (KOH), and trypsin inhibitor activity (TIA)]. In addition, the *in vitro* digestibility of the protein fraction (IVDP) of the SBM was determined using a two-step test that simulated the digestive processes occurring in the gastrointestinal tract of the chick. On 88% dry matter (DM) basis, the content of CP and CF was higher ($P < 0.001$) for the BRA meals (46.9 and 4.63%) than for the SAF (45.9 and 3.75%), ARG (46.2 and 4.18%), and USA (46.2 and 4.18%) meals. Sucrose and stachyose contents were higher ($P < 0.001$) for the SAF (8.76 and 4.83%) and USA (7.33 and 5.08%) meals than for the ARG (6.86 and 4.67%) and BRA (5.80 and 4.45%) meals. The AA profile (% CP) varied with the origin of the SBM, with Lys being more abundant in the SAF and USA meals than in the ARG and BRA meals ($P < 0.001$). The UA, PDI, KOH, and TIA values were highest for the SAF meals and lowest for the ARG meals, with USA and BRA meals showing

intermediate values. At 1 and 3 h post-incubation, the IVDP was higher ($P < 0.001$) for the USA (77.9 and 82.3%) and the SAF (77.3 and 82.5%) meals than for the ARG (75.9 and 81.0%) and the BRA (76.0 and 81.0%) meals. Independent of the origin of the SBM, significant correlations were detected between IVDP at 3 h post-incubation and TIA ($r = 0.353$; $P < 0.001$) and KOH ($r = 0.217$; $P < 0.05$), but not with UA or PDI. Trypsin inhibitor activity was correlated with KOH ($r = 0.602$; $P < 0.001$) and PDI ($r = 0.368$; $P < 0.001$) but not with UA.

In Experiment 2. We evaluated the chemical composition, protein quality, and the IVDP of commercial SBM samples from the main SBM exporter countries, ARG, BRA, and USA ($n = 9$ per each country). In addition, we studied the apparent metabolizable energy corrected for nitrogen (AMEn) content and the apparent (AID) and standardized (SID) ileal digestibility of the CP and AA of these 27 SBM samples in broilers at 21 d of age. The SBM samples were collected at random from European crushers and feed mills by specialized personnel and analyzed for main components and protein quality indicators. In addition, the IVDP of the SBM was evaluated using 3 accepted methodologies based on enzymatic digestion, that simulated the digestive processes occurring in the gastrointestinal tract of the chicks. On 88% DM basis, the SBM from BRA had more CP (46.9 vs. 46.0 and 45.9%; $P < 0.05$) and less sucrose (5.21 vs. 6.28 and 6.47%; $P < 0.001$) and stachyose (4.20 vs. 4.66 and 4.78%; $P < 0.05$) than the SBM from USA and ARG. Per unit of CP, the contents in Lys, total sulfur AA, and the sum of 5 key AA (Σ 5 AA; Lys, Met, Cys, Thr, and Trp) were higher for the USA meals than for the South American meals ($P < 0.05$). All protein quality indicators were higher for the USA than for the BRA and ARG meals ($P < 0.05$). Reactive Lys (RLys), obtained by the furosine procedure was higher for the ARG meals than the BRA meals, with USA meals being intermediate ($P < 0.05$). However, RLys did not vary among SBM origins

when the homoarginine procedure was used. The origin of the soybeans did not affect the IVDP in any of the 3 *in vitro* methodologies used.

For the *in vivo* assay, broilers received a commercial corn-SBM diet in crumble form from hatch to 16 d of age and then, their corresponding experimental diets for 5 d. The experimental diets resulted from the combination of 53% of a nitrogen-free diet (dextrose, soybean oil, lignocellulose, and vitamins and minerals) and 47% of the corresponding SBM tested, as the unique source of nitrogen. Each of the 27 experimental diets was replicated 6 times and the experimental unit was a cage with 8 broilers. On 88% DM, the AMEn (2,334 vs. 2,282 and 2,277 kcal/kg; $P = 0.062$) tended to be higher for the USA meals than for the ARG and BRA meals. Also, the SID of the protein was greater ($P < 0.001$) for the USA meals than for the BRA meals, with the ARG being intermediate (92.0 vs. 90.9 and 91.4%; respectively). The SID of Lys (93.6 vs. 92.2 and 93.1%; $P < 0.001$) and Σ 5 AA (91.5 vs. 90.3, and 91.1%; $P < 0.05$) were higher for the USA meals than for BRA meals, with the ARG meals being intermediate. Similar differences to those reported for the SID were found for the AID values. Based on the coefficient of variation (%), the AID and SID values of the SBM samples for all key AA except Trp, were less variable for the USA than for the South American meals. From 17 to 21 d of age, birds fed the USA meals ate more, grew faster, and had better feed conversion ratio than birds fed the BRA and ARG meals ($P < 0.01$). Independent of the origin of the soybeans, the AMEn was positive correlated with the SID of CP ($r = 0.523$; $P < 0.01$) and SID of Lys ($r = 0.492$; $P < 0.01$) of the SBM, as well as with the RLys using the homoarginine procedure ($r = 0.466$; $P < 0.05$) of the SBM. The SID of CP and Lys of the SBM were positively correlated with the RLys using the homoarginine procedure ($r = 0.553$ and $r = 0.559$; $P < 0.01$) and with all the key main protein quality indicators of the SBM ($P < 0.10$), except UA. *In vitro* digestibility tests of the CP were

not consistently related to *in vivo* CP and AA digestibility and AMEn of the SBM ($P > 0.05$).

In summary, the chemical composition, protein quality, and IVDP of the SBM were influenced by the origin of the soybeans. Also, the AMEn content and the ileal digestibility of CP and AA of the SBM varied with the origin of the soybeans. Consequently, nutritionists and feed mill managers should use different matrices to evaluate more accurately the nutritional value and protein quality of SBM, specially taking into consideration the differences in digestibility and quality of the protein fraction and energy content of the SBM among different batches and origin of the soybeans in the formulation of poultry diets.

RESUMEN

RESUMEN

La presente Tesis Doctoral está compuesta por 2 Experimentos cuyo objetivo principal fue estudiar la composición química, calidad de la fracción proteica y el contenido energético de la harina de soja (HS) para su uso en piensos de broilers. Además, estudiar correlaciones entre análisis de laboratorio y datos *in vivo* del contenido energético y digestibilidad de la proteína bruta (PB) y aminoácidos (AA) de HS comerciales en broilers.

Experimento 1. Se evaluó la composición química, indicadores de calidad de la proteína y la digestibilidad *in vitro* de la proteína (IVDP) de la HS de diferentes orígenes. En total, 160 muestras de HS de Argentina (ARG), Brasil (BRA), Estados Unidos de América (USA) y Sudáfrica (SAF), en igual número por país ($n = 40$), fueron colectadas de forma aleatoria por personal de control de calidad especializado. Las muestras fueron analizadas para los principales componentes [PB, AA, fibra bruta (FB), fibra neutro detergente, azúcares y minerales] e indicadores de calidad de la proteína [actividad de ureasa (UA), índice de dispersibilidad de la proteína (PDI), solubilidad de la proteína en KOH (KOH) e actividad inhibidora de tripsina (TIA)]. Además, la digestibilidad *in vitro* de la proteína (IVDP) de las HS, fue determinada usando un test que simula los procesos que ocurren en el sistema gastrointestinal del ave. Sobre un 88% de materia seca, la PB y FB fue mayor ($P < 0,001$) en las HS de BRA (46,9 y 4,62%) que en las HS de SAF (45,9 y 3,75%), ARG (46,2 y 4,18%) y USA (46,2 y 4,18%). El contenido de sacarosa y estaquiosa fue mayor ($P < 0,001$) en las HS de SAF (8,76 y 4,83%) y USA (7,33 y 5,08%) en comparación a las HS de ARG (6,86 y 4,67%) y BRA (5,80 y 4,45%). El perfil de aminoácidos (% PB) varió con el origen de la HS, siendo la lisina más abundante en las HS de SAF y USA que en las HS de ARG y BRA ($P < 0,001$). Todos los indicadores de calidad de la proteína fueron mayores para las HS de SAF y menor para las HS de ARG,

con las HS de USA y BRA presentando valores intermedios. La IVDP, utilizando una digestión enzimática (pepsinas y pancreatinas), tras 1 o 3 h post-incubación fue mayor ($P < 0,001$) para las harinas de USA (77,9 y 82,3%) y SAF (77,3 y 82,5%) que para las harinas de ARG (75,9 y 81,0%) y BRA (76,0 y 81,0%). Independientemente del país de origen de la HS, la IVDP a 3 h post-incubación se correlacionó positivamente con la TIA ($r = 0,353$; $P < 0,001$) y KOH ($r = 0,217$; $P < 0,05$), pero no con la UA ni con el PDI. La TIA se correlacionó con KOH ($r = 0,602$; $P < 0,001$) y PDI ($r = 0,368$; $P < 0,001$), pero no con UA.

Experimento 2. Se estudió la composición química, calidad de la proteína y valor nutricional de muestras comerciales de HS procedentes de ARG, BRA y USA (9 por país de origen). Además, se evaluó la energía metabolizable aparente corregida por nitrógeno (EMAn) y la digestibilidad ileal aparente (DIA) y estandarizada (DIE) de la PB y de los AA de las 27 muestras de HS en broilers de 21 d de edad. Las muestras fueron colectadas aleatoriamente, por personal de control de calidad especializado, en plantas de procesamiento y fábricas de piensos europeas. Se analizaron los principales componentes químicos e indicadores de calidad de proteína de las HS. La IVDP de la HS, se determinó mediante 3 metodologías aceptadas (basadas en digestión enzimática), que simulan los procesos digestivos que ocurren en el tracto gastrointestinal de las aves. Sobre un 88% de materia seca, las HS de BRA tuvieron más contenido PB (46,9 vs. 46,0 y 45,9%; $P < 0,05$) pero menor contenido de sacarosa (5,21 vs. 6,28 y 6,47%; $P < 0,001$) y estaquiosa (4,20 vs. 4,66 y 4,78%; $P < 0,05$) que las HS de USA y ARG. El perfil de AA (% PB), varió con el origen de las HS, siendo el contenido en lisina, metionina + cisteína y la suma de los 5 AA claves en piensos de broilers (Σ 5 AA; lisina, metionina, cisteína, treonina y triptófano) mayor en las HS de origen USA que en las HS de origen brasileñas y argentinas. Todos los valores de los indicadores de calidad de la proteína (UA, PDI,

KOH y TIA) fueron más altos en las harinas de USA que en las harinas Sudamericanas ($P < 0,01$). La lisina reactiva (RLys), determinada por el método de la furosina, fue mayor para las HS de ARG que para las HS de BRA, con las HS de USA en posición intermedia ($P < 0,05$). Sin embargo, la RLys determinada por el método de homoarginina no se vio afectada por origen de las habas de soja.

En la parte *in vivo* del Experimento 2, las aves recibieron un pienso comercial basado en maíz-HS en forma migaja de 0 hasta los 16 días de edad. Luego, recibieron sus correspondientes piensos experimentales durante 5 días. Los piensos experimentales resultaron de la combinación del 53% de un pienso libre de nitrógeno (dextrosa, aceite de soja, lignocelulosa y vitaminas y minerales) y el 47% de la correspondiente SBM utilizada, como única fuente de nitrógeno. Cada uno de los 27 piensos experimentales se replicó 6 veces y la unidad experimental fue una jaula con 8 pollos. La EMAn (2.334 vs. 2.282 y 2.277 kcal/kg; $P = 0,062$) tendió a ser mayor en la HS de USA que para las HS de ARG y BRA. La DIE de la proteína (92,0 vs. 90,9 y 91,4%; $P < 0,001$), lisina (93,6 vs. 92,2 y 93,1%; $P < 0,01$) y Σ 5 AA (91,5 vs. 90,3 y 91,1%; $P < 0,05$) fue mayor para las HS de USA que para las HS de BRA, con las HS de ARG con valores intermedios. Para los valores de DIA se encontraron las mismas diferencias reportadas para los valores de DIE. Según el coeficiente de variación (%), los valores de digestibilidad de las HS fueron menos variables para las HS de USA que para las HS sudamericanas. De 17 a los 21 d de edad, las aves alimentadas con las HS de USA comieron más, crecieron más rápido y tuvieron una mejor tasa de conversión alimenticia que las aves alimentadas con las HS de BRA y ARG ($P < 0,01$). Independientemente del origen de las habas de soja, la EMAn de la HS estuvo positivamente correlacionada con la DIE de la PB ($r = 0,523$; $P < 0,01$), lisina ($r = 0,492$; $P < 0,01$), y RLys por el método de la homoarginina ($r = 0,466$; $P < 0,05$) de las HS. La DIE de la PB y lisina de las HS estuvieron positivamente

correlacionadas con la RLys por el método de la homoraginina ($r = 0,553$ y $r = 0,559$; $P < 0,01$) y con los indicadores de calidad de la proteína de las HS ($P < 0,10$), excepto para la UA. Las metodologías *in vitro* para determinar la digestibilidad de proteína de las HS no se correlacionaron con ninguna de las determinaciones *in vivo* (contenido de EMAn y digestibilidad de la PB y AA de las HS).

En resumen, la composición química, calidad de la fracción proteica y la IVDP de las HS varía de acuerdo el país de origen de las habas. Además, el contenido energético y la digestibilidad ileal de la PB y AA de la HS se ven afectados por el origen de las habas de soja. En consecuencia, nutricionistas y encargados de fábricas de piensos deben utilizar diferentes matrices para evaluar de una forma más precisa el valor nutricional y la calidad de la fracción proteína de las HS, especialmente tomando en cuenta las diferencias en digestibilidad y calidad de fracción de la proteína y el contenido energético de los diferentes lotes y origen de habas de soja en la formulación de piensos.

Documento de fe de erratas

23 de enero de 2024

A quien corresponda,

Mediante este documento hacemos constar, que el documento de tesis presenta un fallo de formato a partir de la página 116 hasta la página 133. Dicho fallo se encuentra en el encabezado de estas páginas, donde figura la frase "General Conclusions" en vez de "References".

Por consecuente, quisiera solventar dicho fallo y poder adjuntar el nuevo documento, siendo lo explicado en el párrafo anterior como único cambio de la memoria de Tesis.

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ABBREVIATIONS AND ACRONYMS

AA	Amino acids
AID	Apparent ileal digestibility
AME	Apparent metabolizable energy
AMEn	Apparent metabolizable energy corrected for nitrogen
ANF	Antinutritional factors
ARG	Argentina
BEL	Basal endogenous losses
BRA	Brazil
BW	Body weight
CF	Crude fiber
CHO	Carbohydrates
CP	Crude protein
CV	Coefficient of variation
DIA	Digestibilidad ileal aparente
DIE	Digestibilidad ileal estandarizada
DM	Dry matter
EE	Ether extract
EEh	Ether extract after previous HCl hydrolysis
EMAn	Energía metabolizable aparente corregida por nitrógeno
GE	Gross energy
GMD	Geometric means diameter
GIT	Gastrointestinal tract
GOS	α -galactosidases
GSD	Geometric standard deviation
HS	Harina de soja
IVDP	<i>In vitro</i> digestibility of crude protein
KOH	Protein solubility in KOH
NDF	Neutral detergent fiber

NFD	N-free diet
NSP	Non-starch polysaccharides
PDI	Protein dispersibility index
fRLys	Reactive lysine by the furosine procedure
hRLys	Reactive lysine by the homoarginine procedure
RLys	Reactive lysine
SAF	South Africa
SBM	Soybean meal
SEM	Standard error of the mean
SID	Standardized ileal digestibility
TI	Trypsin inhibitor
TIA	Trypsin inhibitor activity
TSAA	Total sulfur amino acids
UA	Urease activity
USA	United States of America
Σ 5 AA	Sum of the five key amino acids (Lys, Met, Thr, Trp, and Cys)

LITERATURE REVIEW

1. LITERATURE REVIEW

1.1. Introduction

Soybean meal (**SBM**) is the main protein feedstuff used in poultry diets due to its crude protein (**CP**) content, amino acids (**AA**) profile, and consistent supply and availability. In addition, SBM contributes to a great extent (approximately 25%, in corn-SBM diets), to the energy content of poultry diets due to its inclusion in commercial diets (Mateos et al., 2019; Abdollahi et al., 2021). Brazil (**BRA**), United States of America (**USA**), and Argentina (**ARG**) as the main exporter countries worldwide. South Africa (**SAF**), the largest soybean producer of Africa (FAO, 2023), does not export SBM to Europe and possible reason is that the information available on the chemical composition and nutritional value of the SBM produced in this country is very limited. The chemical composition, ileal digestibility of the CP and AA, and energy content of commercial SBM are influenced by numerous factors, including soil and seed characteristics, geographical area of production, and procedure used for oil extraction (Westgate et al., 2000; Ravindran et al., 2014; Ibáñez et al., 2020; Aguirre et al., 2022).

Trypsin inhibitors (**TI**), the main antinutritional factors (**ANF**) present in raw soybeans, are inactivated by heat, a process that increases the nutritional value of the SBM and its use in poultry diets. Under- and over-processing of the soybeans affects the digestibility of the AA and consequently, energy utilization. Under-processing results in an excess of the TI that affects AA digestibility. An excess of heat, however, reduces the digestibility of heat-labile AA such as Arg, Cys, and more specifically Lys, through the formation of Maillard reactions (Fontaine et al., 2007; Pahm et al., 2008). The heat process quality of the SBM is currently measured by laboratory procedures such as urease activity (**UA**), protein dispersibility index (**PDI**), protein solubility in KOH (**KOH**), TI

activity (**TIA**), reactive Lys (**RLys**). Therefore, *in vitro* digestibility of the CP (**IVDP**) tests can be used to evaluate the quality of the protein fraction of the SBM. However, the information about robust correlations between these procedures and *in vivo* data (CP and AA digestibility and energy content) to predict the quality of the SBM is scarce.

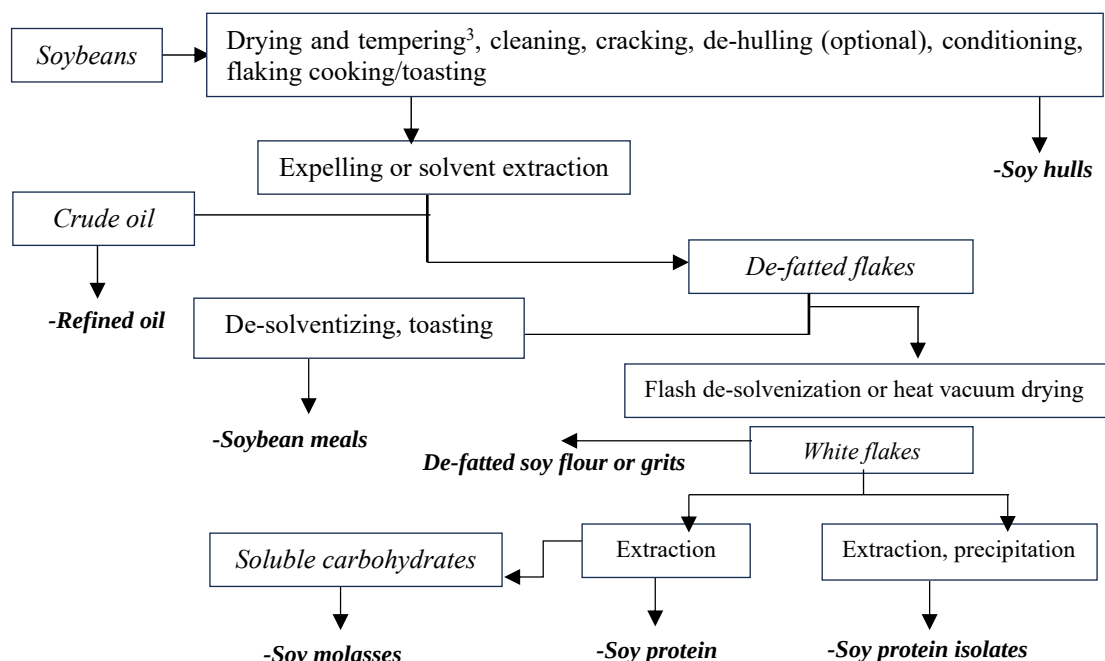
Crude protein, moisture, and fiber content are the main variables used by the feed compound industry to estimate the nutritional value of the SBM, factors that, together with the cost, determine its use in poultry diets. The energy of the SBM is often estimated from Tables of Feed Ingredient Composition published by Scientific Institutions (NRC, 1994; Rostagno et al., 2017; Premier Atlas, 2019; FEDNA, 2021; AMINODat, 2023) and prediction equations (WPSA, 1989; CVB, 2021). However, the energy values of commercial SBM estimated with these sources, are inconsistent, which limits its utilization in practice. Originally, the energy content of the SBM was based on the chemical composition, with CP ($N \times 6.25$), ether extract (**EE**), and N-free extract as main variable (WPSA, 1989; NRC, 1994). However, the characteristics and digestibility of the protein and of the carbohydrates (**CHO**) fraction according to the quality and origin, main components of the soybeans on the energy content of the SBM, are not well considered. In this respect, prediction equations created in recent years, use digestible protein and sugar content of the SBM as parameters for the prediction of the energy content of the SBM (CVB, 2021; Fondevila et al., 2021). But most of the time, the digestible protein content is estimated by near-infrared spectroscopy analyses, and the sugar content does not differentiate among the origin of the soybeans and value of this variable (sucrose *vs.* stachyose and raffinose). Consequently, data provided from *in vitro* and *in vivo* trials are gaining interest to validate the energy content of Tables of Feed Ingredient Composition values for SBM produced in different geographical areas worldwide (Adedokun et al.,

2008; Ravindran et al., 2014; Bryan et al., 2018; Zaefarian et al., 2021; Siegert et al., 2023).

1.2. Soybean Products

There are many soybean products used in the feed industry, these products are obtained from the separation or extraction of different components of the soybeans and depend on the process applied (Figure 1). Conventional SBM, which is solvent-extracted dehulled SBM, is produced using a solvent, that usually is hexane (Hammond et al., 2005; van Eys and Ruiz, 2021). The crude oil is refined and separated into lecithin and refined oil that is used in human as well as in animal nutrition. Part of the soy hulls separated at the beginning of the process is added back in variable amounts to the SBM after oil extraction determining at great extent the CP content of the resulting SBM. To remark that the additional soy hulls added back have not been thermally processed and thus, it contains certain amounts of TI.

Figure 1. Manufacturing soybean products^{1, 2}



¹ Adapted from van Eys and Ruiz (2021).

² Boxes with regular script are processes and with italics are intermediary products. Bold and italics script are final soy products.

³ Only in the case of solvent extraction

The quality of the equipment used in the processing plant as well as the methodology and the conditions of the extraction of the oil (moisture, temperature, and duration) will affect the nutritive value of the commercial SBM (Hammond et al., 2005; Karr-Lilienthal et al., 2005; Ravindran et al., 2014; Dunmire et al., 2023). Also, poor storage and transport conditions of the soybeans and the resulting SBM, especially under excessive temperatures, might result in an accumulation of the moisture contained in the product in certain areas of the container decreasing the quality of the SBM. Consequently, it is essential to monitor these factors to preserve SBM quality, especially when the storage and transport conditions of the product are deficient (Coradi et al., 2020).

1.3. Origin of the Soybeans

Brazil, USA, and ARG are the main suppliers of the SBM worldwide. From 2017 to 2023, Europe imported as an average 30 MMT of soybeans equivalent, from which 45, 19, and 17% correspond to BRA, ARG, and USA, respectively (European Commission, 2023). Variations in soil, seed type, agricultural practices, environmental conditions during the growing and harvesting seasons, and processing and storage characteristics of the soybeans will influence in the chemical composition and quality of the SBM (Westgate et al., 2000; Ravindran et al., 2014; García-Rebollar et al., 2016; Galkanda-Arachchige et al., 2021; Aguirre et al., 2022). Particularly, the latitude of the planting area in relation to the day-length and the temperature during the growing period of the crop are key factors that influence the composition of the original soybeans (Stefanello et al., 2016).

1.4. Chemical Composition and Nutritional Value of the Soybean Meals

1.4.1. Proximal Composition

The effects of the soybean origin on the chemical composition and nutritional values of the SBM have been studied by several authors (Ravindran et al., 2014; García-Rebollar

et al., 2016; Lagos and Stein, 2017; Galkanda-Arachchige et al., 2021; Aguirre et al., 2022). However, in the industry these information, based on the differences among soybean origins, is not usually used to adjust the SBM matrix for feed formulation. In general, these studies observed that the SBM from BRA contained more CP, crude fiber (CF), neutral detergent fiber (NDF), and raffinose but less sucrose and stachyose content than the SBM from USA and ARG. A meta-analytical study (Ibáñez et al., 2020) on the relation between the country of origin of the soybeans (ARG, BRA, USA, and India) and chemical composition, protein quality, and nutritional values of the SBM, also, confirmed that the origin of the soybeans had significant effects on most of the chemical variables studied of the SBM (Table 1). Crude protein of the SBM depends on the seed genotype and the location of the crop field (Figure 2) but also on the quality of the de-hulling process (Wolf et al., 1982; Ravindran et al., 2014; Wijewardanaa et al., 2019; Alsajri et al., 2020; Aguirre et al., 2022). The oil content of the SBM depends at a great extent on the characteristics of the oil extraction process, including the solvent (e.g., hexane or petroleum ether) used and the amount of gums, soupstocks, or other lipid materials added during the process (Hammond et al., 2005; Ravindran et al., 2014; García-Rebollar et al., 2016).

Sucrose is one of the most important fractions of the SBM, with increased interest for the energy contribution for poultry diets. Sucrose content of the SBM varies between 3 and 10% (Ibáñez et al., 2020; Galkanda-Arachchige et al., 2021; Aguirre et al., 2022). The sucrose content of commercial SBM is very variable and factors such as average temperature during the growing season (Wolf et al., 1982; Kumar et al., 2010; Alsajri et al., 2020) and maturity state and weather conditions at harvest (Saldivar et al., 2011; Jaureguy et al., 2013) affect the sucrose content of the SBM. Because of the differences in temperature (e.g., cool vs. tropical area of production), the sucrose content of the

soybeans is higher when the soybeans are produced in cooler areas (García- Rebollar et al., 2016; Lagos and Stein, 2017). In this respect, Wolf et al. (1982) observed that the sucrose and stachyose content of the soybeans was affected by the cultivar temperature, with the raffinose content without effect of the cultivar temperature (Figure 2). Also, Alsajiri et al. (2020) in a study with 2 varieties of soybeans and 5 day/night temperatures reported similar results (Figure 3).

Table 1. Influence of the origin of the soybeans on the chemical composition (%) of the soybean meals¹ (88% dry matter)

Trait	n ²	Soybeans origin ³				SEM1 ⁴	SEM2 ⁵	P-value
		ARG	BRA	USA	IND			
Ash	44	6.54 ^b	6.29 ^c	6.54 ^b	7.48 ^a	0.137	0.571	<0.001
Crude protein	58	45.5 ^c	47.0 ^a	46.4 ^b	46.3 ^{a,b,c}	0.451	1.980	<0.001
Ether extract	46	1.66 ^a	1.78 ^a	1.67 ^a	1.11 ^b	0.175	0.562	<0.001
Sucrose	34	6.41 ^b	5.24 ^c	6.99 ^a	4.19 ^d	0.309	0.983	<0.001
Stachyose	30	4.15 ^b	3.80 ^c	4.77 ^a	3.97 ^{a,b,c}	0.461	0.741	<0.001
Raffinose	27	1.15 ^b	1.33 ^a	0.95 ^c	1.70 ^{a,b}	0.226	0.372	<0.001
Crude fiber	40	4.32 ^c	5.03 ^b	3.88 ^d	6.55 ^a	0.318	1.31	<0.001
NDF ⁶	34	9.57 ^b	10.8 ^a	8.69 ^c	13.0 ^a	0.931	2.84	<0.001

a, b, c, d Means within a row lacking of a common superscript differ significantly ($P < 0.05$).

¹ Data from Ibáñez et al. (2020).

² Number of means per origin compared in all the studies.

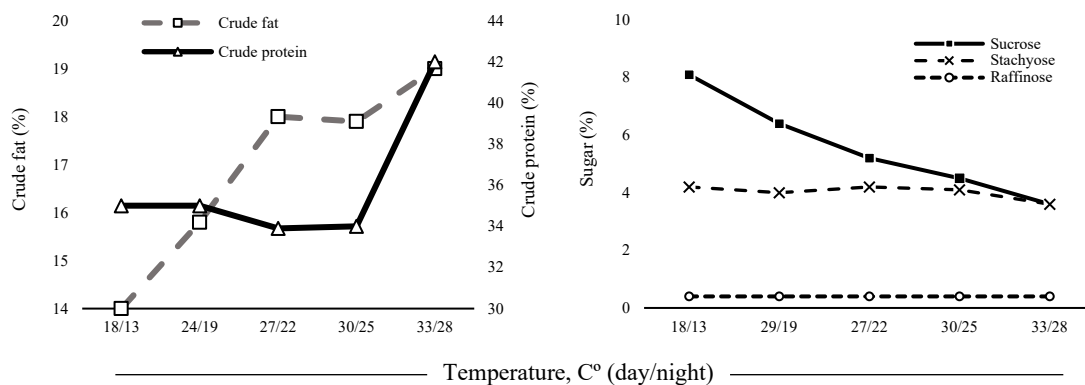
³ ARG = Argentina, BRA = Brazil, USA = United States of America, and IND = India.

⁴ SED1 = Standard error of the mean between India and the 3 other origins.

⁵ SED2 = Standard error of mean among Argentina, Brazil, and USA meals.

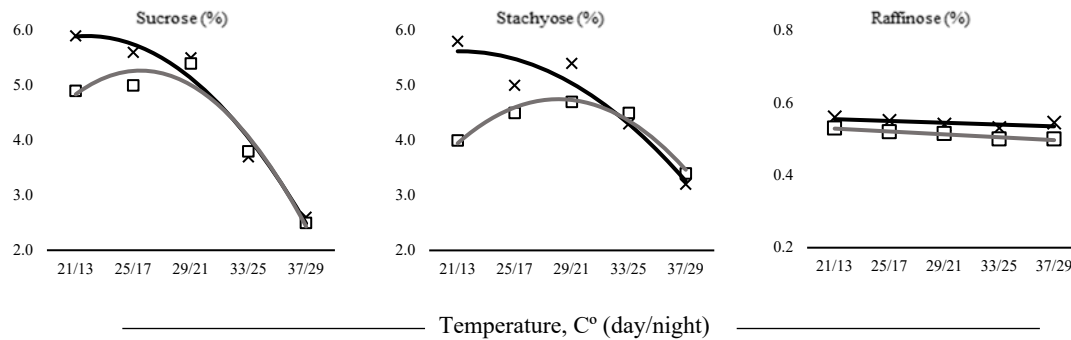
⁶ Neutral detergent fiber.

Figure 2. Effect of the cultivar temperatures on chemical composition (% dry matter) of the soybean meals



¹ Data from Wolf et al. (1982).

Figure 3. Effect of the cultivar temperatures on sugar content (% dry matter) of the two soybeans varieties¹



¹ Data from Alsajiri et al. (2020).

Additionally, sucrose content of the soybeans decreases with the stage of maturity and when poor weather conditions retard the harvesting of the crop, fermentation and germination processes might take place, reducing the level of sucrose in the soybeans (Peer and Leeson, 1985; Saldivar et al., 2011; Jauregui et al., 2013; Talegón et al., 2023). In this respect, Talegón et al. (2022) analyzed commercial raw beans from USA and BRA that arrived in Europe ports between 2021 and 2023 and these authors observed differences among countries on chemical composition and quality of the soybeans (Table 2). Although there is a clear difference in sucrose content, depending on the country of origin of the soybeans, the sucrose is not commonly analyzed and taken into account in the energy contribution of the SBM.

Table 2. Chemical composition (% as fresh) of the raw soybeans, according to the origin and quality¹

	USA (n = 14)		Brazil (n = 29)		SD ⁴	P-value ⁵	
	HQ ²	Damaged ³	HQ ²	Damaged ³		Origin	Quality
Dry matter	88.4	89.9	89.0	89.6	0.98	NS	< 0.05
Crude protein	35.0	36.4	34.9	36.6	1.06	NS	< 0.001
Sucrose	6.2	4.3	4.9	3.7	0.860	< 0.01	< 0.001
Stachyose	3.6	2.4	2.6	1.3	0.500	< 0.001	< 0.001
Raffinose	1.1	0.8	1.1	0.7	0.004	< 0.001	< 0.001

¹ Data from Talegón et al. (2023).

² HQ = high quality soybeans.

³ Damaged = heat damaged, mottled purple, immature green, moldy, and sprouted grains.

⁴ Standard deviation.

⁵No significant.

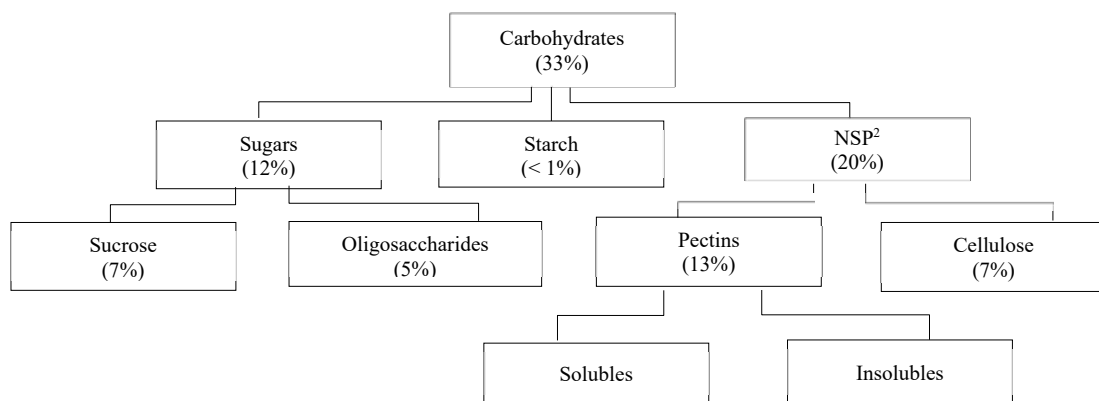
The primary oligosaccharides found in SBM are the α -galactosidases (**GOS**), which are stachyose, raffinose, and small amount of verbascose. The GOS represent approximately 4 to 6% of the SBM composition but can range up to 9% (Carré et al., 1995; Grieshop et al., 2003; García-Rebollar et al., 2016; Ibáñez et al., 2020). Stachyose, composed of two α -D-galactose units, one α -D-glucose unit, and one D-fructose unit, is the most abundant oligosaccharide present in SBM. Stachyose accumulates in the seed during the late phase of the maturation process and serves for the transport and storage of CHO in mature seeds (Obendorf et al., 2009; Wijewardana et al., 2019). Birds are not able to digest the GOS because they lack of the endogenous α -1,6 galactosidase enzyme production for their hydrolysis. The role of the GOS present in the SBM as an ANF is a subject of debate. Different authors (Coon, 1990; Leske et al., 1993; Choct et al., 2010) consider that GOS act as ANF in non-ruminant diets, because when fermented in the large intestine produce flatulence and increase the multiplication and growth of the existing microflora. In addition, reduce dietary energy and GOS impairs water reabsorption, which can negatively impact nutrient utilization and litter conditions (Bedford, 1995; Perryman and Dozier, 2012; Teague et al., 2023). However, GOS are easily fermented by the microorganism present in the gastrointestinal tract (**GIT**), producing short chain fatty acids which contribute with measurable amounts to the energy requirements of the animal.

Simple sugars (e.g., glucose) and starch are present in small amount (< 1%) in the SBM and both are well utilized by poultry. Significant levels of glucose occur only in immature seeds or as result of fast degradation of the oligosaccharide molecules (Svejstl et al., 2015). Usually, none of these CHO are determined in the laboratory when evaluating the energy content of SBM and are not taken into account in feed formulation. In addition, the methodology used to determine the starch content will be important to increase our

accuracy. For example, using the polarimetry methodology, starch content ranges from 5 to 6% (WPSA, 1989; Rostagno et al., 2017; Premier Atlas, 2019). However, when the amyloglucosidase/ α -amilase method is used (a more adequate procedure), starch content ranges from 0.5 to 1.0% (Mateos et al., 2019; AMINODat, 2023).

The fiber content (CF and NDF) of commercial SBM depends on the amount of hulls removed from the soybeans prior to the oil extraction process or added back to the meal after processing, but also on the climate conditions during the growing and harvesting seasons (Medic et al., 2014; Ibáñez et al., 2020; Aguirre et al., 2022). The higher concentration of fiber in SBM reflects both its concentration due to removal of soybean oil during processing, and the fact that soluble fiber is quantified in total dietary fiber and not in NDF. The specific structures of dietary fibers are not well understood and the information available is very limited. The classification of the CHO of the SBM, according to Choct et al. (2010), is shown in Figure 4. Non-starch polysaccharides (NSP; 16% pectins and 7% cellulose) are the main portion of this fraction, followed by sucrose, stachyose, and raffinose with 12%, and starch with a content below 1%.

Figure 4. Classification of the carbohydrate contents of the soybean meals¹



¹ Adapted from Choct et al. (2010).

² Non-starch polysaccharides.

Differences have been observed in soybean cotyledon and hull polysaccharides. Total NSP are the sum of the water-soluble and water-insoluble NSP and include the sum of cellulosic and non-cellulosic polysaccharides (arabinose, xylose, mannose, galactose, glucose, and uronic acids). The NSP of the SBM range from 159 to 217 g/kg, which a 22% of the NSP correspond to the soluble fraction and the 78% to the insoluble fraction (Table 3).

Table 3. Non-starch polysaccharides (NSP) of the soybean meals¹ (g/kg dry matter)

	Irish and Belnave (1993)	Bach Knudsen (1997)	Bueno et al. (2018)
Number of samples	6	10	6
Soluble NSP			
Rhamnose	1	1	-
Fucose	-	-	-
Arabinose	5	9	5
Xylose	1	2	2
Mannose	2	5	2
Galactose	6	16	9
Glucose	2	6	7
Galacturonic acid	11	25	11
Total Soluble NSP	27	63	35
Insoluble NSP			
Rhamnose	2	2	-
Fucose	3	-	-
Arabinose	24	17	16
Xylose	17	17	13
Mannose	7	8	5
Galactose	39	25	22
Glucose	3	1	46
Galacturonic acid	25	23	23
Cellulose	44	62	-
Total Insoluble NSP	165	155	124
Insoluble:soluble ² NSP	6.11	2.46	3.54
Total NSP	192	217	159

Note: Dash indicated that no data were reported.

¹ Average values.

² Insoluble to soluble NSP ratio.

1.4.2. Mineral Composition

The mineral content of the SBM is influenced by the origin of the soybeans, mainly due to differences in the composition and acidity of the planting soil and the use of

fertilizers during the germination and growing periods. Thus, the higher mineral concentration and acidity produce soybeans containing less P, Ca, and K but more Fe content (García-Rebollar et al., 2016; Lagos and Stein, 2017; Aguirre et al., 2022). To remark that occasionally, certain amounts of limestone are added to the SBM as a flow agent consequently, the Ca content of the SBM will increase (Ravindran et al., 2014; García-Rebollar et al., 2016). Also, the variability in mineral content reported is related to soil characteristics, fertilization rate, and plant availability to absorb the minerals (Westgate et al., 2000; Huerta and Martin, 2002).

1.5. Protein Quality Indicators of the Soybean Meals

Because of cost and complexity, TI and Maillard reactions are not usually analyzed under commercial conditions, and protein quality is estimated indirectly using laboratory procedures such as UA, PDI, and KOH. High values of these protein quality indicators are indicative of under-processing whereas low values reflect over-cooking. The values recommended by the industry for protein quality indicators and RLys are shown in Table 4.

Table 4. Industry recommendation of the protein quality indicators and reactive lysine of the soybean meals¹

Method	Values
Urease activity (Δ pH)	0.00 to 0.10
Protein solubility index (%)	15 to 30
Protein solubility in KOH (%)	75 to 85
Trypsin inhibitor activity (mg/g)	< 2.2 to 3.5
Reactive Lys (%)	≥ 88

¹ van Eys and Ruiz (2021).

In general, the SBM from USA presented higher values for the protein quality indicators used in the industry compared to the SBM from South America (Ravindran et al., 2014; García-Rebollar et al., 2016; Lagos and Stein, 2017; Aguirre et al., 2022). These observations are related to the quality of the original soybeans (Ibáñez et al., 2020;

Cámara et al., 2022; Talegón et al., 2023). For example, soybeans collected with a moisture content above 13%, will require extra drying at the farm before storage (Coradi et al., 2016, 2020). Any excess of heat, prior to crushing the soybeans will reduce the final PDI and KOH values of the resulting SBM.

1.5.1. Urease Activity

Urease activity is widely used as an indicator of the presence of TI in SBM although this method might not be appropriate to detect over-processed meals (García-Rebollar et al., 2016; Ibáñez et al., 2020; Aguirre et al., 2022). The soybeans content variables quantities of urease, a thermolabile enzyme which its destruction due to the heat process, which presents a similar dynamic to the TI destruction (Balloun, 1980; Waldroup et al., 1985). Urease activity is based on measured the pH increase, that results from the NH_3 production for urease, enzyme present in SBM without process or under-processed. Depending on the protocol used, the endpoint is determined differently. Using the AOCS (2019) method, the endpoint is determined by measuring the increase in pH of the samples whereas the European Union method, the endpoint reflects the amount of acid required to maintain a constant static pH. However, both methods differ slightly (van Eys and Ruiz, 2021). This methodology measured indirectly the heat treatment process that is applied to the SBM, consequently, the denaturation of the ANF. This is the most common method used by the industry due to the cost and simplicity.

1.5.2. Protein Dispersibility Index

The PDI measures the N solubility of the SBM sample in an aqueous medium and makes it possible to more precisely determine the optimal level of processing of SBM than the UA or KOH tests (Batal et al., 2000). The procedures applied during its analysis in the laboratory can affect the measurement. To notice that storage time tends to reduce PDI values while the effect is scarce on KOH values and practically null on TI (Table 5).

For this reason, the PDI values will tend to be lower when is analyzed in the country of destination than in the country of origin (Serrano et al., 2013).

Table 5. Storage time effect on the protein quality indicators of the soybean meals¹

Method ²	Storage time (weeks)					SEM ³ (n = 7)	P-value ⁴
	0	24	48	80	136		
UA (mg N/g)	0.03	0.02	0.02	0.02	0.02	0.011	NS
PDI (%)	19.5	16.5	14.9	12.7	9.8	1.11	< 0.05
KOH (%)	84.8	84.2	82.2	82.9	80.9	1.08	< 0.001
TIA (mg/g)	2.4	2.4	2.3	2.3	2.3	0.19	NS

¹ Data from Serrano et al. (2013).

² UA = Urease activity; PDI = protein dispersibility index; KOH = protein solubility in KOH; TIA = trypsin inhibitor activity.

³ Standard error of the mean.

⁴ NS = no significant; $P < 0.05$ = linear effect.

1.5.3. Protein Solubility in KOH

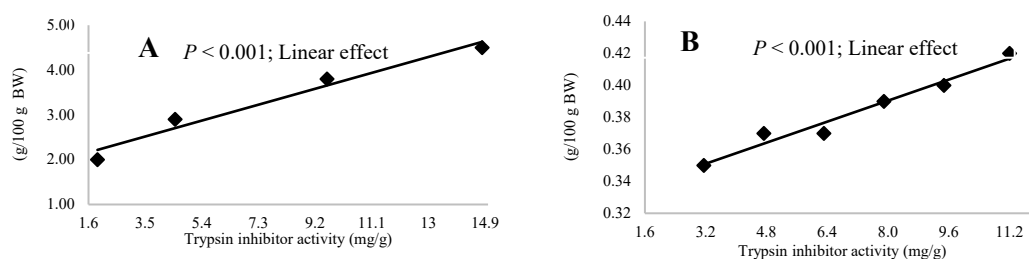
The solubility of globulins, the main storage proteins of soybeans, is high in raw soybeans but decreases with thermal processing. The procedure measures the decrease in solubility of the soybean protein fraction as a result of thermal processing. It should be taken into account that the handling of the samples during their analysis in the laboratory (e.g., grinding, agitation, temperature, pH of the solution, time, etc.) can influence the result (Whittle and Araba, 1992). The KOH test allows us to measure the solubility of SBM protein in a 0.2% KOH solution and thus indirectly determine the degree of denaturation of the protein fraction. This is an effective procedure for determining overheating heat treatment. However, Anderson-Haferman et al. (1992) and Hemetsberger et al. (2021) reported that KOH test as the sole parameter to estimate sufficient heat treatment of the SBM is less effective.

1.5.4. Trypsin Inhibitor Activity

Trypsin inhibitors are peptides that form stable complexes with the trypsin and chymotrypsin enzymes produced in the pancreas. Both enzymes play an important role in the digestion of the protein fraction of the feeds in the GIT of the animals. The presence

of TI inactivates these 2 pancreatic enzymes and, in response to the inhabitation of these enzymes the pancreas increases its size and number of cells to increase the secretion of digestive enzymes (Perilla et al., 1997; Pacheco et al., 2014; Kuenz et al., 2022). When trypsin is inhibited by TI, cholecystokinin production is enhanced, resulting in an increase of production of pancreatic digestive enzymes leading to pancreatic hypertrophy (Liener, 1981; Perilla et al., 1997; Clarke and Wiseman, 2005). The Kunitz inhibitors ($\approx 70\%$ in raw soybeans) and the Bowman-Birk inhibitors ($\approx 30\%$ in raw soybeans) are the 2 most important TI present in raw soybeans (Liener, 1981; Goebel and Stein, 2011). Fortunately, heat processing reduces the negative effects of these ANF on animal performance (Di Pietro and Liener, 1989; Clarke and Wiseman, 2005). In this respect, Clarke and Wiseman (2007) and Pacheco et al. (2014) studied the effect of the TI (mg/g) of full-fat soybeans on pancreas size in broilers at 26 and 16 d of age, respectively (Figure 5). The direct analysis for TI has been the most desirable assay to evaluate SBM quality and processing conditions. However, the analyses of TI have been time consuming, required expensive equipment, produced inconsistent results probably due to differences in methodologies (Parsons, 2000), such as Hamerstrand et al. (1981) vs. Ba 12-75 (AOCS, 2017) vs. ISO (2012) and have been impractical for day-to-day monitoring of SBM quality and adequacy of heat treatment.

Figure 5. Effect of trypsin inhibitor on pancreas weight in broilers^{1,2}



¹Figure A, broilers at 26 d of age (Clarke and Wiseman, 2007);

²Figure B, broilers at 16 d of age (Pacheco et al., 2014).

1.5.5. *Reactive Lysine*

The RLys procedure has previously been used to measure heat damage in protein sources caused by Maillard reactions. The Maillard reaction complexes formed between the reducing sugars and the AA of the SBM in the presence of heat. The reactions involve the aldehyde (or ketone) of the sugar and the amino groups of the protein. The multiple reaction chain has several progressive steps: a) initial reaction with the formation of Amadori compounds, b) ring formation which adds the flavour and aroma characteristics of the process, and c) melanoid production with the typical browning reaction. It is important to understand that the Maillard reactions require the presence of reducing sugars (monosaccharides, lactose, and maltose). The homoarginine and furosine procedures are methodologies used to measure heat damage in feed ingredients (Fontaine et al., 2007; Pahm et al., 2008; Erbersdobler and Somoza, 2017). The furosine procedure is suited for proteins that have undergone moderate heating and when Amadori products are the sole source of unreactive Lys (Chiang, 1983; Erbersdobler and Somoza, 2017) and the homoarginine procedure is effective in measuring RLys in proteins that have undergone moderate or severe heating (Hurrell and Carpenter, 1981). The homoarginine procedure consists in transforming the unbound Lys to homoarginine through a guanidination reaction.

1.6. **In Vitro Digestibility of the Crude Protein of the Soybean Meals**

The CP digestibility of the SBM can be determined *in vivo* or *in vitro*. *In vivo* methods are expensive and time-consuming and require the use of live birds which limits their use in practice (Losada et al., 2009; Frikha et al., 2012; Ravindran et al., 2014; Mateos et al., 2019). *In vitro* methods simulate the digestive processes that occur in the GIT of the birds and are simple, faster, and more convenient in practice than *in vivo* methods (Boisen and Eggum, 1991; Bedford and Classen, 1993). However, the

information available on the IVDP fraction of the SBM, is scarce (de Coca-Sinova et al., 2008; Frikha et al., 2012; Siegert et al., 2023). Despite some fundamental differences between the digestion in pigs and poultry, almost all current *in vitro* poultry models are based on pigs (Boisen and Fernández, 1995, 1997; Woyengo et al., 2015) and used for the generation of *in vitro* data for poultry (Losada et al., 2009, 2010). The IVDP might be a good predictor of the *in vivo* CP digestibility of the SBM but in some cases do not correlate with the *in vivo* CP and AA digestibility of the SBM (Frikha et al., 2012; Siegert et al., 2023). However, as a tool to see differences in IVDP of the SBM, this provides valuable information. In this respect, Frikha et al. (2012) reported significant differences on IVDP among soybean origins. In contrast, Siegert et al. (2023) did not find significant differences on IVDP testing SBM from 5 origins.

1.7. Amino Acids Content and Digestible of the Soybean Meals

In general, SBM from BRA have more CP content, consequently more AA content than the SBM from USA and ARG, opposite that the general believes that the highest fiber content the lowest CP content. However, per unit of CP, BRA meals have similar or less AA than USA and ARG meals, consequently, the nutritional value of the BRA meals in relation to their protein fractions are not necessarily higher than the other origins. In this respect, García-Rebollar et al. (2016) reported that per unit of CP, the sum of the 5 key AA (Σ 5 AA; Lys, Met, Thr, Trp, and Cys) was higher for the USA and ARG meals than for the BRA meals. Similar results were observed by Ibáñez et al. (2020) and AMINODat (2023). There is not a clear explanation for the differences in the AA profile of the SBM observed according to the country of origin of the soybeans (Lagos and Stein, 2017; Aguirre et al., 2022). The AA profile of the soybean might depend on the variety used, the location of the area of production (Karr-Lilienthal et al., 2005; Stefanello et al., 2016; Lagos and Stein, 2017; Galkanda-Arachchige et al., 2021), the weather conditions

during harvest (Jaureguy et al., 2013), the drying of the seeds at farm level, the quality of the raw soybeans prior to process (Cámara et al., 2022; Talegón et al., 2023), and the heat applied during the crushing process (Fontaine et al., 2007; Gonzalez-Vega et al., 2011; Abdohalli et al., 2022; Dunmire et al., 2023). In this respect, because of the pluviometry, high temperature, and weather conditions the soybeans produced in tropical areas cannot be harvested on time and remain for long periods in the field. Under these circumstance, part of the beans will germinate, affecting the content and the AA profile of the raw soybeans (Peer and Leeson, 1985; Talegón et al., 2023). In addition, under high humidity and temperature conditions the seeds are contaminated by the microbial population which in turn will increase the incidence of fermentation process, reducing the amount of real protein and modifying the AA profile of the soybeans (Paulsen et al., 1981; Coradi et al., 2016). Moreover, soybeans produced in areas close to the equator, are usually harvested with higher moisture content than beans produced at higher latitude. Under these circumstances, soybeans with a high moisture content have to be dried before storage at the farm. If the heat applied is excessive or inadequate (e.g., burning wood vs. air flow at low temperature) the AA profile of the corresponding SBM will be affected (Coradi et al., 2016). The digestibility of the AA varied with the origin of the soybeans. In this respect, Frikha et al. (2012) reported significant differences on apparent (**AID**) and standardized (**SID**) ileal digestibility of the CP and AA of the SBM among soybean origins (ARG, BRA, and USA), in an *in vivo* broiler assay. Similarly, Ravindran et al. (2014), reported significant differences on SID of the CP and AA of the SBM among soybean origins, using 55 SBM (from ARG, BRA, USA, and India) samples tested in broilers. However, Siegert et al. (2023), did not find significant differences in AA digestibility of the SBM among soybean origins (USA, ARG, BRA, India, and Europe) in an *in vivo assay* with laying hens.

The SID of the CP and AA of the SBM used by the industry, are most times based on Feed Composition Tables (Table 6). Also, the SID of the CP and AA of the SBM have been studied by different authors and important variability exist among these sources (Table 7). Consequently, understanding the type of data to evaluate the AA digestibility of the SBM is necessary. The reasons for the variability reported are numerous and include factors explained before such as the geographical area of production, harvest conditions in which the soybeans tested were produced, heat processing conditions, and the research protocol used in the determination (de Coca-Sinova et al., 2008; Perryman and Dozier, 2012; Sheikhhasan et al., 2020; Ahasic et al., 2023; Ali et al., 2023).

Table 6. Standardized ileal digestibility (%) of the crude protein (CP) and selected amino acids of the soybean meals in broilers¹

Institution	Year	Country	CP	Lys	Met	TSAA ²	Thr	Trp
WPSA ³	1989	Europe	87.0	-	-	-	-	-
NARO	2009	Japan	85.0	-	-	-	-	-
RPRI ⁴	2014	Russia	-	87.2	85.5	84.4	84.0	84.0
Rostagno et al.	2017	Brazil	91.4	92.4	93.6	89.0	87.6	90.9
Premier Atlas	2019	UK	89.0	88.0	90.3	81.5	82.7	89.8
INRAE	2021	France	-	88.0	89.0	82.0	83.1	89.1
CVB	2021	Netherlands	85.0	88.0	90.0	82.3	83.0	89.0
FEDNA ⁴	2021	Spain	88.0	89.0	90.0	86.0	86.0	86.0
AMINODat	2023	Germany	-	89.0	90.0	84.0	83.0	89.0

Note: Dash indicated that no data were reported.

¹ Values in table correspond to average value reported by the institutions.

² Total sulfur amino acids.

³ Apparent ileal digestibility values.

⁴ Apparent fecal digestibility values.

Table 7. Standardized ileal digestibility (%) of the crude protein (CP) and selected amino acids of the soybean meals in poultry^{1, 2}

Reference	n ³	Soybeans origin ⁴	CP	Lys	Met	Cys	Thr	Trp
Adedokun et al. (2008)	1	USA	-	89.5	91.5	81.8	87.3	-
de Coca-Sinova et al. ⁵ (2008)	6	ARG, BRA, USA	80.2	82.6	85.6	58.8	70.8	-
Perryman and Dozier ⁵ (2012)	2	USA	-	90.1	91.1	79.9	83.6	89.8
Frikha et al. (2012)	22	ARG, BRA, USA	92.2	91.6	94.8	83.7	91.7	-
Ravindran et al. (2014)	55	ARG, BRA, USA, IND	79.0	82.0	84.0	58.0	75.0	-
Leung and Kiarie (2020)	1	IND	-	90.6	92.9	87.3	91.4	-
Sung et al. (2023)	6	USA	92.9	92.6	94.5	87.3	89.2	-
Ahasic et al. (2023)	1	USA	-	87.2	88.0	78.5	85.6	95.2
Siegert et al. (2023)	18	ARG, BRA, USA, IND, Europe	-	90.1	90.8	77.6	84.2	-
Ali et al. (2023)	1	USA	89.0	91.9	94.7	84.5	87.9	92.4

Note: Dash indicated that no data were reported.

¹ Values correspond to the average value reported by the authors.

² Broilers data, except for Ahasic et al. (2023) = rooster and Siegert et al. (2023) = laying hens.

³ Number of soybean meal samples used.

⁴ ARG = Argentina, BRA = Brazil, USA = United States of America, and IND = India.

⁵ Authors reported apparent ileal digestibility value.

1.8. Energy Content of the Soybean Meals

Dietary energy represents a major cost in feed formulation. Consequently, the energy content of the SBM is of relevant interest in poultry feeding. The energy content of the SBM depends primarily on the quality of the protein fraction (de Coca-Sinova et al., 2008; Ravindran et al., 2014; CVB, 2021) as well as sucrose (Karr-Lilienthal et al., 2005; Ravindran et al., 2014), EE (Bolton, 1957; CVB 2021), and oligosaccharides (Coon et al., 1990; Carré et al., 1995) contents.

Most commercial poultry diets are formulated according to the energy values published for ingredients by scientific Institutions (e.g., WPSA, 1989; Rostagno et al., 2017; Premier Atlas, 2019; CVB, 2021; FEDNA, 2021) or companies involved in animal nutrition (AMINODat, 2023). However, the chemical composition and energy values reported in these publications for SBM are very variable (Table 8), limiting its use in practical feed formulation (Mateos et al., 2019; Zaefarian et al., 2021).

Table 8. Chemical composition (%) and apparent metabolizable energy corrected for nitrogen (AMEn; kcal/kg) content of the soybean meals in broilers (88% dry matter)

Institution	Year	Country	CP ¹	EEh ²	NDF ³	Sugars ⁴	Starch ⁵	AMEn
WPSA	1989	Europe	47.5	0.9	13.0	9.0	6.2	2,230
NARO	2009	Japan	45.0	1.9	8.5	-	-	2,400
NRC ⁶	2012	USA	46.7	2.8	8.0	15.1	1.8	2,230
RPRI	2014	Russia	46.0	1.4	11.7	4.2	1.0	2,495
Rostagno et al.	2017	Brazil	47.7	1.8	14.8	-	3.0	2,350
Premier Atlas	2019	UK	46.5	2.7	8.6	10.0	1.0	2,364
INRAE	2021	France	46.2	1.7	13.0	9.3	0.8	2,260
CVB	2021	Netherlands	46.8	2.4	8.4	9.2	1.1	2,180
FEDNA	2021	Spain	47.0	1.7	9.5	6.7	0.8	2,324
AMINODat	2023	Germany	46.5	2.1	11.3	9.0	0.6	2,341

Note: Dash indicated that no data were reported.

¹ Crude protein.

² Ether extract after previous HCl hydrolysis, except for WPSA, 1989 and Rostagno et al., 2017.

³ Neutral detergent fiber

⁴ NRC, 2012 = sucrose, stachyose, and raffinose and FEDNA, 2021 = sucrose values.

⁵ Enzymatic method, except WPSA, 1989 (polarimetric method).

⁶ AMEn value from NRC, 1994.

Also, data from *in vivo* assays published in scientific journals, presents an important variation in the apparent metabolizable energy corrected for nitrogen (**AMEn**) content of the SBM. Consequently, the criteria used by the feed industry to purchase SBM based primarily on their CP content, might be re-examined (Table 9). For example, on 88% dry matter (**DM**) basis, the range of values obtained and published for AMEn value of commercial SBM in broilers based on *in vivo* trials varies between 1,698 and 2,340 kcal/kg (Bertechini et al., 2018; Abdollahi et al., 2021; Siegert et al., 2023; Sung et al., 2023), whereas values reported in Tables of Feed Ingredient Composition vary between 2,180 and 2,365 kcal/kg (Rostagno et al., 2017; Premier Atlas, 2019; CVB 2021).

Table 9. *In vivo* apparent metabolizable energy corrected for nitrogen (AMEn; kcal/kg) content of the soybean meals in poultry^{1, 2} (88% dry matter)

Reference	n ³	Soybeans origin ⁴	AMEn
Perryman and Dozier (2012)	2	USA	2,090
Ravindran et al. ⁵ . (2014)	55	ARG, BRA, USA, IND	1,980
Bertechini et al. (2018)	3	BRA	2,340
Leung and Kiarie (2020)	1	IND	2,228
Abdollahi et al. (2021)	28	ARG, BRA, USA, IND	1,698
Khalil et al. (2021)	1	USA	2,097
Sung et al. (2023)	6	USA	2,244
Ahasic et al. (2023)	1	USA	2,518
Siegert et al. (2023)	18	ARG, BRA, USA, IND, Europe	1,998

Note: Dash indicated that no data were reported.

¹ Values correspond to the average means reported by the authors.

² Broilers data, except for Ahasic et al. (2023) = rooster and Siegert et al. (2023) = laying hens.

³ Number of soybean meal samples used.

⁴ ARG = Argentina, BRA = Brazil, USA = United States of America, and IND = India.

⁵ Authors reported apparent metabolizable energy without nitrogen correction.

More recently, the energy values for SBM and other ingredients are obtained using prediction equations based on the availability of nutrients for poultry (CVB, 2021). However, the prediction equations available to estimate the AMEn of the SBM are based on different variables and in many cases, they do not take into account the content in ANF, the quality of the protein fraction, and differences in content and composition of the CHO fraction, which are known to vary widely according to the origin of the soybeans

(Karr-Lilienthal et al., 2005; Ibáñez et al., 2020; Sheikhhasan et al., 2020; Aguirre et al., 2022). Consequently, depending on the variables used for the prediction, the estimated energy content of the SBM will vary (CVB, 2021; Fondevila et al., 2021). In this respect, Fondevila et al. (2021) used different predictions equations, with the same chemical composition of the SBM (data from Ravindran et al., 2014), and depending on the origin of the soybeans and equations used the AMEn content of the SBM vary between 2,058 and 2,332 kcal/kg (88% DM). Consequently, an improvement in the methodologies to estimate the energy content of the SBM is needed (Table 10).

Table 10. Apparent metabolizable energy corrected for nitrogen (AMEn) for poultry of soybean meals according to the origin of the soybeans and prediction equation used^{1,2}

Soybeans origin ³	ARG	BRA	USA	IND	Average
Chemical composition ⁴					
Crude protein	46.3	47.7	46.7	45.9	46.6
Starch + sucrose	7.95	6.73	8.97	5.81	7.37
EEh ⁵	1.83	2.03	1.61	1.08	1.64
Stachyose + raffinose	6.00	6.00	6.00	6.00	6.00
	AMEn (kcal/kg; 88% dry matter)				
WPSA (1989)	2,238	2,267	2,242	2,159	2,227
Mariano et al. (2012)	2,195	2,191	2,180	1,668	2,058
Rostagno et al. (2017)	2,281	2,315	2,286	2,204	2,271
Tangendjaja (2020)	2,155	2,170	2,152	1,999	2,119
CVB broilers (2021)	2,097	2,140	2,137	1,972	2,087
CVB rosters (2021)	2,159	2,175	2,155	1,997	2,122
FEDNA (2021)	2,354	2,368	2,394	2,210	2,332

¹ Data from (Fondevila et al., 2021).

² Average of the SBM independent of the origin of the soybeans.

³ ARG = Argentina, BRA = Brazil, USA = United States of America, and IND = India.

⁴ Chemical composition and ileal digestibility data from Ravindran et al. (2014).

⁵ Ether extract analyzed after previous HCl hydrolysis.

For comparative purposes, the apparent metabolizable energy (AME) value of poultry diets is frequently corrected for a zero N balance (AMEn) to eliminate the variation associated with the amount of N that is deposited as protein tissue and not oxidized in the body to provide energy (Farrell et al., 1991; Lopez and Leeson, 2008). The correction of AME values to zero N retention minimizes variability across assays for feed ingredients (Lopez and Leeson, 2008). It was proposed that a correction value of

8.22 kcal/g of N retained should be applied to AME values because this is the energy required by the bird to expel nitrogen as uric acid (Hill and Anderson, 1958). However, the concept of N retention is questionable due to fact that AME values are penalized when N is efficiently converted to lean muscle growth, a process for which modern broilers have been genetically selected. Dale and Fuller (1984) found that the AME value of high protein sources such as SBM were influenced the most when corrected for N retention compared with low-protein ingredients such as cereals. Lopez and Leeson (2008) confirmed the findings by Dale and Fuller (1984) and further concluded that correcting for N retention reduced the AME values of a conventional SBM from 7 to 12%. Therefore, it is advantageous to report N retention values as well as both AME and AMEn values to provide readers with awareness for interpreting energy values.

OBJECTIVES AND HYPOTHESIS

2. OBJECTIVES AND HYPOTHESIS

Soybean meal is the main protein source use in non-ruminant diets animal, with almost a 70% of the total of the protein sources production. The nutrient composition of SBM has been typically associated with low variability characterized by a relatively high CP content and AA profile and digestibility. However, the chemical composition and the nutritive value of SBM are influenced by numerous factors. Consequently, the feed industry is interested in valuable information to predict the nutritional value of the SBM in the diets. Different procedures to monitor the quality of the feed ingredients are considered day-to-day by the nutritionist and quality control department, with the aim of reducing time and cost and improving the feed ingredients evaluation. Usually, feed industry analyzes moisture, CP, and fiber content of the SBM and the UA methodology in order to estimate the nutritional value of the SBM. However, based only on this information, the evaluation of the quality and nutritional value of SBM might be not convenient. Also, factors such as the original quality and origin of the soybeans, are frequently not taking into account.

The general objective of this Doctoral Thesis was to study the influence of the soybean origin on the chemical composition, quality and digestibility of the protein fraction, energy content, and nutritional values of commercial SBM, based on fully characterization of the SBM, to adjust the formulation matrices for poultry diets using the origin and quality of the SBM as main factors. Moreover, to study the potential of the laboratory data (proximal analyses, protein quality indicators, and *in vitro* tests of the CP digestibility) to predict the nutritional values (*in vivo* CP and AA digestibility and energy content) of the SBM, improving the control quality day-to-day of the SBM.

The main hypothesis in this Doctoral was that chemical composition, quality of the protein fraction, and nutritional values of the SBM is affected by the country of the origin of the soybeans. Consequently, the formulation matrices of the SBM for poultry diets should be update periodically, based on the analytical data and the country of origin of the soybeans to improve the accuracy of the nutritional value of the SBM and reduce cost in feed formulation.

2.1. Specific Objectives

2.1.1 Experiment 1

To study the effect of the origin of the soybeans on the chemical composition, protein quality, and *in vitro* digestibility of the protein of commercial SBM (ARG, BRA, USA, and SAF) to improve the evaluation of the nutritional values of the SBM in feed formulation. Also, to study the correlations between the chemical composition, protein quality indicators, and *in vitro* protein digestibility of the SBM samples.

2.1.2 Experiment 2

To study the influence of the soybean origin, of the 3 major SBM exports (ARG, BRA, and USA), on chemical composition, digestibility, and quality of the protein fraction by *in vitro* assays, and *in vivo* energy content and CP and AA digestibility in broilers at 21 d of age in commercial SBM. Moreover, to study the correlations between the *in vivo* determinations (energy content and ileal digestibility of CP and AA of the SBM, and broiler performance) and the laboratory analyses of the commercial SBM samples.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Experiment 1

3.1.1. *Soybean Meals Collection*

Commercial samples of SBM from ARG, BRA, USA, and SAF ($n = 40$ per origin) were collected at random by specialized quality control personnel. The samples from ARG, BRA, and USA were obtained either at the arrival of the vessels to European ports or at the crushing plants from 2018 to 2020. All the ARG samples were crushed in the country of origin, loaded in cargoes at Rosario port and unloaded in Spain ($n = 31$), Italy ($n = 5$) and Portugal ($n = 4$). The BRA samples (soybeans or SBM) were loaded at Paranaguá and Santos ports and unloaded at Spain ($n = 19$ for soybeans and 10 for SBM), France ($n = 2$ for SBM), Italy ($n = 3$ for soybeans and 2 for SBM) and Portugal ($n = 4$ for soybeans) ports. All USA soybeans were loaded at New Orleans port and processed in Spain ($n = 29$), Italy ($n = 6$) and Portugal ($n = 5$). The SAF samples were collected from 6 crushing plants that operated in the country in the same years. Consequently, this study did not intend to compare the average quality of SBM of the countries analyzed or of particular crushing plants within each country but to add new information on the differences in quality of SBM potentially available in the European Union based on the origin of the soybeans. All samples were identity preserved and the date of collection, crushing plant (or port of departure and arrival of the vessels) and the technician in charge of the sampling, were recorded.

3.1.2. *Laboratory Analysis*

3.1.2.1. *Proximal composition and chemical analyses of the soybean meals.*

Samples were analyzed for moisture by oven-drying (method 930.15), ash with a muffle furnace (method 942.05) and N by Kjeldahl (method 988.05) as described by the AOAC

International (2019). Crude protein was calculated as $N \times 6.25$. The AA content of the SBM was determined by ion-exchange chromatography (Hewlett-Packard 1100, Waldbronn, Germany) after acid hydrolysis, following the AOAC International (2019) procedure. Briefly, samples were hydrolyzed in 6 N 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, vol/wt) was used to reduce AA losses in the presence of CHO. Protein hydrolysates and AA calibration mixture were derivatized with o-phthalaldehyde. For the determination of Met and Cys, separate samples were oxidized with performic acid before hydrolysis and measured as Met sulfone and cysteic acid, respectively. Tryptophan content was determined after alkaline hydrolysis for 20 h at 110 °C. Ether extract (**EEh**) was analyzed after 3 N HCl acid hydrolysis (method 159 Am 5-04) as indicated by the AOCS (2017) using an Ankom XT10 extraction system 160 (Ankom Technology Corp. Macedon, NY, USA). The CF was determined by sequential extraction with diluted acid and alkali (method 962.09; AOAC International, 2019) and NDF as described by van Soest et al. (1991). Sucrose and oligosaccharides (stachyose and raffinose) were analyzed as indicated by de Coca-Sinova et al. (2008) and the starch using a thermostable amyloglucosidase/ α -amylase (method 996.11; AOAC International, 2019). Macro-minerals and trace elements were determined as described by Hermida et al. (2006) and phytate P using a Megazyme kit (Megazyme Inc., Bray, Ireland). All samples were processed and analyzed in duplicate in the same laboratory by the same technician. For comparison purposes, all data are expressed on 88% DM basis.

3.1.2.2 Protein quality indicators of the soybean meals. Urease activity (mg N/g) was determined as indicated by Boletín Oficial del Estado (1995), PDI according to method Ba 10-65 (AOCS, 2017) using a Hamilton blender (Model G936, VOS Instrument, Zaltbommel, The Netherlands) and KOH as described by Araba and Dale

(1990). Trypsin inhibitor activity was measured following the Hamerstrand et al. (1981) procedure and expressed in mg/g.

3.1.2.3. In vitro crude protein digestibility of the soybean meals. The test outlined by Bedford and Classen (1993) that simulates the processes that occurred in the GIT of the birds (pepsin and pancreatic digestibility) was used to estimate the IVDP of the SBM in 30 samples chosen at random per country. Briefly, a representative sample (0.4 g) was ground in a laboratory mill (Retsch Model ZM-200, Stuttgart, Germany) equipped with a 0.75-mm screen, mixed with 5 mL of distilled water and incubated during 45 min at 39°C in a solution containing 10 mL of pepsin (2,500 U/mg, Sigma Merck no. 7190, Whitehouse Station, NJ, USA) and 0.12 N of HCl solution. Then, 0.18 mL of NaOH and 13.5 mL of the pancreatin solution (8 × USP, Sigma P-1790, St. Louis, MO, USA) was added and the mixture was incubated for 1 and 3 h at a pH of 7.8 and 39°C. The samples were placed on ice and 5 mL trichloroacetic acid were added. The tubes were equilibrated by weight, centrifuged ($12,700 \times g$) and filtered, and the N content was determined by Kjeldahl.

3.1.3. Statistical Analysis

The data were analyzed as a completely randomized design with country of origin of the soybeans as main effect, using the GLM procedure (SAS Institute, 2008). Year of harvest, area of production, and condition of the crushing procedure were not included in the model. Consequently, the inherent variability caused by soybeans genotype, agricultural practices, environmental conditions at harvest and storage at the farm and heat applied during the oil extraction process, was assumed to be part of the experimental error. When the effect of the origin of the soybeans on any of the variables studied was significant, means were compared using the Tukey test. In addition, the Pearson

correlation (r) analyses (SAS, 2008) was used to determine the correlation among all the variables studied. Differences observed were considered significant at $P \leq 0.05$.

3.2. Experiment 2

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, 2013).

3.2.1. Soybean Meals Collection

In total, 27 commercial SBM samples from ARG, BRA, and USA, in equal number, were used in this study. Representative samples (40 kg each) of the SBM were collected by specialized quality control personnel at the crushing plants (Barcelona, Bilbao, and Cartagena) or at the arrival of the cargoes to Spanish ports (Huelva, Marin, and Tarragona). The samples were collected from March 2021 to January 2022 and the trial was conducted from February to May 2022. Samples were conveniently identified and kept at 4°C until the preparation of the experimental diets. All samples from ARG were crushed in the country of origin and loaded for exports at Rosario port. The BRA soybeans were loaded at the Paranaguá and Santos ports and the USA soybeans at New Orleans port.

3.2.2. Husbandry, Diets, and Experimental Design

In total 1,344 one-day-old, Cobb 500 male broilers were used. The birds were vaccinated at hatch against main common diseases (Infectious Bronchitis, Marek, and Gumboro Disease) according to accepted commercial practices. At arrival to the experimental facilities, the chicks were weighed, housed in 2 identical environmentally controlled barns (block effect), and randomly allotted in groups of 8 to 168 cages. Each cage (145 × 45 × 40 cm; Alternative Design, Siloam Springs, AR, USA) was provided

with an open-trough feeder and 2 low-pressure nipple drinkers. The light program consisted of 23 h of light from 1 to 7 d of age and 18 h from day 8 to the end of the experiment. Room temperature was kept at 33°C during the first 3 d of life and then it was reduced gradually until reaching 22°C at 21 d of age. Chicks had free access to feed and water throughout the experiment. From 0 to 16 d of age, all broilers were fed a common standard commercial diet based on cereals and SBM (3,000 kcal AMEn/kg, 1.23% SID Lys, 0.98% Ca, and 0.47% digestible P) in crumble form. Then, each replicate received its corresponding experimental diet in mash form for 5 days. The experimental diets consisted of 53% of a N-free diet (**NFD**) based on dextrose, soy oil, lignocellulose, and minerals, and 47% of each of the evaluated SBM samples as the unique source of N. Titanium dioxide (0.94%) was included in the NFD as an indigestible marker (Table 11). Each of the 27 experimental diets were replicated 6 times and the experimental unit was a cage with 8 broilers. In addition, 6 extra replicates with 8 chickens each, were fed the NFD exclusively, to determine the basal endogenous losses (**BEL**) of CP and AA and these values were used to estimate the SID of CP and AA of the SBM.

Table 11. Composition of the experimental diets (% , as fed basis). Experiment 2

	N-free diet	Test diet ¹
Ingredient		
Soybean meal	-	47.0
Dextrose	82.8	43.9
Soy oil	6.60	3.50
Lignocellulose ²	3.77	2.00
Calcium carbonate	1.67	0.88
Monocalcium phosphate	2.75	1.44
Vitamin and mineral premix ³	0.75	0.40
Sodium chloride	0.72	0.38
Titanium dioxide	0.94	0.50
Determined analyses		
Gross energy kcal/kg	3,540	- ⁴
Dry matter	92.9	- ⁴

¹ The experimental diets differed exclusively, in the soybean meal tested.

² Natural lignocellulose (J. Rettenmaier & Söhne, Holzmühle, Germany).

³ Provided the following per kilogram of diet: vitamin A (transretinyl acetate), 10,000 IU; vitamin D₃ (cholecalciferol), 4,800 IU; vitamin E (dl- α -tocopheryl acetate), 45 mg; vitamin K₃,

3.0 mg; vitamin B₁, 3.0 mg; vitamin B₂, 9.0 mg; vitamin B₆, 4.5 mg; vitamin B₁₂ (cyanocobalamin), 40 µg; niacin, 51 mg; pantothenic acid (d-calcium pantothenate), 16.5 mg; folic acid, 1.8 mg; biotin, 150 µg; choline chloride, 100 mg; betaine, 200 mg; iron (FeSO₄·H₂O), 54 mg; iodine [Ca(IO₃)₂], 1.2 mg; selenium (Na₂SeO₃), 0.3 mg; copper (CuSO₄), 12 mg; manganese (MnO), 90 mg; zinc (ZnO), and 66 mg; butyl hydroxytoluene, 25 mg.

⁴ Values for all nutrients varied according to the experimental soybean meal tested.

3.2.3. *Energy Content of the Soybean Meals*

Representative samples (80 ± 10 g each day) of the excreta were collected from each cage the last 2 days of the trial (20 and 21 d of age). The samples were cleaned from any contamination of feed or feathers, oven-dried at 60°C for 72 h, and then, were ground using a laboratory mill (Retsch Model Z-I, Stuttgart, Germany) provided with a 0.5-mm screen, and stored at -4°C until further analyses. Diets and excreta samples were analyzed for DM, gross energy (**GE**), N, and titanium (Ti) contents.

3.2.4. *Ileal Digestibility of the Crude Protein and Amino Acids of the Soybean Meals*

At the end of the trial, after the collection of the excreta samples, all the birds were euthanized by CO₂ asphyxiation and of the lower half of the ileum (defined as the section of the small intestine extending from the vitelline Meckel's diverticulum to 4 cm anterior to the ileocecal junction) were removed by gently flushing of the contents with distilled water into plastic containers. Then, the ileal digesta of the 8 birds per cage were pooled, frozen at -20°C, and freeze-dried. Prior to chemical analyses, the samples were ground to pass through a 0.5-mm screen and stored in air-tight containers at room temperature until further analyses of DM, N, AA, and Ti.

3.2.5. *Laboratory Analysis*

3.2.5.1. Physical properties of the soybean meals. Particle size distribution and mean particle size, expressed as geometric mean diameter ± geometric standard deviation (**GMD ± GSD**), were determined in 100 g samples using a Retsch shaker (Retsch, Stuttgart, Germany) provided with 8 sieves ranging in mesh from 5,000 to 40 µm, as

outlined by the ASAE (2003). In addition, the color [L^* (luminosity), a^* (redness), b^* (yellowness)] of the samples were evaluated using a spectrophotometer (CM-700d; Konica Minolta, Osaka, Japan).

3.2.5.2. Chemical composition of the soybean meals. The SBM samples were analyzed for proximal composition, minerals, and AA contents using the same procedures described in the Experiment 1. Additionally, SBM samples were analyzed for GE using an adiabatic bomb calorimeter (model 356, Parr Instrument Company, Moline, IL, USA) and for NSP using an assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α -amylase, protease, and amyloglucosidase (Englyst et al., 1994).

3.2.5.3. Protein quality indicators and reactive lysine and of the soybean meals. Urease activity, based on pH changes (method Ba 9–58) was determined according to AOCS (2017). The PDI, KOH, and TIA of the SBM were determined as described in the Experiment 1. The RLys was determined based a) on the transformation of Lys to its AA analog homoarginine (**hRLys**) by means of guanidination with o-methylisourea as outlined by Fontaine et al. (2007) and b) furosine (**fRLys**) content, after 6 N HCl hydrolysis on a reversed-phase high-performance liquid chromatography instrument as indicated by Pahm et al. (2008).

3.2.5.4. Experimental diets, excreta, and ileal content. Samples of diets, excreta, and ileal content were analyzed for moisture and CP ($N \times 6.25$) as described in the Experiment 1. The GE of the experimental diets and excreta and the AA content of the ileal content samples was analyzed as described above for the SBM samples. Titanium content was determined as proposed by Short et al. (1996).

All samples were analyzed in duplicate in the same laboratory by the same technician. For comparison purposes, all data are expressed on 88% DM basis.

3.2.5.5. *In vitro* digestibility of the crude protein of the soybean meals. The IVDP was determined using 3 accepted methodologies based on enzymatic digestion.

a) The IVDP 1 of the SBM samples was estimated using a test outlined by Boisen and Fernández (1995) that simulates the processes that occurred in the GIT of the birds after pepsin (P-7000, Sigma-Aldrich) and pancreatin (P-1750, Sigma-Aldrich) incubation. Briefly, samples were ground in a laboratory mill (Retsch Model ZM-200, Stuttgart, Germany) equipped with a 1.00-mm screen and 1.0 g of sample were weighed in 250 mL polypropylene Erlenmeyer flasks. Then, 25 mL of phosphate buffer (0.1 M, pH 6.0) and 10 mL of 0.2 M HCl were added to each flask in this order and solution pH was adjusted to 2.0 with 1.0 M HCl and NaOH as needed. A pepsin solution (25 g/L) was prepared, and 1.00 mL of this solution was added to each flask. Finally, flasks were closed and put into an incubator (OVAN, Barcelona, Spain) at 39°C and stirred at 99 rpm for 2 h. After incubation, the resulting mixture was removed from the incubator and 10 mL of a phosphate buffer (0.2 M, pH 6.8) and 5 mL of a 0.6 M NaOH solution were added. The pH was adjusted to 6.8 and then, 1 mL of a freshly prepared pancreatin solution (100 g/L) was added. Flasks were closed and incubated again under continuous stirring at 99 rpm and 39°C for 4 h. Samples were analyzed in quadruplicate by the same technician.

b) The IVDP 2. The same procedure described for the IVDP 1 was conducted but extended by an additional incubation with a multi-enzyme complex containing cellulases (Viscozyme L, Sigma-Aldrich) to simulate the digestion at ileal and cecal level of the birds. Briefly, after the incubation with pancreatin, 10 mL of a 0.2 M EDTA solution were added, the pH was adjusted to pH 4.8, and 0.5 mL of Viscozyme were incorporated to the mixture. Flasks were closed and incubated under the same conditions for 8 h. Samples were made in quadruplicate for IVDP 2. At the end of each procedure, the residual substrates were filtered after ethanol and acetone washing, oven-dried at 105°C for 24 h,

and stored until further analysis. Residual substrates were analyzed for DM and CP contents to calculate IVDP of the SBM by considering the total disappearance of these nutrients in the residual substrate after incubation, as indicated by Boisen and Fernández (1997).

c) The IVDP 3. *In vitro* test was determined as indicated in the Experiment 1 of the current document, outlined by Bedford and Classen (1993).

3.2.6. Calculations

3.2.6.1. *Energy content of the soybean meals.* The AME (kcal/kg) of the experimental diets and the corresponding SBM were determined by indirect method whereby the test ingredient is fed as a part of a compete diet as describe by Veluri and Olukosi (2020):

$$\text{AME diet (kcal/kg)} = \text{GE}_{\text{diet}} - [\text{GE}_{\text{excreta}} \times (\text{Ti}_{\text{diet}}/\text{Ti}_{\text{excreta}})]$$

Where GE_{diet} and $\text{GE}_{\text{excreta}}$ are the analyzed GE values (kcal/kg) and Ti_{diet} and $\text{Ti}_{\text{excreta}}$ are the analyzed Ti values (%).

$$\text{AME SBM (kcal/kg)} = \text{AME diet} - \frac{(\text{AME of N-free diet} \times 0.53)}{0.47}$$

The AMEn was then calculated by correction for zero N retention by assuming 8.22 kcal per g of N retained in the body (Hill and Anderson, 1958).

$$\text{AMEn (kcal/kg)} = \frac{\text{AME} - (8.22 \times \text{N retention})}{1,000}$$

3.2.6.2. *Ileal digestibility of protein and amino acids of the soybean meals.* The AID of CP and AA of the diets was calculated from the ratio of nutrient (CP or AA) to Ti relative to the corresponding ratio in the ileal digesta, using the following formula.

$$\text{AID (\%)} = \frac{(\text{nutrient/Ti})_{\text{diet}} - (\text{nutrient /Ti})_{\text{ileal}}}{(\text{nutrient/Ti})_{\text{diet}}} \times 100$$

Where $(\text{nutrient/Ti})_{\text{diet}}$ = ratio of dietary components (CP or AA) to Ti in the diet, and $(\text{nutrient /Ti})_{\text{ileal}}$ = ratio of the same dietary components to Ti in the ileal digesta. In the calculations, the CP or AA of the diets refers to the CP and AA of the SBM tested, because SBM was the unique source of N and AA in the experimental diets.

The BEL of CP and AA were calculated, as mg of CP or AA flow per kg of DM intake, from the birds fed the NFD, as indicated by Moughan et al. (1992).

$$\text{BEL (mg/kg DM intake)} = \frac{\text{nutrient in ileal content (mg/kg)} \times \text{diet Ti (mg/kg)}}{\text{ileal content Ti (mg/kg)}}$$

The AID data of the SBM for CP and AA were standardized by using the BEL values.

$$\text{SID (\%)} = \text{AID (\%)} + \frac{\text{BEL of nutrient (mg/kg DM intake)}}{\text{nutrient (mg/kg DM)}}$$

Where, SID = standardized ileal digestibility of the nutrient; AID = apparent ileal digestibility of the nutrient; and nutrient = concentration of the CP or AA in the SBM sample tested.

3.2.6.3. Growth performance. Body weight (**BW**) of the birds and feed disappearance were recorded by replicate at 16 and 21 d of age. From these data, BW gain, feed intake, and feed conversion ratio were determined.

3.2.7. Statistical Analysis

Data on the chemical analyses the SBM and on the *in vivo* trial, were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Institute, 2008), with SBM origin as main effect. For the *in vivo* data, SBM sample within each origin, was used as a nested effect. When the effect of SBM origin on any of the variables studied was significant, average means were compared using the Tukey test. Results in tables are presented as means and differences were considered significant at $P < 0.05$. In

addition, the Pearson correlation (r) analyses (SAS, 2008) was performed to determine the correlation among the variables studied and the differences observed were considered significant at $P \leq 0.05$.

RESULTS

4. RESULTS

4.1. Experiment 1

The results of the Experiment 1 of this Doctoral thesis are published in The Animal feed Science and Technology Journal (2022, vol. 293 n°115473).

4.1.1. Chemical Composition of the Soybean Meals

The chemical composition of the SBM was affected by the country of origin of the soybeans (Table 12). On an 88% DM basis, the SAF meals had the lowest ash and the highest EEh content of all the meals ($P < 0.001$). Sucrose (8.76 and 7.33 vs. 6.86 and 5.80%; $P < 0.001$) and stachyose (4.83 and 5.08 vs. 4.67 and 4.45%; $P < 0.001$) contents were higher and raffinose (1.11 and 1.12 vs. 1.25 and 1.36%; $P < 0.001$) was lower for the SAF and USA meals than for the ARG and BRA meals. Starch content was low ($0.60 \pm 0.09\%$) and was not affected by the origin of the soybeans. The CP and CF content (%) was higher ($P < 0.001$) for the BRA meals (46.9 and 4.63) than for the SAF (45.9 and 3.75), ARG (46.2 and 4.18), and USA (46.2 and 4.18) meals. Also, NDF was higher for the BRA meals than for the SAF meals, with the ARG and USA meals being intermediate (9.26, 8.23, 8.79, and 8.72%, respectively; $P < 0.001$). Independent of the origin of the soybeans, the greatest coefficient of variation (CV) among the variables studied was observed for EEh, raffinose and sucrose values (CV = 30.5, 20.9, and 18.4%, respectively) and the lowest (CV = 2.50%) for CP (data not shown). When the data were sorted by the country of origin of the soybeans, the highest variability for most components (CP, NDF, sucrose, and raffinose) was observed for the BRA meals.

4.1.2. Amino Acids Content and Profile of the Soybean Meals

The contents of Lys and of $\sum 5$ AA in poultry diets of the SBM were not affected by soybean origin, in spite of the significant differences in CP content detected (Table

13). Per unit of protein, the profile of the $\sum 5$ AA was more abundant for the SAF meals than for the BRA meals, with the USA and ARG meals being intermediate (14.3, 14.0, 14.2, and 14.2%, respectively; $P < 0.001$) (Table 14). In fact, Lys profile (% CP) was higher for the SAF and USA meals than for the BRA and ARG meals (6.18, 6.13, 6.11, and 6.08% respectively; $P < 0.001$).

4.1.3. *Macro-Minerals and Trace minerals of the Soybean Meals*

The macro-mineral contents of the SBM varied with the country of origin of the soybeans, with the SAF meals having less Ca and more P ($P < 0.001$) for the SAF meals (0.23 and 0.69%) than for the USA (0.35 and 0.67%), ARG (0.35 and 0.67%), and BRA (0.35 and 0.64%) meals (Table 15). Potassium content was lower for the BRA and USA meals than for the ARG and the SAF meals ($P < 0.001$). Trace mineral content varied with the country of origin of the soybeans but except for Fe (143, 101, 104, and 101 mg/kg for the BRA, ARG, USA, and SAF meals respectively, $P < 0.001$), the differences were of limited practical interest.

4.1.4. *Protein Quality Indicators of the Soybean Meals*

The SAF SBM showed the highest values for all protein quality indicators, whereas the lowest values were for the ARG SBM ($P < 0.001$). However, all values were generally within acceptable values for protein quality. Irrespective of the origin of the soybeans, the variability was highest for UA (CV = 125%) and lowest for KOH (CV = 6.15%), data not shown. When the data were sorted by the country of origin of the meals, the lowest variability for all protein quality indicators, except UA, was observed for the USA meals (Table 16).

4.1.5. *In Vitro* Digestibility of the Crude Protein of the Soybean Meals

The IVDP at 1 h (77.9 and 77.3 vs. 75.9 and 76.0%; $P < 0.001$) and 3 h (82.3 and 82.5 vs. 81.0 and 81.0%; $P < 0.001$) post-incubation, were greater for the USA and the SAF meals than for the ARG and the BRA meals (Table 17). When the data were sorted by the country of origin of the soybeans, the highest variability was observed for the SAF meals.

4.1.6. *Correlations between Chemical Composition, Protein Quality, and In Vitro Digestibility of Crude Protein of the Soybean Meals*

The correlations among proximal analyses, protein quality indicators, and IVDP values were numerous and therefore, only the most relevant, based on practical interest, are presented (Table 18). Independent of the origin of the soybeans, CP was negatively correlated with sucrose ($r = -0.397$; $P < 0.001$) and NDF ($r = -0.340$; $P < 0.001$) contents. Sucrose content was positively correlated with oligosaccharides ($r = 0.324$; $P < 0.001$) and EEh ($r = 0.353$; $P < 0.001$) contents. The most important correlations detected among protein quality indicators, were between TIA and KOH ($r = 0.602$; $P < 0.001$) and between TIA and PDI ($r = 0.368$; $P < 0.001$). Urease activity showed a weak correlation with PDI ($r = 0.257$; $P < 0.01$) but not with KOH or TIA. As expected, the highest correlation among all variables presented was between IVDP at 1 h and 3 h post-incubation ($r = 0.834$; $P < 0.001$). Furthermore, significant correlations were observed between IVDP at 1 and 3 h post-incubation and TIA ($r = 0.364$ and 0.353 , respectively; $P < 0.001$) and KOH ($r = 0.227$ and 0.217 , respectively; $P < 0.05$) but not with PDI or UA.

Table 12. Chemical composition (%) of the soybean meals (88% dry matter). Experiment 1

Soybean origin ¹	Average				SEM ²		Range				Coefficient of variation (%)			
	ARG	BRA	USA	SAF	(n =40)	P-value	ARG	BRA	USA	SAF	ARG	BRA	USA	SAF
Dry matter	88.6 ^b	88.8 ^b	88.5 ^b	90.0 ^a	0.126	<0.001	86.5-90.9	87.3-91.2	85.8-89.9	88.7-91.8	0.94	0.96	0.85	0.81
Ash	6.88 ^a	6.74 ^a	6.69 ^a	6.07 ^b	0.049	<0.001	6.29-7.34	5.98-7.81	6.07-7.59	5.61-6.61	4.21	5.54	4.88	3.76
Crude protein	46.2 ^b	46.9 ^a	46.2 ^b	45.9 ^b	0.172	<0.001	44.3-48.0	44.2-49.8	44.2-48.9	43.4-47.5	1.70	2.95	2.23	2.35
EEh ³	1.80 ^b	1.90 ^b	1.94 ^b	2.68 ^a	0.084	<0.001	0.89-2.87	1.02-2.78	1.09-3.02	1.57-3.87	30.4	27.4	26.5	20.3
Starch	0.63	0.58	0.61	0.59	0.018	0.170	0.45-0.98	0.39-0.91	0.35-1.09	0.39-0.98	15.7	17.7	23.8	17.7
Sucrose	6.86 ^c	5.80 ^d	7.33 ^b	8.76 ^a	0.125	<0.001	5.17-8.23	4.18-7.28	4.73-9.03	6.76-9.77	10.3	14.1	12.4	8.19
Stachyose	4.67 ^{bc}	4.45 ^c	5.08 ^a	4.83 ^{ab}	0.082	<0.001	3.42-5.84	3.30-5.94	3.99-6.00	3.33-5.94	11.6	11.5	8.29	12.3
Raffinose	1.25 ^a	1.36 ^a	1.12 ^b	1.11 ^b	0.037	<0.001	0.99-1.73	1.00-2.19	0.83-1.87	0.77-1.62	14.3	18.9	20.4	23.4
Oligosaccharides ⁴	5.92 ^{ab}	5.81 ^b	6.20 ^a	5.93 ^{ab}	0.088	0.017	4.43-7.16	4.43-7.16	5.33-7.27	4.10-7.06	10.6	10.3	7.17	9.20
Crude fiber	4.18 ^b	4.63 ^a	4.18 ^b	3.75 ^b	0.090	<0.001	3.43-5.61	3.53-6.68	3.57-5.14	2.99-4.76	14.0	16.8	9.7	11.9
NDF ⁵	8.79 ^{ab}	9.26 ^a	8.72 ^{ab}	8.23 ^b	0.202	<0.001	6.89-12.3	7.03-14.2	6.93-10.7	6.30-9.98	14.9	16.6	10.5	15.5
Σ of components ⁶	89.1 ^b	89.0 ^b	89.6 ^{ab}	90.2 ^a	0.202	<0.001	86.8-92.5	87.1-91.3	87.3-92.4	88.0-92.6	1.57	1.18	1.53	1.36

^{a, b, c, d} Means within a row, without a common superscript, differ significantly ($P < 0.05$).

¹ ARG = Argentina, BRA = Brazil, USA = United States of America, and SAF = South Africa.

² Standard error of the mean.

³ Ether extract after previous HCl hydrolysis.

⁴ Stachyose + raffinose.

⁵ Neutral detergent fiber.

⁶ Includes moisture, ash, crude protein, EEh, starch, sucrose, stachyose, raffinose, and NDF.

Table 13. Amino acids (AA) content (%) of the soybean meals (88% dry matter). Experiment 1

Soybeans origin ¹	Average				SEM ²		Range				Coefficient of variation (%)			
	ARG	BRA	USA	SAF	(n = 40)	P-value	ARG	BRA	USA	SAF	ARG	BRA	USA	SAF
Indispensable AA														
Arg	3.34 ^b	3.41 ^a	3.38 ^{ab}	3.39 ^{ab}	0.015	0.022	3.23-3.49	3.19-3.64	3.15-3.65	3.09-3.56	1.90	3.31	2.81	3.09
His	1.20	1.21	1.20	1.20	0.005	0.155	1.17-1.24	1.13-1.29	1.13-1.29	1.09-1.25	1.63	3.00	2.74	2.50
Ile	2.10 ^b	2.15 ^a	2.09 ^b	2.07 ^b	0.009	<.0001	2.04-2.19	2.02-2.29	1.97-2.25	1.91-2.16	1.73	3.46	2.89	2.62
Leu	3.52 ^b	3.58 ^a	3.51 ^b	3.48 ^b	0.015	<.0001	3.43-3.68	3.36-3.84	3.31-3.79	3.19-3.61	1.70	3.40	2.83	2.57
Lys	2.82	2.86	2.84	2.84	0.011	0.186	2.74-2.93	2.69-3.04	2.69-3.08	2.64-2.95	1.49	3.15	2.54	2.63
Met	0.62 ^{ab}	0.62 ^b	0.62 ^{ab}	0.63 ^a	0.002	0.023	0.59-0.64	0.57-0.65	0.59-0.68	0.58-0.65	1.73	3.14	2.52	2.37
Phe	2.35 ^b	2.41 ^a	2.35 ^b	2.32 ^b	0.010	<.0001	2.28-2.46	2.25-2.57	2.21-2.53	2.15-2.41	1.70	3.52	2.85	2.58
Thr	1.80 ^{ab}	1.82 ^a	1.79 ^b	1.79 ^b	0.007	0.024	1.76-1.85	1.71-1.92	1.71-1.93	1.64-1.87	1.36	3.04	2.50	2.43
Trp	0.63	0.64	0.63	0.63	0.003	0.351	0.61-0.65	0.60-0.67	0.60-0.68	0.57-0.66	1.38	3.18	2.41	2.72
Val	2.20 ^{ab}	2.23 ^a	2.20 ^b	2.18 ^b	0.009	<.0001	2.14-2.29	2.09-2.37	2.08-2.36	2.01-2.26	1.48	3.11	2.59	2.46
Dispensable AA														
Ala	2.00 ^{ab}	2.03 ^a	1.99 ^b	1.98 ^b	0.010	<.0001	1.95-2.08	1.90-2.15	1.88-2.14	1.81-2.05	1.54	3.10	2.80	2.44
Asp	5.24 ^b	5.36 ^a	5.25 ^b	5.22 ^b	0.020	<.0001	5.12-5.47	5.01-5.73	4.97-5.66	4.81-5.44	1.62	3.51	2.72	2.70
Cys	0.66 ^b	0.67 ^{ab}	0.67 ^{ab}	0.68 ^a	0.003	0.010	0.63-0.68	0.61-0.74	0.62-0.74	0.63-0.71	1.97	3.96	2.92	2.69
Glu	8.25 ^b	8.44 ^a	8.30 ^b	8.26 ^b	0.035	<.0001	7.98-8.60	7.93-9.05	7.79-9.00	7.64-8.56	1.64	3.46	2.71	2.61
Gly	1.94 ^b	1.98 ^a	1.93 ^b	1.93 ^b	0.007	<.0001	1.88-2.02	1.86-2.09	1.79-2.09	1.84-2.00	1.52	2.78	2.89	1.93
Pro	2.30 ^b	2.35 ^a	2.32 ^{ab}	2.31 ^b	0.009	<.0001	2.21-2.37	2.21-2.50	2.21-2.50	2.12-2.39	1.76	3.26	2.44	2.55
Ser	2.31 ^b	2.35 ^a	2.31 ^b	2.30 ^b	0.009	<.0001	2.25-2.41	2.22-2.51	2.19-2.48	2.15-2.39	1.58	3.15	2.66	2.31
Total sulfur AA	1.28 ^b	1.29 ^{ab}	1.29 ^{ab}	1.30 ^a	0.005	0.020	1.22-1.33	1.19-1.37	1.21-1.41	1.21-1.36	1.75	3.27	2.59	2.46
Σ Five key AA ³	6.54	6.60	6.54	6.56	0.027	0.392	6.39-6.73	6.21-7.01	6.24-7.09	6.06-6.84	1.36	3.14	2.61	2.55

^{a, b, c} Means within a row, without a common superscript, differ significantly ($P < 0.05$).

¹ ARG = Argentina, BRA = Brazil, USA = United States of America, and SAF = South Africa.

² Standard error of the mean.

³ Lys, Met, Cys, Thr, and Trp.

Table 14. Amino acids (AA) profile (% crude protein) of the soybean meals. Experiment 1

Soybeans origin ¹	Average				SEM ²		Range				Coefficient of variation (%)			
	ARG	BRA	USA	SAF	(n = 40)	P-value	ARG	BRA	USA	SAF	ARG	BRA	USA	SAF
Indispensable AA														
Arg	7.24 ^c	7.26 ^{bc}	7.31 ^{ab}	7.37 ^a	0.015	<0.001	7.11-7.53	7.11-7.53	7.12-7.73	7.10-7.65	1.32	1.08	1.49	1.30
His	2.60 ^a	2.58 ^b	2.59 ^{ab}	2.60 ^a	0.005	<0.001	2.56-2.70	2.51-2.62	2.52-2.73	2.53-2.67	1.37	0.82	1.51	0.82
Ile	4.56 ^b	4.59 ^a	4.52 ^c	4.51 ^c	0.007	<0.001	4.49-4.68	4.46-4.71	4.44-4.70	4.37-4.57	0.93	1.06	1.14	0.81
Leu	7.63 ^a	7.63 ^a	7.59 ^{ab}	7.58 ^b	0.011	<0.001	7.55-7.84	7.46-7.83	7.40-7.85	7.28-7.77	0.86	0.93	1.16	0.79
Lys	6.11 ^{bc}	6.08 ^c	6.13 ^b	6.18 ^a	0.011	<0.001	5.95-6.39	5.85-6.30	5.99-6.43	6.04-6.38	1.26	1.17	1.31	1.03
Met	1.34 ^b	1.31 ^c	1.34 ^b	1.36 ^a	0.003	<0.001	1.28-1.38	1.25-1.36	1.29-1.38	1.32-1.40	1.51	1.31	1.51	1.28
Phe	5.09 ^b	5.13 ^a	5.07 ^b	5.04 ^c	0.007	<0.001	5.03-5.23	5.00-5.27	4.98-5.28	4.92-5.22	0.79	1.12	1.12	0.83
Thr	3.91 ^a	3.87 ^b	3.87 ^b	3.90 ^a	0.005	<0.001	3.83-4.03	3.76-3.94	3.74-3.97	3.75-3.96	1.05	0.84	0.97	0.94
Trp	1.36 ^b	1.36 ^b	1.37 ^b	1.38 ^a	0.002	<0.001	1.34-1.41	1.30-1.39	1.33-1.40	1.31-1.41	1.11	1.02	1.00	1.25
Val	4.77 ^a	4.76 ^{ab}	4.74 ^b	4.74 ^b	0.006	<0.001	4.73-4.91	4.67-4.87	4.67-4.90	4.59-4.85	0.83	0.78	0.98	0.73
Dispensable AA														
Ala	4.34 ^a	4.32 ^b	4.29 ^c	4.30 ^{bc}	0.006	<0.001	4.26-4.48	4.23-4.42	4.18-4.43	4.14-4.44	0.98	0.77	1.09	0.89
Asp	11.4 ^b	11.5 ^a	11.4 ^b	11.4 ^b	0.015	<0.001	11.2-11.7	11.2-11.7	11.1-11.7	11.0-11.7	0.74	0.96	1.01	0.76
Cys	1.43 ^c	1.43 ^c	1.45 ^b	1.47 ^a	0.005	<0.001	1.37-1.52	1.34-1.58	1.35-1.51	1.43-1.52	2.07	2.64	1.99	1.32
Glu	17.9 ^b	18.0 ^a	17.9 ^b	18.0 ^a	0.024	<0.050	17.7-18.3	17.6-18.5	17.6-18.4	17.5-18.6	0.77	0.95	1.01	0.81
Gly	4.20 ^{ab}	4.22 ^a	4.19 ^b	4.21 ^{ab}	0.006	<0.001	4.16-4.35	4.14-4.34	4.10-4.34	4.18-4.35	1.02	0.81	1.11	0.68
Pro	4.99	5.01	5.02	5.02	0.009	0.082	4.88-5.19	4.93-5.14	4.91-5.28	4.85-5.15	1.56	0.95	1.18	0.78
Ser	5.00 ^{ab}	5.01 ^{ab}	4.98 ^b	5.02 ^a	0.006	<0.001	4.89-5.16	4.92-5.13	4.85-5.08	4.91-5.15	0.90	0.75	0.89	0.62
Total sulfur AA	2.77 ^{bc}	2.75 ^c	2.80 ^{ab}	2.84 ^a	0.007	<0.001	2.66-2.89	2.59-2.90	2.65-2.94	2.76-2.90	1.47	1.79	1.85	1.23
Σ Five key AA ³	14.2 ^b	14.0 ^c	14.2 ^b	14.3 ^a	0.022	<0.001	13.9-14.7	13.5-14.2	13.8-14.5	13.8-14.7	1.13	0.88	0.93	0.95

^{a, b, c} Means within a row, without a common superscript, differ significantly ($P < 0.05$).

¹ ARG = Argentina, BRA = Brazil, USA = United States of America, and SAF = South Africa.

² Standard error of the mean.

³ Lys, Met, Cys, Thr, and Trp.

Table 15. Mineral content of the soybean meals (88% dry matter). Experiment 1

Soybeans origin ¹	Average				SEM ²		Range				Coefficient of variation (%)			
	ARG	BRA	USA	SAF	(n = 40)	P-value	ARG	BRA	USA	SAF	ARG	BRA	USA	SAF
Macro-mineral (%)														
Ca	0.35 ^a	0.35 ^a	0.35 ^a	0.23 ^b	0.008	<0.001	0.28-0.43	0.28-0.43	0.23-0.52	0.18-0.30	11.8	12.4	23.2	14.4
Total P	0.67 ^{ab}	0.64 ^b	0.67 ^{ab}	0.69 ^a	0.006	<0.001	0.60-0.76	0.58-0.71	0.58-0.72	0.61-0.76	5.33	4.60	5.60	5.71
Phytate P	0.37 ^b	0.36 ^b	0.37 ^b	0.38 ^a	0.003	<0.001	0.33-0.39	0.29-0.40	0.33-0.39	0.33-0.41	5.02	6.00	7.00	4.06
Phytate:total P	0.55	0.57	0.55	0.56	0.006	0.077	0.44-0.61	0.48-0.64	0.48-0.67	0.50-0.62	7.43	6.10	8.20	5.70
K	2.21 ^a	2.12 ^b	2.14 ^b	2.21 ^a	0.016	<0.001	2.04-2.38	1.98-2.36	1.76-2.44	2.03-2.42	4.25	3.70	6.30	4.30
Mg	0.30 ^b	0.33 ^a	0.29 ^b	0.27 ^c	0.003	<0.001	0.27-0.34	0.28-0.40	0.24-0.36	0.25-0.30	4.74	8.10	9.10	4.52
Na	0.06 ^a	0.05 ^a	0.06 ^a	0.04 ^b	0.003	<0.001	0.02-0.10	0.01-0.09	0.02-0.11	0.02-0.12	25.9	31.6	36.4	60.4
Trace mineral (mg/kg)														
Zn	44 ^b	53 ^a	51 ^a	51 ^a	0.857	<0.001	39-59	45-76	42-77	47-64	11.7	11.3	15.5	7.51
Mn	43 ^a	32 ^b	33 ^b	32 ^b	0.862	<0.001	34-56	23-52	22-58	28-39	12.1	22.6	18.4	6.15
Fe	101 ^b	143 ^a	104 ^b	104 ^b	5.334	<0.001	75-250	70-305	70-186	77-140	29.9	36.9	25.6	17.2
Cu	15 ^a	14 ^b	15 ^a	13 ^b	0.279	<0.001	12-18	8-22	12-17	11-19	8.4	19.6	8.2	11.9

^{a, b, c} Means within a row, without a common superscript, differ significantly ($P < 0.05$).

¹ ARG = Argentina, BRA = Brazil, USA = United States of America, and SAF = South Africa.

² Standard error of the mean.

Table 16. Protein quality indicators of the soybean meals. Experiment 1

Soybeans origin ¹	Average				SEM ²		Range				Coefficient of variation (%)			
	ARG	BRA	USA	SAF	(n = 40)	P-value	ARG	BRA	USA	SAF	ARG	BRA	USA	SAF
UA ³ (mg N/g)	0.01 ^b	0.01 ^b	0.01 ^b	0.04 ^a	0.003	<0.001	0-0.05	0-0.07	0-0.05	0-0.08	152.6	167.2	151.8	58.0
PDI ⁴ (%)	11.0 ^c	12.9 ^b	14.6 ^{ab}	15.7 ^a	0.492	<0.001	7.0-16.5	7.1-25.0	11.1-21.5	8.8-26.7	20.4	24.7	16.3	26.4
KOH ⁵ (%)	74.6 ^c	77.6 ^b	80.4 ^a	81.0 ^a	0.656	<0.001	60.0-81.6	65.9-85.2	70.6-85.7	66.3-88.9	6.70	5.16	3.55	5.35
TIA ⁶ (mg/g)	1.89 ^b	2.36 ^a	2.61 ^a	2.62 ^a	0.106	<0.001	1.00-2.48	1.10-3.94	1.56-3.77	1.36-5.01	22.3	25.1	18.9	38.0

^{a, b, c} Means within a row, without a common superscript, differ significantly ($P < 0.05$).

¹ ARG = Argentina, BRA = Brazil, USA = United States of America, and SAF = South Africa.

² Standard error of the mean.

³ Urease activity (88% dry matter).

⁴ Protein dispersibility index.

⁵ Protein solubility in KOH.

⁶ Trypsin inhibitor activity (88% dry matter).

Table 17. *In vitro* protein digestibility (%) of the soybean meals. Experiment 1

Soybeans origin ¹	Average				SEM ²		Range				Coefficient of variation (%)			
	ARG	BRA	USA	SAF	(n = 30)	<i>P</i> -value	ARG	BRA	USA	SAF	ARG	BRA	USA	SAF
1 h	75.9 ^b	76.0 ^b	77.9 ^a	77.3 ^{ab}	0.437	<0.001	73.7-78.2	73.1-79.4	72.5-82.5	71.4-83.2	1.70	2.18	3.04	4.43
3 h	81.0 ^b	81.0 ^b	82.3 ^a	82.5 ^a	0.355	<0.001	78.6-83.7	77.7-83.8	79.4-85.1	77.7-88.1	1.91	1.93	1.73	3.48

^{a, b} Means within a row, without a common superscript, differ significantly ($P < 0.05$).

¹ ARG = Argentina, BRA = Brazil, USA = United States of America, and SAF = South Africa.

² Standard error of the mean.

Table 18. Correlations among chemical composition (88% dry matter), protein quality indicators, the *in vitro* digestibility of the crude protein (IVDP) of the soybean meals¹. Experiment 1

	Chemical composition ²					Protein quality indicator ³				IVDP ⁴	
	CP	EEh	Sucrose	Oligos.	NDF	UA	PDI	KOH	TIA	1 h	3 h
Chemical composition											
CP	1										
EEh	-0.245	1									
Sucrose	-0.397***	0.353***	1								
Oligosaccharides	-0.050	0.055	0.324***	1							
NDF	-0.340***	-0.028	-0.315***	-0.345***	1						
Protein quality indicator											
UA	-0.284**	0.470***	0.384***	0.185	0.034	1					
PDI	0.142	0.202*	0.437***	0.288**	-0.361***	0.257**	1				
KOH	-0.043	0.058	0.373***	0.366***	-0.393***	0.176	0.555***	1			
TIA	0.007	-0.088	0.232**	0.299	-0.188	0.165	0.368***	0.602***	1		
IVDP											
1 h	-0.015	-0.120	0.212*	0.265*	-0.192	-0.118	0.119	0.227*	0.364***	1	
3 h	-0.047	-0.072	0.231*	0.253	-0.170	0.108	0.158	0.217*	0.353***	0.834***	1

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

¹ Argentina, Brazil, United States of America, and South Africa origin (n = 30 per origin).

² CP = crude protein, EEh = ether extract after previous HCl hydrolysis, Oligos. = oligosaccharides (stachyose and raffinose), and NDF = neutral detergent fiber.

³ UA = urease activity (mg N/g), PDI = protein dispersibility index (%), KOH = protein solubility in KOH (%), and TIA = trypsin inhibitor activity (mg/g).

⁴ Values at 1 and 3 h of post-incubation (%).

4.2. Experiment 2

The results of Experiment 2 of this Doctoral thesis are under review for publishing in The Poultry Science Journal.

4.2.1. Chemical Composition and Physical Properties of the Soybean Meals

Country of origin of the soybeans affected the chemical composition of the SBM (Table 19). The BRA meals had more CP (46.9 vs. 45.9 and 46.0%; $P < 0.05$) but less sucrose (5.21 vs. 6.47 and 6.28%; $P < 0.001$) and stachyose (4.20 vs. 4.78 and 4.66%; $P < 0.05$) than the ARG and USA meals. The EEh content was higher for the USA meals than for the ARG meals, with the BRA meals being intermediate (2.51 vs. 2.10 and 2.40%, respectively; $P < 0.05$). The GMD of the SBM was not affected by the origin of the soybeans. However, the GDS was lower for the USA meals than for the ARG meals, with the BRA meals being intermediate (1.99, 2.15, and 2.10 μm , respectively; $P < 0.05$). The BRA meals had lower L^* and higher a^* values than for the USA and ARG meals but the differences were not significant ($P > 0.05$). The total NSP of the SBM was higher for the BRA meals than for the USA and ARG meals but the differences were not significant (161 vs. 154 and 154 g/kg). The ratio insoluble to soluble NSP (3.93, 3.33, and 3.41) was numerically higher for the USA meals than for the ARG and BRA meals (Table 20). The macro-mineral content of the SBM was not affected by the country of origin of the soybeans. However, most of the trace mineral contents varied according to SBM origin (Table 21).

4.2.2. Amino Acids Content and Profile of the Soybean Meals

The AA content and profile of the SBM varied with the country of origin of the soybeans (Table 22). The Arg, Ile, Leu, and the $\Sigma 5$ AA contents were higher for the BRA meals than for the ARG meals with the USA meals being intermediate ($P < 0.05$). Per unit of protein, however, the abundance of the total sulfur AA (TSAA; 2.80 vs. 2.69 and 2.77%; $P < 0.01$) was higher for the USA meals than for the ARG meals, with the BRA meals being

intermediate. Also, the $\sum 5$ AA (14.4 vs. 14.2 and 14.2%; $P = 0.062$) tended to be higher for the USA meals than for the South American meals (Table 23). When the data were sorted by the country of origin of the soybean, the lowest variability in AA content and profile was observed for the USA meals for most AA.

4.2.3. Protein Quality Indicators of the Soybean Meals

Urease activity (0.189 vs. 0.178 and 0.061 Δ pH; $P < 0.05$), PDI (14.1 vs. 11.6 and 10.1%; $P < 0.01$), KOH (77.6 vs. 71.5 and 70.8%; $P < 0.01$), and TIA (2.16 vs. 1.48 and 1.75 mg/g; $P < 0.01$) were higher for the USA meals than for the BRA and ARG meals. However, the fRLys was higher for the ARG meals than for the BRA meals, with USA meals being intermediate (94.6 vs. 91.5 and 94.0%, respectively; $P < 0.024$), but no differences were detected when the hRLys procedure was used (Table 24). In all these determinations, the CV was higher for the BRA meals than for the ARG and USA meals (7.71 vs. 7.38 and 5.22%) for KOH, (25.4 vs. 18.0 and 19.1 mg/g) for TIA, (1.96 vs. 1.39 and 1.25%) for hRLys, and (3.29 vs. 2.23 and 1.79%) for fRLys.

4.2.4. In Vitro Digestibility of the Crude Protein of the Soybean Meals

The IVDP of the SBM was not affected by the origin of the soybean in any of the methodologies used (Table 25). The inclusion of an extra phase (cellulolytic digestion) after pepsin and pancreatin incubation (IVDP 2), increased CP digestibility compared to that obtained using IVDP 1. When the IVDP 3 was used, the USA meals tended to have higher CP digestibility ($P = 0.078$) at 3 h post-incubation than the ARG and BRA meals (81.7 vs. 81.3 and 80.2%, respectively).

Table 19. Chemical composition (%) and physical properties of the experimental soybean meals (88% dry matter). Experiment 2

Soybeans origin ¹	Average			SEM ²		Range			Coefficient of variation (%)		
	ARG	BRA	USA	(n = 9)	P-value	ARG	BRA	USA	ARG	BRA	USA
Gross energy (kcal/kg)	4,110	4,130	4,140	0.140	0.336	4,060-4,200	4,040-4,180	4,090-4,190	1.09	1.10	0.73
Dry matter	89.4	88.8	88.3	0.343	0.095	88.4-91.1	87.5-90.5	85.7-89.2	1.13	1.09	1.25
Ash	6.40	6.40	6.43	0.066	0.943	6.05-6.60	6.10-6.65	6.22-6.86	2.85	3.18	3.22
Crude protein	45.9 ^b	46.9 ^a	46.0 ^b	0.237	0.008	44.8-46.8	45.9-48.9	45.2-46.4	1.62	1.92	0.85
EEh ³	2.10 ^b	2.40 ^{ab}	2.51 ^a	0.107	0.034	1.39-2.39	1.69-2.76	1.90-3.02	14.1	13.1	13.9
Starch	0.49	0.42	0.47	0.043	0.534	0.30-0.78	0.29-0.60	0.30-0.69	32.8	24.4	25.0
Sucrose	6.47 ^a	5.21 ^b	6.28 ^a	0.208	0.001	5.45-7.08	4.48-6.18	4.73-6.84	9.28	10.6	11.1
Stachyose	4.78 ^a	4.20 ^b	4.66 ^a	0.139	0.016	4.27-5.20	3.35-4.78	3.99-5.14	6.72	12.8	7.65
Raffinose	1.69	1.85	1.54	0.091	0.075	1.44-2.07	1.42-2.4	1.32-1.87	14.0	20.6	9.8
Crude fiber	4.52	4.55	4.61	0.112	0.831	4.25-4.96	4.08-5.23	4.10-5.34	4.74	8.61	8.14
Neutral detergent fiber	11.2	11.7	11.2	0.297	0.404	10.4-12.7	10.1-13.0	9.7-12.6	6.05	9.54	7.45
Σ Components ⁴	90.1	90.2	90.0	0.330	0.945	88.6-91.3	88.6-91.3	88.9-92.4	0.90	1.04	1.32
Particle size distribution ⁵											
GMD (μm)	1,056	1,139	1,087	62.2	0.617	823-1,383	784-1,302	819-1,315	19.4	15.0	14.8
GSD (μm)	2.15 ^a	2.10 ^{ab}	1.99 ^b	0.04	0.032	1.96-2.28	1.86-2.36	1.90-2.10	4.72	7.03	4.98
Minolta color ⁶											
Luminosity (L*)	69.3	66.5	69.6	1.02	0.062	62.3-72.8	62.1-68.3	66.3-73.9	5.81	3.03	3.40
Redness (a*)	7.76	8.61	7.40	0.40	0.099	5.77-9.62	6.73-10.2	5.70-9.50	13.5	12.6	18.3
Yellowness (b*)	29.1	28.0	27.9	1.29	0.760	23.7-33.0	20.8-37.3	25.6-30.6	10.5	19.3	5.58

^{a, b} Means within a row, without a common superscript, differ significantly ($P < 0.05$).

¹ ARG = Argentina, BRA = Brazil, and USA = United States of America.

² Standard error of the mean.

³ Ether extract after previous HCl hydrolysis.

⁴ Includes moisture, ash, crude protein, EEh, starch, sucrose, stachyose, raffinose, and NDF.

⁵ GMD = geometric mean diameter, GSD = geometric standard deviation.

⁶ Color characteristics of soybean meals.

Table 20. Non-starch polysaccharides (NSP) content (g/kg) of the soybean meals (88% dry matter). Experiment 2

Soybeans origin ¹	Average			SEM ² (n = 9)	P-value	Range			Coefficient of variation (%)		
	ARG	BRA	USA			ARG	BRA	USA	ARG	BRA	USA
Soluble NSP											
Rhamnose	0.73	1.07	0.65	0.139	0.098	0-1	1-2	0-1	61	43	52
Fucose	0.75	0.94	0.70	0.105	0.249	0-1	1-1	0-1	43	30	49
Arabinose	7.08	7.19	6.05	0.395	0.102	5-9	6-9	4-8	17	14	22
Xylose	2.66	2.41	2.04	0.234	0.195	1-4	1-4	1-3	27	31	31
Mannose	4.11	5.36	3.92	0.465	0.078	2-6	3-8	3-6	28	35	25
Galactose	11.7	11.8	10.7	0.838	0.581	6-18	10-15	6-14	28	13	23
Glucose	3.51	2.64	1.40	0.590	0.056	1-7	0-5	0-4	58	72	91
Galacturonic acid	6.18	5.76	6.61	0.724	0.713	4-9	2-9	5-9	36	48	18
Total Soluble NSP	36.8	37.0	31.9	1.92	0.121	25-45	30-46	26-40	19	15	14
Insoluble NSP											
Rhamnose	3.05	2.88	3.27	0.137	0.098	2-4	2-4	3-4	16	13	11
Fucose	2.11	1.89	2.12	0.106	0.249	1-3	2-2	2-2	20	14	10
Arabinose	16.1	16.1	16.5	0.460	0.102	13-18	14-18	15-19	10	8	8
Xylose	9.81	12.0	10.9	0.566	0.195	8-11	8-16	10-12	10	22	8
Mannose	3.42	4.57	4.29	0.296	0.078	3-4	3-8	3-5	11	30	15
Galactose	32.3	29.3	31.5	0.913	0.581	25-36	26-33	29-34	13	7	4
Glucose	31.0	37.6	36.6	1.71	0.056	27-35	27-50	32-41	8	21	8
Galacturonic acid	19.2	20.2	17.3	1.00	0.713	14-22	15-26	13-21	15	15	18
Total Insoluble NSP	117	124	123	3.29	0.121	105-130	108-149	111-133	6	11	6
Insoluble:soluble ³ NSP	3.33	3.41	3.93	0.240	0.183	2.51-5.28	2.67-4.41	2.95-4.88	27	16	17
Total ⁴ NSP	154	161	154	3.55	0.268	145-163	138-183	141-165	4	10	4

¹ ARG = Argentina, BRA = Brazil, and USA = United States of America.² Standard error of the mean.³ Insoluble to soluble NSP ratio.⁴ Soluble and insoluble glucuronic acid were below < 0.1 g/kg.

Table 21. Mineral content of the soybean meals (88% dry matter). Experiment 2

Soybeans origin ¹	Average			SEM ²		Range			Coefficient of variation (%)		
	ARG	BRA	USA	(n = 9)	P-value	ARG	BRA	USA	ARG	BRA	USA
Macro-mineral (%)											
Ca	0.30	0.31	0.35	0.017	0.182	0.26-0.37	0.27-0.38	0.26-0.51	12.5	13.9	20.1
P	0.64	0.62	0.64	0.009	0.185	0.60-0.67	0.58-0.64	0.59-0.69	4.18	3.74	4.92
K	2.19	2.07	2.12	0.044	0.159	1.98-2.50	2.01-2.18	1.76-2.27	7.28	2.59	7.20
Mg	0.28	0.30	0.29	0.007	0.306	0.28-0.30	0.27-0.34	0.28-0.34	3.06	8.11	9.14
Na	0.05	0.03	0.04	0.009	0.301	0.01-0.12	0.01-0.07	0.02-0.08	69.5	76.8	51.0
Trace mineral (mg/kg)											
Zn	50 ^b	62 ^a	60 ^{ab}	2.62	0.013	44-58	50-76	49-72	11.4	17.1	10.7
Mn	38 ^a	30 ^b	33 ^{ab}	1.81	0.024	30-44	24-38	26-41	13.0	20.1	16.2
Fe	101	123	100	6.53	0.049	80-122	95-199	86-125	21.5	25.6	11.6
Cu	14	13	14	0.534	0.051	14-16	9-15	12-15	5.64	18.7	9.16

^{a, b} Means within a row, without a common superscript, differ significantly ($P < 0.05$).

¹ ARG = Argentina, BRA = Brazil, and USA = United States of America.

² Standard error of the mean.

Table 22. Amino acids (AA) content (%) of the soybean meals (88% dry matter). Experiment 2

Soybeans origin ¹	Average			SEM ² (n = 9)	P-value	Range			Coefficient of variation (%)		
	ARG	BRA	USA			ARG	BRA	USA	ARG	BRA	USA
Crude protein	45.9 ^b	46.9 ^a	46.0 ^b	0.275	0.023	44.3-46.4	45.0-48.4	44.5-46.3	1.75	2.29	1.17
Indispensable AA											
Arg	3.37 ^b	3.48 ^a	3.39 ^b	0.021	0.002	3.31-3.41	3.34-3.60	3.35-3.45	1.11	2.75	1.10
His	1.21	1.23	1.20	0.009	0.117	1.15-1.24	1.18-1.29	1.16-1.24	2.29	2.76	1.83
Ile	2.14 ^b	2.23 ^a	2.14 ^b	0.014	<0.001	2.10-2.18	2.12-2.29	2.08-2.19	1.44	2.30	1.81
Leu	3.52 ^b	3.65 ^a	3.52 ^b	0.022	<0.001	3.44-3.60	3.48-3.78	3.42-3.62	1.32	2.24	1.75
Lys	2.83	2.88	2.87	0.016	0.055	2.77-2.87	2.76-2.94	2.84-2.93	1.20	2.35	1.11
Met	0.61 ^b	0.62 ^a	0.63 ^a	0.005	0.006	0.56-0.63	0.59-0.65	0.62-0.64	3.39	2.63	0.80
Phe	2.34 ^b	2.44 ^a	2.38 ^b	0.017	0.001	2.29-2.40	2.33-2.52	2.29-2.48	1.63	2.21	2.61
Thr	1.81	1.84	1.83	0.009	0.107	1.78-1.84	1.78-1.88	1.77-1.86	1.23	1.78	1.39
Trp	0.63	0.63	0.63	0.003	0.982	0.62-0.64	0.60-0.66	0.63-0.65	1.28	2.35	0.91
Val	2.24 ^{ab}	2.30 ^a	2.22 ^b	0.016	0.007	2.18-2.32	2.24-2.37	2.16-2.29	2.00	2.21	2.16
Dispensable AA											
Ala	2.04 ^{ab}	2.08 ^a	2.03 ^b	0.010	0.010	2.01-2.09	2.01-2.14	1.97-2.09	1.16	1.69	1.57
Asp	5.26 ^b	5.44 ^a	5.35 ^{ab}	0.030	0.001	5.12-5.39	5.25-5.62	5.20-5.46	1.70	1.87	1.50
Cys	0.63 ^b	0.68 ^a	0.65 ^b	0.006	<0.001	0.59-0.66	0.66-0.70	0.63-0.68	3.70	2.28	2.23
Glu	8.39 ^b	8.70 ^a	8.54 ^b	0.046	<0.001	8.22-8.55	8.35-9.00	8.29-8.67	1.31	2.02	1.39
Gly	1.96 ^b	2.03 ^a	1.99 ^b	0.008	<0.001	1.93-1.98	1.96-2.07	1.94-2.01	0.80	1.52	1.15
Pro	2.32 ^b	2.41 ^a	2.35 ^b	0.014	0.001	2.27-2.37	2.33-2.48	2.27-2.44	1.37	1.81	2.15
Ser	2.28 ^b	2.36 ^a	2.31 ^b	0.012	0.001	2.23-2.34	2.28-2.43	2.25-2.35	1.50	1.80	1.47
Total sulfur AA	1.23 ^b	1.30 ^a	1.28 ^a	0.010	<0.001	1.15-1.28	1.26-1.34	1.26-1.32	3.24	2.21	1.28
Σ Five key AA ³	6.51 ^b	6.65 ^a	6.62 ^a	0.030	0.006	6.41-6.60	6.45-6.78	6.54-6.75	0.99	1.96	0.89

^{a, b} Means within a row, without a common superscript, differ significantly ($P < 0.05$).

¹ ARG = Argentina, BRA = Brazil, and USA = United States of America.

² Standard error of the mean.

³ Lys, Met, Cys, Thr, and Trp.

Table 23. Amino acids (AA) profile of the soybean meals (% crude protein). Experiment 2

Soybeans origin ¹	Average			SEM ² (n = 9)	P-value	Range			Coefficient of variation (%)		
	ARG	BRA	USA			ARG	BRA	USA	ARG	BRA	USA
Indispensable AA											
Arg	7.34	7.42	7.37	0.038	0.377	7.26-7.45	7.21-7.64	7.24-7.51	0.89	2.23	1.20
His	2.63	2.62	2.62	0.021	0.846	2.55-2.71	2.52-2.75	2.51-2.68	2.11	2.75	2.12
Ile	4.66 ^{ab}	4.76 ^a	4.65 ^b	0.030	0.032	4.50-4.84	4.61-4.90	4.50-4.75	2.07	1.97	1.66
Leu	7.69	7.78	7.65	0.041	0.086	7.59-7.99	7.57-7.97	7.48-7.85	1.69	1.59	1.47
Lys	6.17	6.13	6.25	0.041	0.125	5.95-6.38	5.93-6.34	6.13-6.36	2.45	2.18	1.14
Met	1.32 ^b	1.33 ^b	1.37 ^a	0.012	0.010	1.25-1.39	1.27-1.38	1.35-1.41	3.27	2.81	1.37
Phe	5.11	5.21	5.17	0.033	0.093	5.03-5.19	5.06-5.34	4.95-5.42	1.03	1.55	2.70
Thr	3.95	3.92	3.97	0.018	0.131	3.85-4.07	3.84-4.01	3.92-4.02	1.75	1.37	0.71
Trp	1.38 ^a	1.35 ^b	1.38 ^a	0.007	0.005	1.35-1.40	1.30-1.39	1.36-1.40	1.26	1.92	1.07
Val	4.89	4.90	4.83	0.031	0.253	4.75-4.99	4.73-5.03	4.66-4.96	1.49	1.98	2.26
Dispensable AA											
Ala	4.46	4.43	4.42	0.017	0.235	4.40-4.57	4.37-4.54	4.35-4.53	1.28	1.18	1.06
Asp	11.5	11.6	11.6	0.048	0.051	11.3-11.7	11.4-12.0	11.5-11.8	1.08	1.45	1.18
Cys	1.37 ^b	1.44 ^a	1.42 ^a	0.013	0.002	1.30-1.41	1.36-1.50	1.37-1.47	2.74	2.84	2.38
Glu	18.3 ^b	18.6 ^a	18.6 ^a	0.071	0.029	18.1-18.6	18.2-19.2	18.3-18.8	0.78	1.52	1.02
Gly	4.28	4.32	4.32	0.018	0.272	4.22-4.38	4.23-4.45	4.28-4.38	1.15	1.63	0.77
Pro	5.07	5.14	5.10	0.027	0.234	4.97-5.16	5.06-5.24	4.90-5.29	1.30	1.11	2.13
Ser	4.97	5.03	5.03	0.022	0.134	4.87-5.12	4.96-5.19	4.92-5.07	1.38	1.54	1.02
Total sulfur AA	2.69 ^b	2.77 ^{ab}	2.80 ^a	0.021	0.006	2.55-2.78	2.64-2.88	2.75-2.86	2.63	2.62	1.60
Σ Five key AA ³	14.2	14.2	14.4	0.071	0.062	13.8-14.5	13.8-14.6	14.2-14.6	1.62	1.84	0.80

^{a, b} Means within a row, without a common superscript, differ significantly ($P < 0.05$).

¹ ARG = Argentina, BRA = Brazil, and USA = United States of America.

² Standard error of the mean.

³ Lys, Met, Cys, Thr, and Trp.

Table 24. Protein quality indicators and reactive lysine of the soybean meals. Experiment 2

Soybeans origin ¹	Average			SEM ² (n = 9)	P-value	Range			Coefficient of variation (%)		
	ARG	BRA	USA			ARG	BRA	USA	ARG	BRA	USA
UA ³ (Δ pH)	0.061 ^b	0.178 ^{ab}	0.189 ^a	0.033	0.021	0.02-0.10	0.06-0.29	0.03-0.42	44.1	42.8	80.7
PDI ⁴ (%)	10.1 ^b	11.6 ^{ab}	14.1 ^a	0.778	0.005	8.20-14.9	8.30-13.8	10.1-19.0	20.2	12.5	22.6
KOH ⁵ (%)	70.8 ^b	71.5 ^b	77.6 ^a	1.658	0.010	65.3-79.2	59.6-76.8	73.1-85.7	7.38	7.71	5.22
TIA ⁶ (mg/g)	1.75 ^{ab}	1.48 ^b	2.16 ^a	0.124	0.003	1.29-2.26	1.10-2.41	1.56-2.76	18.0	25.4	19.1
Reactive Lys (%)											
Homoarginine	88.5	88.0	88.0	0.460	0.624	86.7-90.7	85.9-92.0	86.1-89.7	1.39	1.96	1.25
Furosine	94.6 ^a	91.5 ^b	94.0 ^{ab}	0.778	0.024	91.7-97.5	87.8-97.4	89.9-95.6	2.23	3.29	1.79

^{a, b} Means within a row, without a common superscript, differ significantly ($P < 0.05$).

¹ ARG = Argentina, BRA = Brazil, and USA = United States of America.

² Standard error of the mean.

³ Urease activity.

⁴ Protein dispersibility index.

⁵ Protein solubility in KOH.

⁶ Trypsin inhibitor activity (on 88% dry matter).

Table 25. *In vitro* digestibility of the crude protein (IVDP, %) of the soybean meals. Experiment 2

Soybeans origin ¹	Average			SEM ² (n =9)	P-value	Range			Coefficient of variation (%)		
	ARG	BRA	USA			ARG	BRA	USA	ARG	BRA	USA
<i>In vitro</i> assay											
IVDP 1 ³	81.1	80.4	80.8	0.642	0.745	77.7-83.4	78.8-83.7	77.2-83.7	2.23	1.63	2.06
IVDP 2 ⁴	84.9	86.0	86.1	0.649	0.332	82.1-87.8	84.7-88.6	82.7-90.6	2.15	1.52	2.92
IVDP 3 ⁵											
At 1 h	75.9	74.6	75.9	0.652	0.279	73.9-77.9	70.7-78.9	73.6-77.6	1.68	3.62	1.81
At 3 h	81.3	80.2	81.7	0.477	0.078	79.9-83.2	78.3-83.5	79.8-83.3	1.39	2.22	1.42

¹ ARG = Argentina, BRA = Brazil, and USA = United States of America.

² Standard error of the mean.

³ Boisen and Fernández (1995).

⁴ Boisen and Fernández (1997).

⁵ Bedford and Classen (1993) at 1 h or 3 h post-incubation.

4.2.5. Energy Content of the Soybean Meals

At 21 d of age, the AMEn of the SBM tended to be higher (2,334 vs. 2,277 and 2,282 kcal/kg; $P = 0.062$) and more uniform (CV = 5.68 vs. 6.60 and 7.78%) for the USA meals than for the BRA and ARG meal (Table 26). Similarly, the AME content of the SBM tended to be higher for the USA meals than for the BRA and ARG meals (2,500 vs. 2,444 and 2,444 kcal/kg; $P = 0.080$).

4.2.6. Ileal Digestibility of Protein and Amino Acids of the Soybean Meals

At 21 d of age, the AID (87.3 vs. 86.4 and 86.7%; $P = 0.054$) and the SID (92.0 vs. 90.9 and 91.4%; $P < 0.05$) of the protein fraction of the SBM were higher for the USA meals than for the BRA, with the ARG meals being intermediate. The USA meals presented higher SID of Arg (94.7 vs. 93.6 and 94.0%; $P < 0.05$), Lys (93.6 vs. 92.2 and 93.1%; $P < 0.05$), and the Σ 5 AA (91.5 vs. 90.3 and 91.1%; $P < 0.05$) than for the BRA meals, with the ARG being intermediate. Similar differences were observed when the AID values were compared. In addition, the AID and SID values of all key AA, except Trp, which was not affected, were more uniform (% CV) for the USA meals than for the South American meals (Tables 27 and 28).

4.2.7. Growth Performance

From 17 to 21 d of age, feed intake (71.4 vs. 70.3 and 69.2 g/d; $P < 0.001$) and BW gain (37.5 vs. 35.2 and 33.9 g/d; $P < 0.001$) were higher and feed conversion ratio (1.91 vs. 2.01 and 2.05 g/g; $P < 0.001$) was better in broilers fed the USA meals than in broilers fed the BRA or the ARG meals (Table 29). Also, AMEn intake (kcal/d) and the consumption of all key AA except Trp (mg/d) in ileal digestibility were greatest for broilers fed USA meals. No mortality was reported in this period.

4.2.8. Correlations between the Laboratory Analyses of the Soybean Meals and *In Vivo* Determinations

Independent of the origin of the SBM, the correlations detected between the chemical composition, protein quality indicators, IVDP, and the AMEn content and the *in vivo* ileal digestibility of CP and AA of the SBM, were numerous and therefore, only the most relevant for the industry are presented (Table 30 and Figures 4 and 5). The most important correlations among protein quality indicators were observed between KOH and PDI ($r = 0.597$; $P < 0.001$) and TIA and KOH ($r = 0.386$; $P < 0.05$). Urease activity did not show any correlation. Correlation between the 2 procedures (homoarginine and furosine) used for RLys determination was detected ($r = 0.450$; $P < 0.05$) but no correlation of any of the 2 RLys procedures with any protein quality indicator was detected. In the case of the *in vitro* tests on CP digestibility of the SBM, no correlations among these 3 methodologies and other variables studied were detected (data not shown).

When *in vivo* data was studied, positive correlations were detected between the SID of CP and Lys of the SBM and a) AMEn content ($r = 0.523$ and $r = 0.492$; $P < 0.01$), b) hRLys ($r = 0.553$ and $r = 0.559$; $P < 0.01$), c) PDI ($r = 0.403$ and $r = 0.414$; $P < 0.05$), d) TIA ($r = 0.360$ and $r = 0.389$; $P < 0.10$), and e) KOH value ($r = 0.324$ and $r = 0.361$; $P < 0.10$). Also, the AMEn content of the SBM was positively correlated with hRLys value ($r = 0.466$; $P < 0.01$). No correlations between UA and any of the variables studied, were detected. None of the 3 methodologies used to determine de *in vitro* CP digestibility was correlated with any of the *in vivo* determinations ($P > 0.05$; data not shown). Finally, the BW gain and feed conversion ratio were correlated with PDI ($r = 0.495$ and -0.522 ; $P < 0.01$) and KOH ($r = 0.454$ and -0.424 ; $P < 0.05$). As expected, the highest correlations among *in vivo* variables detected were between a) AID and SID of CP and of AA ($P < 0.001$), b) AME

and AMEn content ($P < 0.001$), and c) AME and AMEn with GE ($r = 0.590$ and $r = 0.576$; $P < 0.01$) of the SBM (Table 31).

Table 26. Energy content (kcal/kg) of the soybean meals in broilers at 21 days of age (88% dry matter). Experiment 2

Origin ¹	Average			SEM ² (n = 9)	P-value	Range			Coefficient of variation (%)		
	ARG	BRA	USA			ARG	BRA	USA	ARG	BRA	USA
AME ³	2,444	2,444	2,500	19.6	0.080	1,944-2,776	2,025-2,691	2,196-2,805	7.93	6.68	5.87
AMEn ⁴	2,282	2,277	2,334	18.4	0.062	1,812-2,590	1,899-2,496	2,059-2,598	7.78	6.60	5.68
Diference ⁵	162	167	165	2.14	0.218	105-196	124-202	130-219	12.7	12.0	11.3

¹ ARG = Argentina, BRA = Brazil, and USA = United States of America.

² Standard error of the mean.

³ Apparent metabolizable energy.

⁴ Apparent metabolizable energy corrected for nitrogen.

⁵ Difference in kcal/kg between AME and AMEn.

Table 27. Apparent ileal digestibility (%) of crude protein and amino acids (AA) of the soybean meals in broilers at 21 days of age. Experiment 2

Soybeans origin ¹	Average			SEM ² (n = 9)	P-value	Range			Coefficient of variation (%)		
	ARG	BRA	USA			ARG	BRA	USA	ARG	BRA	USA
Crude protein	86.7	86.4	87.3	0.280	0.054	81.9-91.0	80.9-91.1	80.6-91.0	2.66	2.88	2.66
Indispensable AA											
Arg	91.6 ^{ab}	91.4 ^b	92.3 ^a	0.236	0.013	86.4-94.9	85.9-94.8	88.9-94.6	1.97	2.00	1.53
His	90.9 ^{ab}	90.2 ^b	91.3 ^a	0.250	0.012	85.5-94.3	85.8-93.6	87.1-94.2	2.11	2.04	1.77
Ile	88.9	89.0	89.6	0.262	0.149	83.7-92.2	83.9-92.3	85.5-92.8	2.21	2.26	1.82
Leu	89.0	89.2	89.6	0.262	0.290	84.3-92.1	84.1-92.8	85.5-92.8	2.17	2.23	1.92
Lys	90.3 ^{ab}	89.5 ^b	90.8 ^a	0.260	0.001	85.0-93.8	84.0-92.4	87.2-93.7	2.07	2.30	1.80
Met	92.2	92.3	92.5	0.216	0.489	88.6-95.1	89.0-94.9	89.4-94.8	1.93	1.59	1.48
Phe	90.1 ^a	90.2 ^a	91.0 ^b	0.228	0.021	84.5-93.0	86.1-93.2	87.1-93.6	2.00	1.79	1.64
Thr	83.8	83.3	84.2	0.346	0.187	76.5-88.4	77.9-87.5	78.7-87.7	3.24	2.91	2.72
Trp	88.5	87.9	88.3	0.287	0.306	83.5-92.5	83.9-91.4	81.2-91.6	2.40	2.24	2.40
Val	88.2	88.0	88.7	0.258	0.193	83.2-91.4	83.8-90.9	84.9-91.6	2.26	2.15	1.87
Dispensable AA											
Ala	88.3	88.0	88.6	0.247	0.200	83.1-91.5	84.3-91.1	83.6-91.5	2.14	1.94	1.94
Asp	86.5 ^{ab}	85.8 ^b	87.1 ^a	0.340	0.023	78.2-90.4	79.1-90.4	82.0-90.3	2.94	2.97	2.97
Cys	72.3	71.4	73.3	0.807	0.255	56.3-83.3	59.9-83.9	60.4-83.3	8.87	7.56	7.56
Glu	90.9 ^b	90.7 ^b	91.6 ^a	0.244	0.022	84.3-94.0	85.4-93.2	87.8-94.0	2.08	1.98	1.98
Gly	84.9 ^{ab}	83.8 ^b	85.2 ^a	0.338	0.007	77.1-89.4	78.1-88.6	79.0-88.9	2.96	3.03	3.03
Pro	86.8	86.4	87.5	0.325	0.052	79.8-91.4	81.1-92.3	81.4-91.0	2.91	2.72	2.72
Ser	86.7	86.3	87.2	0.303	0.155	79.8-90.6	81.7-90.7	82.0-90.7	2.73	2.45	2.45
Total sulfur AA	82.0	81.4	82.8	0.494	0.169	72.2-88.7	74.3-88.1	74.9-88.2	4.85	4.08	4.08
Σ Five key AA ³	86.7 ^{ab}	86.0 ^b	87.2 ^a	0.316	0.035	80.7-91.0	81.1-89.3	82.5-90.4	2.81	2.67	2.67

^{a, b} Means within a row, without a common superscript, differ significantly ($P < 0.05$).

¹ ARG = Argentina, BRA = Brazil, and USA = United States of America.

² Standard error of the mean.

³ Lys, Met, Cys, Thr, and Trp.

Table 28. Standardized ileal digestibility¹ (%) of protein and amino acids (AA) of the soybean meals in broilers at 21 days of age. Experiment 2

Soybean origin ²	Average			SEM ³ (n = 9)	P-value	Range			Coefficient of variation (%)		
	ARG	BRA	USA			ARG	BRA	USA	ARG	BRA	USA
Crude protein	91.4 ^{ab}	90.9 ^b	92.0 ^a	0.282	0.001	86.6-95.7	85.5-95.6	85.3-95.6	2.52	2.78	2.52
Indispensable AA											
Arg	94.0 ^{ab}	93.6 ^b	94.7 ^a	0.237	0.008	88.7-97.3	88.1-97.0	91.3-97.0	1.92	1.96	1.48
His	93.6 ^{ab}	92.9 ^b	94.0 ^a	0.248	0.006	88.2-96.9	88.4-96.1	89.8-96.8	2.02	1.97	1.71
Ile	92.5	92.4	93.1	0.262	0.075	87.3-95.7	87.2-95.6	89.1-96.3	2.11	2.20	1.75
Leu	92.4	92.4	93.0	0.263	0.198	87.7-95.5	87.3-96.0	88.9-96.1	2.08	2.16	1.84
Lys	93.1 ^{ab}	92.2 ^b	93.6 ^a	0.260	0.001	87.8-96.6	86.7-95.2	90.0-96.4	2.01	2.23	1.74
Met	96.6	96.6	96.8	0.212	0.716	93.0-99.4	93.2-99.3	93.7-99.0	1.75	1.52	1.41
Phe	93.0 ^b	93.0 ^b	93.8 ^a	0.229	0.015	87.4-95.8	88.8-95.9	90.0-96.4	1.93	1.75	1.57
Thr	90.2	89.6	90.5	0.349	0.142	82.8-94.7	84.3-93.8	85.1-93.9	3.03	2.73	2.52
Trp	92.2	91.7	92.1	0.288	0.317	87.2-96.3	87.9-95.2	85.0-95.4	2.29	2.15	2.28
Val	92.7	92.4	93.2	0.256	0.066	87.6-95.9	88.1-95.3	89.5-96.0	2.12	2.05	1.74
Dispensable AA											
Ala	92.5	92.1	92.8	0.249	0.106	87.3-95.6	88.4-95.2	87.6-95.6	2.05	1.88	1.88
Asp	89.5 ^{ab}	88.7 ^b	90.1 ^a	0.342	0.016	81.2-93.5	81.9-93.3	85-93.2	2.83	2.89	2.89
Cys	80.5	79.0	81.3	0.796	0.146	64.5-91.2	67.7-91.5	68.4-91.3	7.83	6.82	6.82
Glu	93.3 ^{ab}	93.0 ^b	94.0 ^a	0.245	0.016	86.7-96.5	87.7-95.6	90.2-96.4	2.03	1.95	1.95
Gly	90.3 ^{ab}	89.0 ^b	90.6 ^a	0.340	0.003	82.5-94.8	83.3-93.9	84.4-94.2	2.78	2.89	2.89
Pro	90.5 ^{ab}	89.9 ^b	91.2 ^a	0.327	0.029	83.4-95.1	84.5-95.8	85.1-94.6	2.81	2.63	2.63
Ser	91.3	90.7	91.7	0.304	0.088	84.3-95.1	85.9-95.1	86.6-95.1	2.59	2.36	2.36
Total sulfur AA	88.4	87.5	88.9	0.487	0.112	78.5-94.9	80.4-94.0	81.1-94.2	4.38	3.77	3.77
Σ Five key AA ⁴	91.1 ^{ab}	90.3 ^b	91.5 ^a	0.316	0.026	85.1-95.3	85.2-93.5	86.8-94.6	2.66	2.55	2.55

^{a, b} Means within a row, without a common superscript, differ significantly ($P < 0.05$).

¹ Apparent digestibility values were standardized using the basal ileal endogenous flow values (g/kg dry matter intake) determined from broiler chickens fed the N-free diet: crude protein, 10.8; Arg, 0.41; His, 0.16; Ile, 0.39; Leu, 0.61; Lys, 0.41; Met, 0.14; Phe, 0.34; Thr, 0.58; Trp, 0.13; Val, 0.51; Ala, 0.44; Asp, 0.81; Cys, 0.24; Glu, 1.04; Gly, 0.55; Pro, 0.43; and Ser, 0.52.

² ARG = Argentina, BRA = Brazil, and USA = United States of America.

³ Standard error of the mean.

⁴ Lys, Met, Cys, Thr, and Trp.

Table 29. Influence of the origin of the soybean meals on broilers performance from 17 to 21 days of age^{1,2}. Experiment 2

Soybeans origin ³	Average			SEM ⁴		Range			Coefficient of variation (%)		
	ARG	BRA	USA	(n = 9)	P-value	ARG	BRA	USA	ARG	BRA	USA
Growth performance											
Feed intake (g/d)	69.2 ^b	70.3 ^{ab}	71.4 ^a	0.513	0.001	52.8-75.0	62.3-77.5	58.9-79.1	5.63	4.80	5.34
BW gain (g/d)	33.9 ^b	35.2 ^b	37.5 ^a	0.466	0.001	27.0-40.8	29.2-43.5	30.7-45.4	9.07	9.45	9.82
Feed conversion ratio (g/g)	2.05 ^b	2.01 ^b	1.91 ^a	0.021	0.001	1.74-2.44	1.67-2.35	1.63-2.16	7.36	7.36	7.75
AMEn intake (kcal/d)	158 ^b	160 ^b	166 ^a	1.82	0.004	124-204	147-208	149-210	9.66	7.49	6.93
SID ⁵ protein intake (g/d)	14.0 ^b	14.4 ^a	14.4 ^a	0.115	0.014	10.8-15.3	13.0-15.9	11.8-15.9	6.53	4.83	5.97
SID ⁵ amino acid intake (mg/d)											
Cys	168 ^b	179 ^{ab}	180 ^a	2.46	0.001	121-205	141-214	129-218	11.6	7.8	10.7
Ile	658 ^b	694 ^a	677 ^a	5.34	0.001	525-726	611-765	555-755	6.15	4.96	5.98
Lys	876 ^b	893 ^{ab}	915 ^a	6.85	0.001	694-961	793-979	744-1013	5.91	4.68	5.91
Met	195 ^b	203 ^a	208 ^a	1.70	0.001	154-221	181-222	171-231	7.27	4.73	6.05
Thr	545 ^b	555 ^{ab}	564 ^a	4.61	0.020	431-600	489-604	454-641	6.43	4.87	6.59
Trp	194	195	198	1.56	0.179	150-213	174-219	163-217	6.17	4.99	6.05
Val	693 ^b	714 ^a	704 ^{ab}	5.84	0.030	532-762	636-774	565-795	6.61	4.61	6.63
Total sulfur amino acids	363 ^b	382 ^{ab}	388 ^a	3.93	0.001	276-425	323-430	301-447	8.85	5.53	7.87

^{a, b} Means within a row, without a common superscript, differ significantly ($P < 0.05$).

¹ The diets contained 53% of the nitrogen-free diet and 47% of the soybean meals evaluated.

² No mortality occurred in this period.

³ ARG = Argentina, BRA = Brazil, and USA = United States of America.

⁴ Standard error of the mean.

⁵ Standardized ileal digestibility values.

Table 30. Correlations among protein quality indicators of the soybean meals¹ and *in vivo* data in broilers at 21 days of age. Experiment 2

	Protein quality indicators ²				Reactive Lys ³		<i>In vivo</i> AMEn
	UA	PDI	KOH	TIA	Homoarg.	Furosine	
Protein quality indicators							
UA (Δ pH)	1						
PDI (%)	0.274	1					
KOH (%)	0.171	0.597***	1				
TIA (mg/g)	0.280	0.375 [†]	0.386*	1			
Reactive Lys (% Lys)							
Homoarginine procedure	-0.143	0.215	0.128	0.154	1		
Furosine procedure	-0.221	-0.050	-0.062	0.171	0.450*	1	
<i>In vivo data</i>							
AMEn (88% DM; kcal/kg)	-0.106	0.224	0.228	0.137	0.466*	0.201	1
Standardized ileal digestibility (%)							
Crude protein	0.097	0.403*	0.324 [†]	0.360 [†]	0.553**	0.037	0.523**
Arg	0.183	0.386*	0.386*	0.364 [†]	0.523**	0.082	0.478*
Cys	-0.036	0.258	0.288	0.264	0.527**	0.165	0.556**
Lys	0.015	0.414*	0.361 [†]	0.389*	0.559**	0.078	0.492**
Met	0.093	0.232	0.227	0.182	0.544**	-0.056	0.564**
Total sulfur AA	-0.012	0.253	0.294	0.274	0.538**	0.135	0.560**
Σ Five key AA ⁵	0.031	0.326 [†]	0.317	0.349 [†]	0.594**	0.115	0.543**
Growth performance							
BW gain (g/d)	0.117	0.495**	0.454*	0.276	-0.136	0.107	0.305
Feed intake (g/d)	0.183	0.335 [†]	0.447*	0.252	-0.037	0.020	0.341 [†]
Feed conversion ratio (g/g)	-0.101	-0.522**	-0.424*	-0.247	0.184	-0.109	-0.254

[†] = $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

¹ Argentina, Brazil, and United States of America origin (n = 9 per each soybean meals origin).

² UA = urease activity, PDI = protein dispersibility index, KOH = Protein solubility in KOH, and TIA = trypsin inhibitor activity

³ Reactive Lys by the homoarginine and furosine procedures.

⁴ Lys, Met, Cys, Thr, and Trp.

Table 31. Correlations among energy content and standardized (SID) ileal digestibility of soybean meals obtained *in vivo* in broilers at 21 days of age¹. Experiment 2

	Energy content ² (kcal/kg)			SID (%)						
	GE	AME	AMEn	CP	Arg	Cys	Lys	Met	TSAA ³	Σ Five AA ⁴
Energy content (kcal/kg)										
GE	1									
AME	0.590**	1								
AMEn	0.576**	0.995***	1							
SID (%)										
Crude protein	0.243	0.495*	0.523**	1						
Arg	0.214	0.447*	0.478**	0.952***	1					
Cys	0.215	0.530**	0.556**	0.864***	0.870***	1				
Lys	0.259	0.452*	0.492**	0.940***	0.947***	0.874***	1			
Met	0.304	0.551**	0.564**	0.889***	0.861***	0.857***	0.825***	1		
Total sulfur AA	0.216	0.534**	0.560**	0.893***	0.893***	0.994***	0.893***	0.896***	1	
Σ Five key AA	0.249	0.510**	0.543**	0.958***	0.957***	0.949***	0.970***	0.905***	0.966***	1

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.¹ Argentina, Brazil, and United States of America (n = 9 per each soybean meals origin).² GE = gross energy; AME = apparent metabolizable energy; AMEn = AME corrected for nitrogen (88% dry matter basis).³ Total sulfur amino acids.⁴ Sum of five key amino acids (Lys, Met, Cys, Thr, and Trp).

Figure 6. Correlations between standardized ileal digestibility (SID) of crude protein (CP) and A) apparent metabolizable energy corrected for nitrogen (AMEn) content and B) reactive Lys by the homoarginine method (hRLys) and between the SID of Lys and C) AMEn content and D) hRLys of the soybean meals (n = 27). Experiment 2

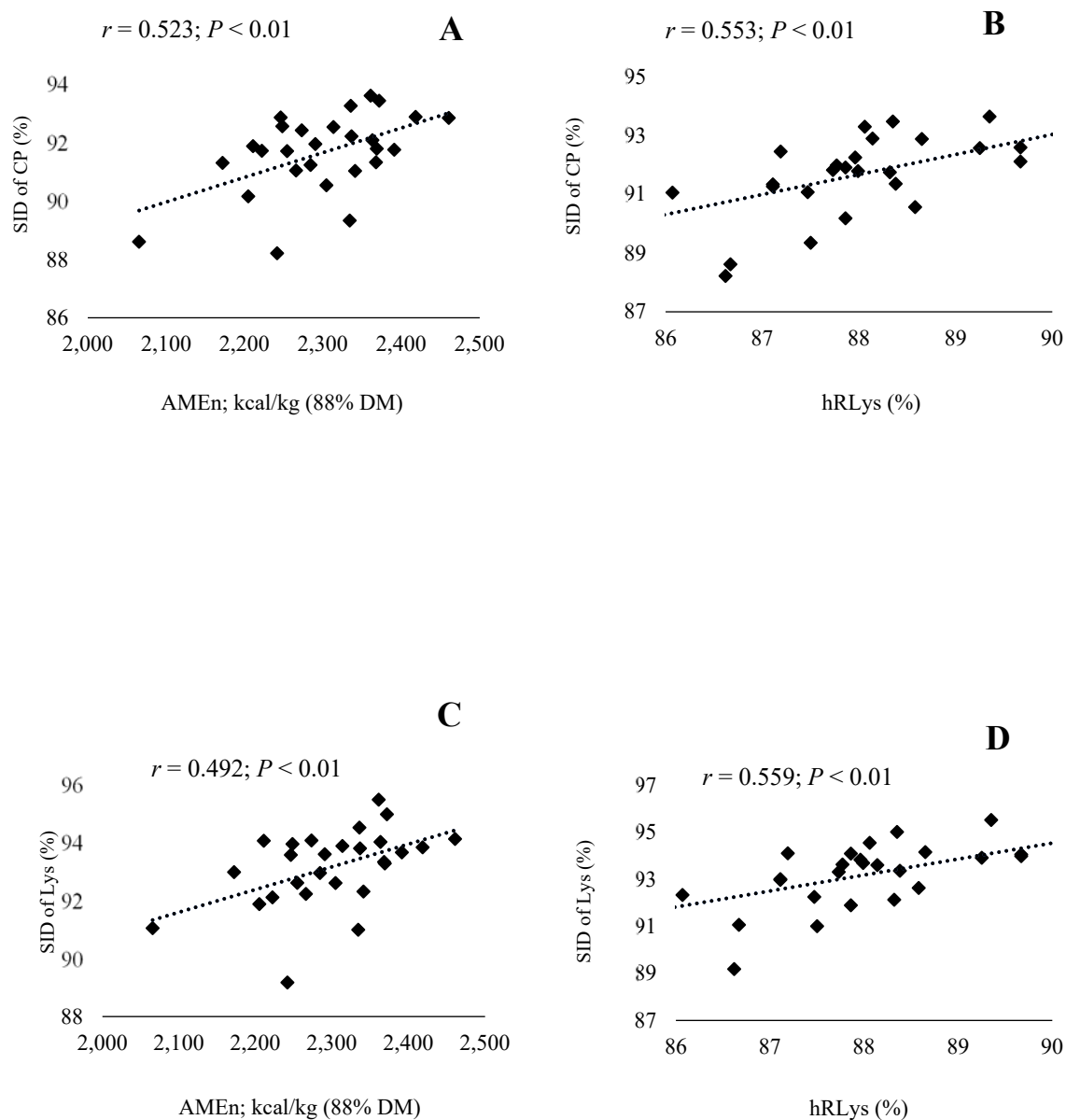
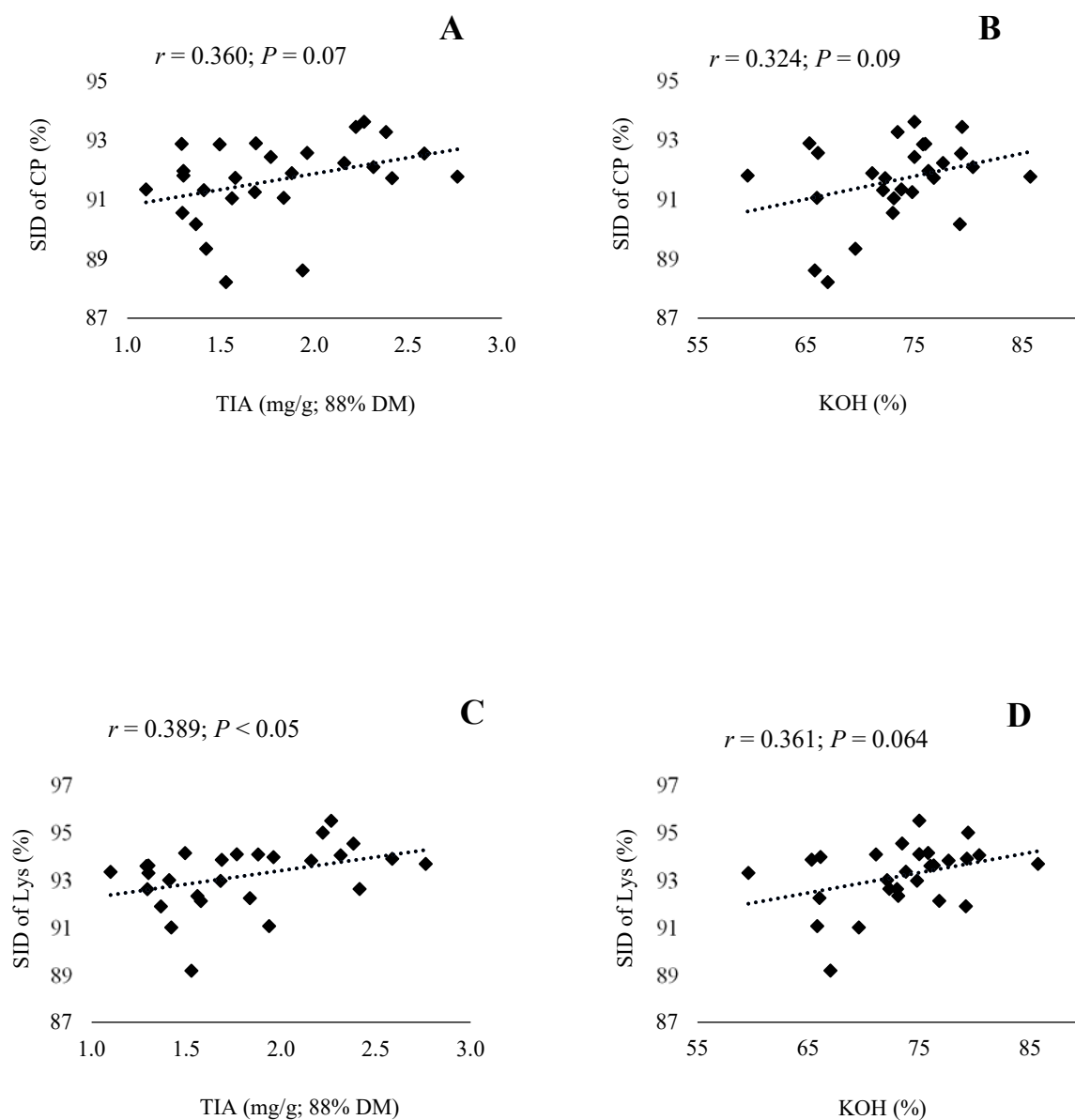


Figure 7. Correlations between the standardized ileal digestibility (SID) of crude protein (CP) and A) trypsin inhibitor activity (TIA) and B) protein solubility in KOH (KOH) and between the SID of Lys and C) TIA and D) KOH of the soybean meals (n = 27). Experiment 2



GENERAL DISCUSSION

5. GENERAL DISCUSSION

5.1. *Chemical Composition of the Soybean Meals*

The chemical composition of the SBM, independent of the origin of the soybeans, in the Experiment 1 and 2 was within the range reported in the literature (Ravindran et al., 2014; García-Rebollar et al., 2016; Lagos and Stein, 2017; Ibáñez et al., 2020; Galkanda-Arachchige et al., 2021).

Crude protein. In Experiments 1 and 2, the BRA meals had the highest CP content, consistent with most published reports comparing SBM (Thakur and Hurburgh, 2007; García-Rebollar et al., 2016; Ibáñez et al., 2020; Aguirre et al., 2022). Factors such as type of seed and soil, latitude and agricultural practices, environmental conditions at harvest, and storage of the soybeans at the farm, affect CP ($N \times 6.25$) content of the soybeans and consequently, that of the resulting SBM (Wilcox and Shibbles, 2001; Karr-Lilienthal et al., 2004; Naeve et al., 2008). As an average, soybeans produced close to the equator had 0.40 to 0.70% more CP than soybeans produced in temperate areas (Stefanello et al., 2016; AMINODat, 2023). Consequently, after oil extraction and depending on the crop year (Corrales et al., 2023), SBM from BRA should contain 0.50 to 0.85% more CP than the SBM produced in cooler areas, consistent with the results reported herein (Experiments 1 and 2).

Ether extract. In general, the EEh content of commercial SBM marketed in Europe, is quite uniform but depends on the solvent utilized for oil extraction, and on the pressure applied during the process, and the potential inclusion of the gums produced at crushing plant to the meal (Hammond et al., 2005; Ravindran et al., 2014). In this respect, the SAF meals had higher EEh content than the USA meals, and both origins had higher EEh content than the South American meals (Experiment 1). In Experiment 2, EEh

content was higher for the USA meals than for the South American meals, suggesting that difference methodologies in the oil extraction process were used.

Carbohydrates. In the Experiment 1, sucrose content was greater for the SAF and USA meals than for the ARG and BRA meals, consistent with data of Lagos and Stein (2017), Ravindran et al. (2014), and Ibáñez et al. (2020). In the Experiment 2, the same tendency of the sucrose content was observed, with the USA and ARG meals with greater sucrose content than the BRA meals. The amount of sucrose in soybeans depends on the area of production, with greater values in soybeans produced in cooler areas (Wolf et al., 1982; Kumar et al., 2010; Alsajri et al., 2020). In this respect, in SAF, soybeans are produced at high altitude (over 1,100 m) with relative low temperatures during the growing season and therefore, a higher sucrose content of the SAF meals than of the ARG and BRA meals was expected (Lagos and Stein, 2017; Aguirre et al., 2022). Moreover, sucrose is easily fermented and when soybeans are harvested in tropical areas, under high humidity and temperature conditions, the content will decrease (Carré et al., 1995; Coradi et al., 2016). Consequently, sucrose content is expected to be lower in SBM from BRA than in SBM from the other 3 origins, in agreement with the data reported herein.

García-Rebollar et al. (2016), Lagos and Stein (2017), and Ibáñez et al. (2020) reported that USA and ARG SBM had more stachyose but less raffinose than BRA SBM, consistent with the results of the current experiments (Experiment 1 and 2). The GOS are not digested by the birds and are often considered as ANF in young birds (Leske and Coon, 1999; Choct et al., 2010). However, soybean GOS have shown beneficial effects on the growth of *Lactobacillus spp* and *Bifidobacterium spp* (Hoover, 1993; Svejstl et al., 2015), acting as bioactive substances (e.g., prebiotics) in humans and animals. Moreover, GOS are easily fermented in the large intestine of the birds, contributing to some extent, depending on the level of inclusion and target species, to increase the energy

content of SBM (Carré et al., 1990, 1995; Coon et al., 1990). Consequently, depending on amount consumed and age, GOS might not be necessarily, an undesirable component of poultry diets (Teague et al., 2023). The starch content of immature soybeans is around 0.40%, a value that decrease rapidly as the seed matures (Yazdi-Samadi et al., 1977; Saldivar et al., 2011), consistent with the starch values reported herein that range from 0.60 and 0.90% for all the meals (Experiments 1 and 2).

In the Experiment 1 and 2, the BRA meals had higher fiber content (CF and NDF) than the meals of the other origins, consistent with data of Thakur and Hurburgh (2007), Ravindran et al. (2014), García-Rebollar et al. (2016), and Ibáñez et al. (2020). In general, the fiber content of commercial SBM depends on the amount of soy hulls removed from the soybeans prior to the oil extraction process as well the amount of soy hulls added back to the meal after processing. Also, the climate conditions during the growing and harvesting seasons can influence on the fiber content of the SBM. In this respect, soybeans produced in tropical areas, have a higher fiber content than soybeans produced in more temperatures areas (Medic et al., 2014), consistent with the results of the current experiments comparing BRA and USA meals. The NSP determined in the 27 commercial samples of the Experiment 2 were not affected among origin of the SBM. This fraction is the less understood of the SBM, the contribution in the nutritional value for poultry diets is poorly, because the complexity of this fraction for the animal digestion. More information is needed to obtain a clear idea of the importance of this fraction of the SBM.

5.2. Macro-Mineral and Trace Mineral Contents of the Soybean Meals

The information available on the mineral content of the SBM of SAF origin is very limited and consequently, it is not possible to compare the data from SAF meals (Experiment 1) with those from other publications. In the Experiment 1, the SAF meals had less Ca, Mg, and Na but more P and K than any of the other meals, reflecting to some

extent, differences in the mineral content and pH of the soil, as well as the rate of fertilization of the crop (Westgate et al., 2000; Huerta and Martin, 2002). Chang (1960) and Gibson and Mullen (2001) reported low Ca level in soybeans produced under relative low temperatures. In this respect, SAF soybeans, that are produced at moderate temperatures (high altitude) during the growing and harvesting seasons, had the highest Ca level values in the current experiment. In addition, small amounts of CaCO_3 might be added to the samples after the oil extraction process as an anticaking agent to improve the flowability of the meals (Batal et al., 2010; Lagos and Stein, 2017). Both in Experiment 1 and 2, the P content was lowest for the BRA meals than for the other meals, consistent with data of Karr-Lilienthal et al. (2004), Lagos and Stein (2017), and Ibáñez et al. (2020). These findings are related with the low soil pH of the main planting areas of Brazil, with high Al and Fe contents. At low pH, the phosphate molecules react with the Al present in the soil to form less soluble compounds, which limits P absorption by the plant and reduces its accumulation in the seed (Brennan et al., 1994; Ibáñez et al., 2020). In the Experiment 1, the K content was greater for the ARG and SAF meals than for the BRA and USA meals, in agreement with data of Ravindran et al. (2014) and Lagos and Stein (2017) comparing ARG, BRA, and USA meals. In all cases, however, the differences in macro-minerals content of the SBM, because of origin, were of limited practical interest.

Trace minerals content varied with the country of origin of the soybeans (Experiment 1 and 2), consistent with data of García-Rebollar et al. (2016) and Ibáñez et al. (2020). The biggest differences were observed for Fe, that was highest for the BRA meals in Experiments 1 and 2. In this respect, Brennan et al. (1994) and Jensen (2010) reported that Fe absorption increased as the pH of the soil decreased, consistent with the soil characteristics (low pH of the soils) of the soybean production areas of Brazil. The soils of the main Brazil are acid, with high Al and Fe contents.

5.3. *Protein Quality Indicators of the Soybean Meals*

The values for all protein quality indicators were within the range reported in the literature in both Experiments 1 and 2 (Ibáñez et al., 2020; Aguirre et al., 2022; Siegert et al., 2023) indicating that in general the SBM were conveniently processed. Independent of the origin of the soybeans, the UA values for the Experiments 1 and 2 are consistent with recommendations by the feed industry for high quality SBM (Waldroup et al., 1985; van Eys and Ruiz, 2021).

The PDI and KOH values recommended by feed industry ranged from 15 to 30% and 70 to 85%, respectively (Balloun, 1980; Araba and Dale, 1990; van Eys and Ruiz, 2021). Independent of the origin of the SBM, the values ranged for PDI from 7.0 to 26.7%, in the Experiment 1 and from 8.2 to 19.0%, in the Experiment 2. In fact, none of the samples analyzed for both experiments had a PDI value above 30%, whereas 85% of the samples had PDI values below 15%. Similarly, KOH values ranged from 60 to 89%, in the Experiment 1 and from 60.0 to 85.7%, in the Experiment 2. Only 10 and 1 samples analyzed of the Experiment 1 and 2, respectively, had KOH value over 85%, whereas 7% and 26% of the samples analyzed of the Experiment 1 and 2, respectively, had KOH values below 70%. The data reported herein were obtained in the same official laboratory for the analyses or commercial ingredients and agree with previous research conducted in the last decade by García-Rebollar et al. (2016), Ibáñez et al. (2020), and Siegert et al. (2023). This information suggest that further studies are needed to confirm or modify current recommended values for SBM processed under commercial practices.

The optimal TIA values recommended by the industry for well processed SBM are not well defined, with high discrepancies among authors. In the Experiment 1, the average TIA value of the SBM was of 2.4 mg/g whereas for the Experiment 2 was of 1.80 mg/g, which are within the range (1.6 and 2.5 mg/g) recommended by van Eys and Ruiz

(2021) and Kuenz et al. (2022). In fact, these last authors in study conducted in broilers with expeller SBM reported that the digestibility of the AA increased as the TIA content of the diet decreased from 5.3 to 0.1 mg/g, which the tested samples had TIA content between 2.7 and 23.6 mg/g, respectively. Similarly, in a study also using expeller SBM as a source of protein, Hoffman et al. (2019) reported that feed efficiency increased as the TIA content of the diet decreased from 0.1 to 7.1 mg/g corresponding to a range of TIA value for the SBM from 0.25 to 23.6 mg/g. In contrast, Heger et al. (2016), concluded that growth performance was not affected in broilers fed diets that contained from 2.0 up to 5.5 mg TIA/g. Similarly, Clarke and Wiseman (2005, 2007) recommended TIA values below 4.0 mg/g in diets based on full-fat soybeans. The reasons for the differences among authors for the best recommended values for KOH, PDI, and TIA for well processed SBM are not apparent. Two possibilities are a) most of the recommendations presented refers to the TIA content of the SBM rather than to TIA consumption by the birds and b) none of works presented, included complementary information on RLys (Maillard reactions) of the soy product used. Moreover, TIA determination has been validated for SBM but no for other soy products, such as full-fat SBM expeller or protein concentrate (van Eys and Ruiz, 2021; Abdollahi et al., 2022). Unfortunately, the authors have found any reports comparing diets based on different samples of SBM varying TIA and RLys values in broilers for the last 10 years. The information provided suggest the need of further studies to confirm, or modify as needed, current values recommended by the industry for SBM processed under current commercial practices.

In the Experiment 2, the hRLys varied from 86 to 92%, and did not vary according to origin of the SBM, consistent with data from (Frikha et al., 2012) who did reported values between 84 and 88%, using the homoarginine test. However, the fRLys varied from 88 to 98%, with the ARG and USA meals with higher values than the BRA meals.

The high values reported for the SID of all the AA, together with the RLys values suggest that the conditions applied during the oil extraction procedure (e.g., T° C, time, moisture, and particle size) were adequate to reduce TIA of the samples without resulting in any damage of the protein fraction of the soybeans. The data reported herein, suggest that SBM with more than 72% for KOH, 12% for PDI, and TIA close to 2 mg/g, and RLys above 88%, might be indicative of adequate processed SBM.

5.4. *In Vitro Crude Protein Digestibility of the Soybean Meals*

In the Experiment 1, the IVDP was higher for the USA and SAF meals than for the ARG and BRA meals at 1 and 3 h of incubation. However, in the Experiment 2, no differences were detected among country of origin of the soybeans using 3 *in vitro* tests to determine the digestibility of the CP of the SBM. *In vitro* assays are used to evaluate faster and cheaper the protein quality of ingredients included SBM than *in vivo* assays (Losada et al., 2010; Bryan et al., 2018). However, *in vitro* CP digestibility results were lower than we expected. For example, Frikha et al. (2012) using a gastric and intestine digestion methods proposed by Boisen and Fernández (1995) reported higher IVDP for the USA meals than for the BRA meals with the ARG meals being intermediate (88.2, 86.4, and 87.5%, respectively). Also, de Coca-Sinova et al. (2008) using the same methodology reported IVDP values from 93.0 to 97.1% in SBM from USA, ARG, and BRA. The reasons for the discrepancies among these higher IVDP values are not known but might be related to the characteristics and quality (e.g., ANF, mineral, and NSP contents) of the SBM evaluated as well as the type of processing of the samples (Boisen and Eggum, 1991; de Coca-Sinova et al., 2008; Frikha et al., 2012).

5.5. *Apparent Metabolizable Energy of the Soybean Meals*

The average AMEn content of the 27 SBM tested (Experiment 2), was of 2,298 kcal/kg (88% DM basis) with a range of 2,277 to 2,334 kcal/kg, a value that is within the

range reported for the most Tables on Feed Ingredient Composition (Mateos et al., 2019). However, the AMEn content of SBM reported in scientific journals are usually below this value. In fact, recent values on the AMEn of SBM (88% DM basis), with a CP content in the range of 45.7 to 46.7%, varied from 1,998 kcal/kg to 2,340 kcal/kg (Ravindran et al., 2014; Bertechini et al., 2018; Leung and Kiarie 2020; Khalil et al., 2021; Sung et al., 2023). In general, none of the authors of these *in vivo* trials gives a clear explanation for the differences observed, and in fact the reasons for the variability are unclear. Probably, the lack of standardization of the conditions applied among experiments, such as the procedure (total excreta collection vs. index), the basal diet, and the methodology (difference vs. regression) used might explain at least in part, the differences observed (Adeola and Ileji, 2009; Loeffler et al., 2013; West, 2018; Veluri and Olukosi, 2020).

The highest AMEn value was observed for the USA meals, consistent with their higher CP digestibility and greater sucrose, oligosaccharides, and EEh content (Experiment 2). As average the USA meals used in the current research, had 1.10% less CP but 1.00% better SID protein, and 1.00, 0.16, and 0.10% more sucrose, oligosaccharides, and EEh content than the BRA meals. Each percent unit of digestible protein provides 35 to 40 kcal extra of AMEn/kg in the SBM (WPSA 1989; CVB, 2021, Fondevila et al., 2021). Similarly, each percent unit of sucrose, a very well digested CHO by poultry, contributes with 30 to 35 kcal AMEn/kg to the energy content of the SBM (Coon et al., 1990; Parsons et al., 2000; Perryman and Dozier, 2012; Fondevila et al., 2021). In addition, the oligosaccharides present in the SBM, although not digested, are easily fermented in the large intestine, contributing with approximately 10 to 12 kcal AMEn/kg per each percent unit extra (Carré et al., 1990, 1995; Coon et al., 1990). Finally, the 0.10% extra EEh of the USA meals contributed approximately with 6 to 8 kcal AMEn/kg (Bolton, 1957; CVB,

2021; AMINODat, 2023). Based on the amount and digestibility of these 4 main energy fractions, the USA SBM should have 30 to 32 more kcal AMEn/kg than the BRA SBM.

Usually, the AME value of poultry diets is corrected to zero N balance (AMEn), to eliminate the variation associated with the amount of N that is deposited as protein tissue *vs.* that oxidized in the body to provide energy (Farrell et al., 1991; Lopez and Leeson, 2008). In the current Experiment, the difference between AME and AMEn values were of 165 ± 10 kcal/kg, consistent with data of Bertecheni et al. (2018) and Khalil et al. (2021) but below data of Leung and Kiarie (2020), Abdohalli et al. (2021), and Sung et al. (2023). The reason for the difference between AMEn and AME reported is unknown but might depends on the nature of the basal diet used (Lopez and Leeson, 2008; Adeola and Ileji, 2009; Veluri and Olukosi et al., 2020).

5.6. *Amino Acids Content and Digestibility of the Soybean Meals*

The abundance and the profile of the AA of the SBM were within the range reported in the literature (Adedokun et al., 2008; García-Rebollar et al., 2016; Ibáñez et al., 2020; Aguirre et al., 2022; Sung et al., 2023). In general, in the Experiment 1 and 2, the BRA SBM have more indispensable AA than the USA and ARG SBM, mostly because of CP content (García-Rebollar et al., 2016; Stefanello et al., 2016; Lagos and Stein, 2017; Galkanda-Arachchige et al., 2021). When the abundance of the AA is studied on CP basis, the differences disappear for some of them AA (e.g., Arg, Lys, and Thr). In fact, the USA meals had better AA profile than the BRA and ARG meals in Experiment 1 and 2, consisting with data reported by García-Rebollar et al. (2016), Lagos and Stein (2017), and Ibáñez et al. (2020).

The ileal digestibility of the CP and AA varied with the country of origin of the soybeans (Experiment 2) and in general, was higher for the USA than for the South American meals, in agreement with data of de Coca et al. (2008), Frikha et al. (2012),

Ravindran et al. (2014), and AMINODat (2023). The reasons for the differences in AA content and the digestibility of the AA of SBM according to the origin, are not known but might be related to the quality and characteristics of the original soybeans (e.g., percentage of damaged seeds prior to processing) and the conditions applied during the oil extraction process. In this respect, Cámara et al. (2022) and Talegón et al. (2023) reported that the percentage of damaged raw soybeans arriving to Spain during the 2021/2023 harvest season, were higher for the BRA soybeans than for the USA soybeans, suggesting that factors such as poor weather conditions at harvest, heat applied to dry the soybeans at the farm before storage, and length and conditions during transport, impact the quality of the original soybeans and of the resulting SBM (Hill et al., 1996; García-Rebollar et al., 2016; Ibáñez et al., 2020; Aguirre et al., 2022). In this respect, raining at high temperature, frequent in tropical areas, might increase the moisture content of the soybeans and delay cropping, increasing the incidence of fermented, germinated, and fungal contaminated grains (Paulsen et al., 1981; Peer and Leeson, 1985; Coradi et al., 2016).

In addition, any excess of heat applied to dry the soybeans will result in more Maillard reactions, reducing the digestibility of the AA, specially Lys, Cys, and Arg (Papadopoulos, 1989; Fontaine et al., 2007; Pahm et al., 2008; Coradi et al., 2016, 2020; Abdollahi et al., 2022). In fact, the ileal digestibility of these 3 AA in the Experiment 2, were higher for the USA meals than for the South American meals, suggesting less aggressive situations during harvesting, storage, and transport, for the USA meals than for the BRA and ARG meals (Frikha et al., 2012; Ravindran et al., 2014; Siegert et al., 2023). The Maillard reactions caused probably being relevant contributor to these differences on the ileal digestibility of the CP and AA reported in the Experiment 2.

5.7. *Growth Performance*

Broilers fed with their respective experimental diets (53% NFD and 47% of their individual experimental SBM) in the Experiment 2, from 17 to 21 d of age, growth performance was better for broilers fed the USA meals than for broilers fed the South American meals, consistent with the higher energy and digestibility of the AA of the USA meals and the greater FI of birds fed these diets. Assuming that the diet is adequate in all essential nutrients and that the energy to essential nutrient ratio is maintained constant, poultry eat to satisfy their energy requirements (NRC, 1994; Brickett et al., 2007; Grobas et al., 1999). Consequently, the AMEn content of the SBM was not responsible for the difference in feed intake observed among treatments, although could be the main reason for the differences in feed conversion ratio. Usually, Lys and TSAA are the AA limiting growth in young broilers (Dozier et al., 2008, Dozier and Payne, 2012; FEDNA 2018). In the current research, Lys content of all the experimental diets was above broilers requirements and therefore, Lys was not the limiting of the birds. Based on the ideal protein concept and the AA profile of the SBM tested, the TSAA content could possibly explain the differences in growth performance observed

5.8. *Correlations between Chemical Composition and Protein Quality Indicators of Soybean Meals and In Vivo Determinations*

Sucrose content was positively correlated with oligosaccharides and negatively with CP contents, consistent with data of Hartwig et al. (1997), Wilcox and Shibles (2001), García-Rebollar et al. (2016), and Bueno et al. (2018). The correlations among protein quality indicators were numerous, with the strongest r coefficients observed between KOH and PDI and between KOH and TIA in the Experiment 1 and 2. Urease activity, however, was not correlated with KOH or TIA, consistent with data of García-Rebollar et al. (2016) and Ibáñez et al. (2020). Urease activity does not have negative

values and therefore, values close to zero indicate adequate processing or severe overcooking of the meals. Consequently, UA values are not adequate to evaluate overprocessing of the SBM. In the Experiment 1, the correlations between IVDP and TIA and KOH were significant. However, no correlations between IVDP and PDI or UA were detected. In contrast, in the Experiment 2, the IVDP tests were not correlated with protein quality indicator studied ($P > 0.05$).

When the *in vivo* determinations were correlated with chemical composition, protein quality indicators, an IVDP of the SBM (Experiment 2), numerous correlations were found. The AMEn content of the SBM determined *in vivo* was positively correlated with the SID of the protein and all indispensable AA, consistent with data of Perryman and Dozier (2012). Also, the relation between AMEn contents and RLys of the soybean meal was positive, confirming the importance of an optimal processing to improve the energy content and AA digestibility of the meals in SBM. In the current research, the SID of the protein fraction was positively correlated with all the protein quality indicators, except UA, in agreement with data of González-Vega et al. (2011), Frikha et al. (2012), and Siegert et al. (2023).

GENERAL CONCLUSIONS

6. GENERAL CONCLUSIONS

1. The chemical composition, protein quality, and nutritive value of the soybean meals vary widely depending on the country of origin of the soybeans.
2. Laboratory data on amino acid profile, sucrose and neutral detergent fiber contents, and *in vitro* digestibility of the protein fraction, favor the use of soybean meals from United States of America and South Africa vs. soybean meals from Argentina and Brazil in poultry diets. However, the lower crude protein content and the high variability on *in vitro* digestibility of the protein fraction values, reduce in part, the advantages of the South Africa meals over the other meals.
3. The *in vivo* energy content and the ileal digestibility of the crude protein and amino acids of the soybean meals, varied with the country of origin of the soybeans, with USA meals showing greater AMEn content, better ileal digestibility of the amino acids, and having more uniform composition and nutritional values, than the South American meals.
4. The *in vitro* tests on the protein digestibility can be useful to detected differences among quality and origin of the soybeans but the lower values found and the weak correlations between *in vivo* data (ileal digestibility of the protein and amino acids and energy content of the soybean meals) make necessary further studies to understand and enhance these techniques in poultry.
5. Data reported herein suggest that protein solubility in KOH might be better indicator for over-processing soybean meals than for the protein dispersibility index. The urease activity test is not adequate to evaluate over processing SBM.
6. The differences in the sucrose content among origin of the soybeans, are simple to detect and might be useful to evaluate the soybean quality and the energy contribution in feed formulation of the soybean meals.

7. Predicting potential variations according to the origin of the soybeans might improve the soybean meals matrix and allows the reduction of security margins in the feed formulation process. Consequently, update periodically the matrix of the soybean meal, based on laboratory data and origin of the soybeans, might be useful to optimize feed formulation cost.

REFERENCES

7. REFERENCES

- Abdollahi, M. R., M. Wiltafsky-Martin, and V. Ravindran. 2021. Application of apparent metabolizable energy versus nitrogen-corrected apparent metabolizable energy in poultry feed formulations: A continuing conundrum. *Animals* 11:2174.
- Abdollahi, M. R., M. Wiltafsky-Martin, F. Zaefarian, and V. Ravindran. 2022. Metabolizable energy and standardized ileal amino acid digestibility of full-fat soybeans for broilers are influenced by wet-heating, expansion temperature and autoclaving time. *Poult. Sci.* 101:102016.
- Adedokun, S. A., O. Adeola, C. M. Parsons, M. S. Lilburn, and T. J. Applegate. 2008. Standardized ileal amino acid digestibility of plant feedstuffs in broiler chickens and turkey poults using a nitrogen-free or casein diet. *Poult. Sci.* 87:2535-2548.
- Adeola, O., and K. E. Ileleji. 2009. Comparison of two diet types in the determination of metabolizable energy content of corn distiller dried grains with soluble for broilers chickens by regression method. *Poult. Sci.* 88:579-585.
- Aguirre, L., L. Cámara, A. Smith, J. J. Arroyo, A. F. de Juan, G. Fondevila, and G. G. Mateos. 2022. Chemical composition, protein quality indicators and in vitro protein digestibility of commercial soybean meals from four different origins. *Anim. Feed Sci. Technol.* 293:115473.
- Ahasic E. M., P. L. Utterback, and C. M. Parsons. 2023. Nutritional valued of soybean meals varying in particle size. *Poult. Sci.* 102:102708.
- Ali M., M. Joseph, M. C. Alfaro-Wisaquillo, G. A. Quintana-Ospina, D. Patiño, L. M. Peñuela-Sierra, T. Vu, R. Mian, E. Taliercio, O. Toomer, and E. O. Oviedo-Rondón.

2023. Standardized ileal amino acid digestibility of high-oleic full-fat soybean meal in broilers. *Poult. Sci.* 103:152.
- Alsajri, F. A., C. Wijewardana, J. T. Irby, N. Bellaloui, L. J. Krutz, B. Golden, W. Gao, and K. R. Reddy. 2020. Developing functional relationships between temperature and soybean yield and seed quality. *Agron. J.* 112:194-204.
- AMINODat 6.2. Evonik Operations GmbH. Nutrition and Care. Hanau-Wolfgang, Germany. <https://aminodat.evonik.com/aminodat6-client/#!/amino-acids>. (Accessed August 2023).
- Anderson-Haferman, J. C., Y. Zhang, C. M. Parsons, and T. Hymowitz. 1992. Effect of heating on the nutritional quality of Kunitz trypsin inhibitor-free and conventional soybeans for chicks. *Poult. Sci.* 71:1700-1709.
- AOAC International (Association of Official Analytical Chemists International). 2019. Official methods of analysis of the AOAC International, 21st ed. AOAC International, Gaithersburg, MD, USA.
- AOCS (American Oil Chemists Society). 2017. Official Methods and Recommended Practices of the AOCS, 7th ed. Am. Oil Chem. Soc., Champaign, IL, USA.
- Araba, M., and N. M. Dale. 1990. Evaluation of protein solubility as an indicator of overprocessing of soybean meal. *Poult. Sci.* 69:76-83.
- ASAE. 2003. Method of determining and expressing fineness of feed materials by sieving. ASAE standard S319.2. Agriculture Engineers Yearbook of Standards. ASAE, St. Joseph, MO, USA.
- Bach Knudsen, K. E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. *Anim. Food Sci. Technol.* 67:319-338.

- Balloun, S. L. 1980. Soybean Meal in Poultry Nutrition. Ovid Bell Press, Fulton, MO, USA.
- Batal, A. B., M. W. Douglas, A. E. Engram, C. M. Parson. 2000. Protein dispersibility index as in indicator of adequately processed soybean meal. Poult. Sci. 79:1592-1596.
- Batal, A. B., N. M. Dale, and U. K. Saha. 2010. Mineral composition of corn and soybean meal. J. Appl. Poult. Res. 19:361-364.
- Bedford, M. R., and H. L. Classen. 1993. An in vitro assay for prediction of broiler intestinal viscosity and growth when fed-rye-based diets in the presence of exogenous enzymes. Poult Sci. 72:137-143.
- Bedford, M. 1995. Mechanism of action and potential environmental benefits from the use of feed enzymes. Anim. Feed. Sci. Technol. 53:145-155.
- Bertechini, A. G., R. K. Kato, L. F. V. B. de Freitas, R. T. C. Da Castro, and H. Mazzuco. 2018. Metabolizable energy values of soybean meals and soybean oil for broilers at different ages. Acta Scien. Anim Sci. 41:44540.
- Boisen, S., and B. O. Eggum. 1991. Critical evaluation of in vitro methods for estimating digestibility in simple-stomached animals. Nutr. Res. Rev. 4:141-162.
- Boisen, S., and J. A. Fernández. 1995. Prediction of the apparent ileal digestibility of protein and amino acids in feedstuffs and feed mixtures for pigs by in vitro analyses. Anim. Feed Sci. Technol. 51:29-43.
- Boisen, S., and A. Fernández. 1997. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. Anim. Feed Sci. Technol. 68:277-286.

- BOE (Boletín Oficial del Estado). 2013. Real Decreto 53/2013 de 1 de febrero por el que se establecen las normas básicas aplicables para la protección de los animales utilizados en experimentación y otros fines científicos, incluyendo la docencia. BOE 34:11370-11421.
- Bolton, W. 1957. The digestibility by adult fowls of wheat fine middlings, maize germ meal, maize gluten feed, soya-bean meal and groundnut meal. *J. Sci. Food Agric.* 8:132-140.
- Brennan, R. F., M. D. A. Bolland, R. C. Jeffery, and D. G. Allen. 1994. Phosphorus adsorption by a range of Western Australian soils related to soil properties. *Commun. Soil Sci. Plant. Anal.* 25:2785-2795.
- Brickett, K. E., J. P. Dahiya, H. L. Classen, and S. Gomis. 2007. Influence of dietary nutrient density, feed form, and lighting on growth and meat yield of broiler chickens. *Poult. Sci.* 86:2172-2181.
- Bryan, D. D. S. L., D. A. Abbott, and H. L. Classen. 2018. Development of an in vitro protein digestibility assay mimicking the chicken digestive tract. *Anim. Nutr.* 4:401-409.
- Bueno, R. D., L. L. Borges, P. I. V. Good God, N. D. Piovesan, A. I. Teixeira, and C. D. Cruz. 2018. Quantification of anti-nutritional factors and their correlations with protein and oil in soybeans. *An. Acad. Bras. Cienc.* 90:205-217.
- Cámara, L., L. Aguirre, A. F. de Juan, J. Ben-Mabrouk, H. Kadardar, G. Talegón, and G. G. Mateos. 2022. Chemical composition and nutritional value of raw soybeans from Brazil and USA arriving to European ports. *Poult. Sci.* 101 (E-Suppl. 1):248. (Abstr.).

- Carré, B., L. Derquet, and B. Leclercq. 1990. The digestibility of cell-wall polysaccharides from wheat (bran or whole grain), soybean meal, and white lupin meal in cockerels, muscovy ducks, and rats. *Poult Sci.* 69:623-633.
- Carré, B., J. Gomez, and A. M. Chagneau. 1995. Contribution of oligosaccharide and polysaccharide digestion, and excreta losses of lactic acid and short fatty acids, to dietary metabolizable energy values in broilers chickens and adult cockerels. *Br. Poult. Sci.* 36:611-629.
- Chang, H. Y. 1960. Relationship of Soybean Oil Quality with Completeness of Extraction. PhD Thesis. Iowa State University. Ames, IA, USA.
- Chiang, G. 1983. A simple and rapid high-performance liquid chromatographic procedure for determination of furosine, a lysinereducing sugar derivative. *J. Agric. Food Chem.* 48:1373-1374.
- Choct, M., Y. Dersjant-Li, J. McLish, and M. Peisker. 2010. Soy oligosaccharides and soluble non-starch polysaccharides: a review of digestion, nutritive and anti-nutritive effects in pigs and poultry. *Asian-Aust. J. Anim. Sci.* 23:1386-1398.
- Clarke, E., and J. Wiseman. 2005. Effects of variability in trypsin inhibitor content of soya bean meals on true and apparent ileal digestibility of amino acids and pancreas size in broiler chicks. *Anim. Feed Sci. Technol.* 121:125-138.
- Clarke, E., and J. Wiseman. 2007. Effects of extrusion conditions on trypsin inhibitor activity of full fat soybeans and subsequent effects on their nutritional value for young broilers. *Br. Poult. Sci.* 48:703-712.

- Coon, C. N., K. L. Leske, O. Akavanichan, and T. K. Cheng. 1990. Effect of oligosaccharide-free soybean meal on true metabolizable energy and fiber digestion in adult roosters. *Poult. Sci.* 69:787-793.
- Coradi, P. C., C. H. P. Fernandes, J. C. Helmich, and A. L. D. Goneli. 2016. Effects of drying air temperature and grain initial moisture content on soybean quality (*Glycine Max* (L.) Merrill). *Eng. Agri.* 36:866-876.
- Coradi, P. C., M. B. de Oliveira, L. de Oliveira Carneiro, G. A. Coelho de Souza, M. C. Elias, A. Brackmann, and P. E. Teodoro. 2020. Technological and sustainable strategies for reducing losses and maintaining the quality of soybean grains in real production scale storage units. *J. Stored Prod. Res.* 87:101624.
- Corrales N. L., P. Vivanco, L. Aguirre, L. Cámara, and G. G. Mateos. 2023. Crop year and country of origin of the beans affect the chemical composition, amino acid profile, and protein quality of commercial soybean meals. *Poult. Sci.* 102 (E-suppl.1):275. (Abstr.).
- CVB (Centraal Veevoeder Bureau). 2021. Feed Table Chemical Composition and Nutritional Values of Feedstuffs. *Veevoedertabel* (Livestock Feed Table). Wageningen. The Netherlands.
- Dale, N., and H. L. Fuller. 1984. Correlation of protein content of feedstuffs with the magnitude of nitrogen correction in true metabolizable energy determinations. *Poult. Sci.* 63:1008-1012.
- de Coca-Sinova, A., D. G. Valencia, E. Jiménez-Moreno, R. Lázaro, and G. G. Mateos. 2008. Apparent ileal digestibility of energy, nitrogen, and amino acids of soybean meals of different origin in broilers. *Poult. Sci.* 87:2613-2623.

- Di Pietro, C. M., and I. E. Liener. 1989. Heat inactivation of the Kunitz and Bowman-Birk soybean protease inhibitors. *J. Agric. Food Chem.* 37:39-44.
- Dozier, W. A., M. T. Kidd, and A. Corzo. 2008. Dietary amino acid responses of broilers. *J. Appl. Poult. Res.* 17:157-167.
- Dozier, W. A., and R. L. Payne. 2012. Digestible Lys requirements of female broilers from 1 to 15 days of age. Dietary amino acid responses of broilers. *J. Appl. Poult. Res.* 21:348-357.
- Dunmire, K. M., M. B. Braun, C. E. Evans, B. D. Fallen, C. R. Stark, and C. B. Paulk. 2023. Determining the apparent ileal digestibility of amino acids for soybean meal from select soybean varieties with varying levels of crude protein. *J. Appl. Poult. Res.* 32:100333.
- Englyst, H. N., M. E. Quigley, and G. J. Hudson. 1994. Determination of dietary fibre as non-starch polysaccharides with gas-liquid chromatographic, high-performance chromatographic or spectrophotometric measurement of constituent sugars. *Analyst (Lond.)* 119:1497-1509.
- Erbersdobler, H. F., and V. Somoza. 2017. Forty years of furosine, forty years of using early Maillard products as indicators of the nutritional quality of foods. *Mol. Nutr. Food Res.* 51:423-430.
- European Commission, 2023. EU Oilseeds and protein crops trade 2022/23 marketing year. Euroestat-Comext. Accessed. September 2023. https://agriculture.ec.europa.eu/data-and-analysis/markets/overviews/market-observatories/crops/oilseeds-and-protein-crops_en.

- FAO (Food and Agriculture Organization of the United Nations) Statistical Data Base, 2023. Food and Agriculture Data. <http://www.fao.org/faostat/en/#data>. (Accessed September 2023).
- Farrell, D. J., E. Thomson, J. J. Du Preez, and J. P. Hayes. 1991. The estimation of endogenous excreta and the measurement of metabolisable energy in poultry feedstuffs using four feeding systems, four assay methods and four diets. *Br. Poult. Sci.* 32:483-499.
- FEDNA (Fundación Española Desarrollo Nutrición Animal). 2018. Necesidades Nutricionales Para Avicultura. G. Santomá and G. G. Mateos, eds. 2nd ed. Fund. Esp. Desarro. Nutr. Anim., Madrid, Spain.
- FEDNA (Fundación Española Desarrollo Nutrición Animal). 2021. FEDNA Tables on the Composition and Nutritional Values of Raw Materials for the Production of Compound Animal Feeds. de Blas, C., P. G. Rebollar, M. Gorrachategui, and G. G. Mateos, eds. 4th ed. Fund. Esp. Desarro. Nutr. Anim., Madrid, Spain.
- Fondevila, G., L. Aguirre, L. Cámara, A. F. de Juan, and G. G. Mateos. 2021. Evaluation of the energy content of soybean meals for poultry on their chemical composition. a review of currently available prediction equations. *FEDNA*. 38:59-86
- Fontaine, J., U. Zimmer, P. J. Moughan, and S. M. Rutherfurd. 2007. Effect of heat damage in an autoclave on the reactive lysine contents of soy products and corn distillers dried grains with solubles. Use of the results to check on lysine damage in common qualities of these ingredients. *J. Agric. Food Chem.* 55:10737-10743.
- Frikha, M., M. P. Serrano, D. G. Valencia, P. G. Rebollar, J. Fickler, and G. G. Mateos. 2012. Correlation between ileal digestibility of amino acids and chemical

- composition of soybean meals in broilers at 21 days of age. *Anim. Feed Sci. Technol.* 178:103-114.
- Galkanda-Arachchige, H. S. C., H. H. Stein, and D. A. Davis. 2021. Soybean meal sourced from Argentina, Brazil, China, India and USA as an ingredient in practical diets for Pacific white shrimp *Litopenaeus vannamei*. *Aquac. Nutr.* 27:1103-1113.
- García-Rebollar, P., L. Cámara, R. P. Lázaro, C. Dapoza, R. Pérez-Maldonado, and G. G. Mateos. 2016. Influence of the origin of the beans on the chemical composition and nutritive value of commercial soybean meals. *Anim. Feed Sci. Technol.* 221:245-261.
- Gibson, L. R., and R. E. Mullen. 2001. Mineral concentrations in soybean seed produced under high day and night temperature. *Can. J. Plant Sci.* 81:595-600.
- Goebel, K. P., and H. H. Stein. 2011. Ileal digestibility of amino acids in conventional and low-Kunitz soybean products fed to weanling pigs. *Asian-Aust. J. Anim. Sci.* 24:88-95.
- González-Vega, J. C., B. G. Kim, J. K. Htoo, A. Lemme, and H. H. Stein. 2011. Amino acid digestibility in heated soybean meal fed to growing pigs. *J. Anim. Sci.* 89:3617-3625.
- Grieshop, C. M., C. T. Kadzere, G. M. Clapper, E. A. Flickinger, L. L. Bauer, R. L. Frazier, and G. C. Fahey. 2003. Chemical and nutritional characteristics of United States soybeans and soybean meals. *J. Agric. Food Chem.* 51:7684-7691.
- Grobas, S., J. Mendez, C. De Blas, and G. G. Mateos. 1999. Influence of dietary energy, supplemental fat, and linoleic acid concentration on performance of laying hens at two ages. *Br. Poult. Sci.* 40:681-687.

- Hamerstrand, G. E., L. T. Black, and J. D. Glover. 1981. Trypsin inhibitors in soy products: modification of the standard analytical procedure. *Cereal Chem.* 58:42-45.
- Hammond, E. G., L. A. Johnson, C. Su, T. Wang, and P. J. White. 2005. Soybean Oil. In: *Bailey's Industrial Oil and Fat Products*, 6th edition, ed. F. Shahidi, J. Wiley and Sons, Inc. p. 577-653.
- Hartwig, E. E., T. M. Kuo, and M. M. Kenty. 1997. Seed protein and its relationship to soluble sugars in soybeans. *Crop Sci.* 37:770-773.
- Heger, J., M. Wiltafsky, and J. Zelenka. 2016. Impact of different processing of full-fat soybeans on broiler performance. *Czech J. Anim. Sci.* 61:57-66.
- Hemetsberger, F., T. Hauser, K. J. Domig, W. Kneifel, and K. Schedle. 2021. Interaction of soybean varieties and heat treatments and its effect on growth performance and nutrient digestibility in broiler chickens. *Animals.* 11:2668.
- Hermida, M., M. González, M. Miranda, and J. L. Rodríguez-Otero. 2006. Mineral analysis in rabbit meat from Galicia (NW Spain). *Meat Sci.* 73:635-639.
- Hill, F. W., and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. *J. Nutr.* 64:587-603.
- Hill, L., K. Bender, G. Bode, K. Beachy, and J. Dueringer. 1996. Quality choices in international soybean markets. *Agribusiness* 12:231-246.
- Hoffmann, D., S. Thurner, D. Ankerst, K. Damme, W. Windisch, and D. Brugger. 2019. Chickens' growth performance and pancreas development exposed to soy cake varying in trypsin inhibitor activity, heat-degraded lysine concentration, and protein solubility in potassium hydroxide. *Poult. Sci.* 98:2489-2499.

- Hoover, D. G. 1993. Bifidobacteria: activity and potential benefits. *Food Technol.* 47:120-124.
- Huerta, A. I., and M. A. Martin. 2002. Soybean production costs: an analysis of the United States, Brazil, and Argentina. In: *Annual Meeting of the Agricultural and Applied Economics Association*. Long Beach, CA, USA. 19621.
- Hurrell, R. F., and K. J. Carpenter. 1981. The estimation of available lysine in foodstuffs after Maillard reactions. *Prog. Food Nutr. Sci.* 5:159-176.
- Ibáñez, M. A., C. de Blas, L. Cámara, and G. G. Mateos. 2020. Chemical composition, protein quality and nutritive value of commercial soybean meals produced from beans from different countries: A meta-analytical study. *Anim. Feed Sci. Technol.* 267:114531.
- INRAE (Institut National de la Recherche Agronomique). 2021. Composition and Nutritive Values of Feeds for Cattle, Sheep, Goats, Pigs, Poultry, Rabbits, Horses and Salmonids. (Accessed September 2023). <https://www.feedtables.com/>.
- Irish, G. G., and D. Balnave. 1993. Non-starch polysaccharides and broiler performance on diets containing soyabean meal as the sole protein concentrate. *Aust. J. Agric. Res.* 44:1483-1499.
- ISO (International Organization for Standardization). 2012. Animal feeding stuffs: determination of trypsin activity of soya products. In: *ISO International Standard 1402:2001*. Geneva, Switzerland.
- Jauregui, L. M., F. L. Rodriguez, L. Zhang, P. Chen, K. Brye, D. Oosterhuis, A. Mauromoustakos, and J. R. Clark. 2013. Planting date and delayed harvest effects on soybean seed composition. *Crop Sci.* 53:2162-2175.

- Jensen, T. L. 2010. Soil pH and the availability of plant nutrients. International Plant Nutrition Institute, Plant Nutrition Today, Norcross, GA, USA.
- Karr-Lilienthal, L. K., C. M. Grieshop, N. R. Merchen, D. C. Mahan, and G. C. Jr. Fahey. 2004. Chemical composition and protein quality comparisons of soybeans and soybean meals from five leading soybean-producing countries. *J. Agric. Food Chem.* 52:6193-6199.
- Karr-Lilienthal, L. K., C. M. Grieshop, G. C. Spears, and G. C. Jr. Fahey. 2005. Amino acid, carbohydrate, and fat composition of soybean meals prepared at 55 commercial U.S. soybean processing plants. *J. Agric. Food Chem.* 53:2146-2150.
- Khalil, M. M., M. R. Abdollahi, F. Zaefarian, and V. Ravindran. 2021. Influence of feed form on the apparent metabolisable energy of feed ingredients for broiler chickens. *Anim. Feed Sci. Technol.* 271:114754.
- Kuenz, S., S. Thurner, D. Hoffmann, K. Kraft, M. Wiltafsky-Martin, K. Damme, W. Windisch, and D. Brugger. 2022. Effects of gradual differences in trypsin inhibitor activity on the estimation of digestible amino acids in soybean expellers for broiler chickens. *Poult. Sci.* 101:101740.
- Kumar, V., A. Rani, L. Goyal, A. K. Dixit, J. G. Manjaya, J. Dev, and M. Swamy. 2010. Sucrose and raffinose family oligosaccharide (RFOs) in soybean seeds as influenced by genotype and growing location. *J. Agric. Food Chem.* 58:5081-5085.
- Lagos, L. V., and H. H. Stein. 2017. Chemical composition and amino acid digestibility of soybean meal produced in the United States, China, Argentina, Brazil, or India. *J. Anim. Sci.* 95:1626-1636.

- Leske, K., C. J. Jeune, and C. N. Coon. 1993. Extraction methods for removing soybean alpha-galactosides and improving true metabolizable energy for poultry. *Anim. Feed Sci. Technol.* 41:73-78.
- Leske, K. L., and C. N. Coon. 1999. Nutrient content and protein and energy digestibilities of ethanol-extracted, low alpha-galactoside soybean meal as compared to intact soybean meal. *Poult. Sci.* 78:1177-1183.
- Leung, H., and E. G. Kiarie. 2020. Standardized ileal digestibility of amino acids and apparent metabolizable energy in corn and soybean meal for organic broiler chicken production in Ontario. *Can. J. Anim. Sci.* 100:447-454.
- Liener, I. E. 1981. Factors affecting the nutritional quality of soya products. *J. Am. Oil Chem. Soc.* 58:406-415.
- Lopez, G., and S. Leeson. 2008. Assessment of the nitrogen correction factor in evaluating metabolizable energy of corn and soybean meal in diets for broilers. *Poult. Sci.* 87:298-306.
- Losada B., P. Garcia-Rebollar, P. Cachaldora, C. Alvarez, J. Méndez, and J. C de Blas. 2009. comparison of the prediction of apparent metabolisable energy content of starchy grains and cereal by-products for poultry from its chemical components, in vitro analysis or near-infrared reflectance spectroscopy. *Spanish J. Agric. Res.* 7:813-823.
- Losada B., P. García-Rebollar, C. Alvarez, P. Cachaldora, M A. Ibanez, J. Mendez, and J. C. de Blas. 2010. The prediction of apparent metabolisable energy content of oil seeds and oil seed by-products for poultry from its chemical components, in vitro analysis or nearinfrared reflectance spectroscopy. *Anim. Feed Sci. Technol.* 160:62-72.

- Mariano, F. C. M. Q., R. R. Lima, P. B. Rodrigues, R. R. Alvarenga y G. A. J. Nascimento. 2012. Prediction equations of energy values of feedstuff using meta-analysis and principal components. *Ciência Rural* 42:1634-1640.
- Mateos, G. G., L. Cámara, G. Fondevila, and R. P. Lázaro. 2019. Critical review of the procedures used for estimation of the energy content of diets and ingredients in poultry. *J. Appl. Poult. Res.* 28:506-525.
- Medic, J., C. Atkinson, and C. R. Hurburgh. 2014. Current knowledge in soybean composition. *J. Am. Oil Chem. Soc.* 91:363-384.
- Moughan, P. J., P. J. Buttery, C. P. Essex, and J. B. Soar. 1992. Evaluation of the isotope dilution technique for determining ileal endogenous nitrogen excretion in the rat. *J. Sci. Food Agric.* 58:165-172.
- Naeve, S. L. and C. S. Huerd. 2008. Year, region, and temperature effects on the quality of Minnesota's soybean crop. *Agron. J.* 100:690-695.
- NARO (National Agriculture and Food Research Organization). 2009. Standard Tables of Feed Composition in Japan Livestock Industry Association. NARO, Tokyo, Japan.
- NRC (National Research Council). 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, USA.
- NRC (National Research Council). 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC, USA.
- Obendorf, R. L., A. D. Zimmerman, Q. Zhang, A. Castillo, S. M. Kosina, E. G. Bryant, E. M. Sensenig, J. Wu, and S. R. Schnebly. 2009. Accumulation of soluble carbohydrates during seed development and maturation of low-raffinose, low-stachyose soybean. *Crop Sci.* 49:329-341.

- Pacheco, W., C. R. Stark, P. R. Ferket, and J. Brake. 2014. Effects of trypsin inhibitor and particle size of expeller-extracted soybean meal on broiler live performance and weight of gizzard and pancreas. *Poult. Sci.* 93:2245-2252.
- Pahm, A. A., C. Pedersen, and H. H. Stein. 2008. Application of the reactive lysine procedure to estimate lysine digestibility in distillers dried grains with solubles fed to growing pigs. *J. Agric. Food Chem.* 56:9441-9446.
- Papadopoulos, M. C. 1989. Effect of processing on high-protein feedstuffs: A review. *Biol. Wastes* 29:123-138.
- Parsons, C. M. 2000. Assessment of nutritional quality of soy products for animals. In: J. K. Drackley, ed. *Soy in Animal Nutrition*. Federation of Animal Science Societies, Savoy, IL, USA. p. 90-105.
- Parsons, C. M., Y. Zhang, and M. Araba. 2000. Nutritional evaluation of soybean meals varying in oligosaccharide content. *Poult. Sci.* 79:1127-1131.
- Paulsen, M. R., W. R. Nave, and L. E. Gray. 1981. Soybean seed quality as affected by impact damage. *ASABE* 24:1577-1582.
- Peer, D. J., and S. Leeson. 1985. Nutrient and trypsin inhibitor content of hydroponically sprouted soya beans. *Anim. Feed Sci. Technol.* 13:203-214.
- Perilla, N. S., M. P. Cruz, F. de Belalcazar, and G. J. Diaz. 1997. Effect of temperature of wet extrusion on the nutritional value of full-fat soyabeans for broiler chickens. *Br. Poult. Sci.* 38:412-416.
- Perryman, K. R., and W. A. Dozier III. 2012. Apparent metabolizable energy and apparent ileal amino acid digestibility of low and ultra-low oligosaccharide soybean meals fed to broiler chickens. *Poult. Sci.* 91:2556-2563.

- Premier Atlas. 2019. Ingredients Matrix. Premier Nutrition Products Ltd, AB Agri Ltd, Rugeley Staffs, UK.
- Ravindran, V., M. R. Abdollahi, and S. M. Bootwalla. 2014. Nutrient analysis, metabolizable energy, and digestible amino acids of soybean meals of different origins for broilers. *Poult. Sci.* 93:2567-2577.
- Rostagno, H. S., L. F. T. Albino, J. L., M. I. Hannas, J. L. Donzele, N. K. Sakomura, F. G. Perazzo, A. Saraiva, M. L. Teixeira, P. B. Rodrigues, R. F. Oliveira, S. L. Barreto, and C. de Oliveira Brito. 2017. Brazilian Tables for Poultry and Swine. Feedstuff Composition and Nutritional Requirements. 4th ed. Universidade Federal de Viçosa. Viçosa, Brazil.
- RPRI (Russian Poultry Research Institute). 2014. Manual for Optimization of Formulation of Compound Feeds for Poultry. Russian Poultry Research Institute. Sergiev Posade, Russian Federation.
- Saldivar, X., Y. Wang, P. Chen, and A. Hou. 2011. Changes in chemical composition during soybean seed development. *Food Chem.* 124:1369-1375.
- SAS Institute. 2008. SAS/STAT® User's guide, version 9.3. SAS Institute Inc., Cary, NC, USA.
- Serrano, M. P., P. G. Rebollar, S. Sueiro, M. Hermida, and G. G. Mateos. 2013. Influence of duration of storage on protein quality traits of soybean meals. *J. Appl. Poult. Res.* 22:423-429.
- Sheikhhasan, B. S., H. Moravej, M. Shivazad, F. Ghaziani, E. Esteve-Garcia, and W. K. Kim. 2020. Prediction of the total and standardized ileal digestible amino acid

contents from the chemical composition of soybean meals of different origin in broilers. *Poult. Sci.* 99:4947-4957.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Anim. Feed Sci. Technol.* 59:215-221.

Siebert, W., S. Kuenz, W. Windisch, and M. Rodehutschord. 2023. Amino acid digestibility and metabolizable energy of soybean meal of different origins in cecectomized laying hens. *Poult. Sci.* 102:102580.

Stefanello, C., S. L. Vieira, H. V. Rios, C. T. Simoes, and J. O. B. Sorbara. 2016. Energy and nutrient utilisation of broilers fed soybean meal from two different Brazilian production areas with and exogenous protease. *Anim. Feed Sci. Technol.* 221:267-273.

Svejstl, R., S. Musilová, and V. Rada. 2015. Raffinose-series oligosaccharides in soybean products. *Scientia Agriculturae Bohemica* 46:73-77.

Sung, J. Y., A. S. Aderibigbe, and O. Adeola. 2023. Amino acid digestibility and net energy concentration in soybean meal for broiler chickens. *Anim. Feed Sci. Technol.* 297:115572.

Talegón, G., H. Kadardar, L. Cámara, L. Aguirre, and G. G. Mateos. 2023. Chemical composition and nutritional value of raw soybeans from Brazil and USA arriving to European ports. *Poult. Sci.* 102 (E-Suppl. 1):279. (Abstr.).

Tangendjaja, B. 2020. Nutrient content of soybean meal from different origins based on near infrared reflectance spectroscopy. *Indones. J. Agric. Sci.* 21:39-47.

- Teague, K. D., G. Tellez-Isaias, J. Chai, V. Petrone-Garcia, C. N. Vuong, A. Blanch, S. H. Rasmussen, K. Brown, Jiangchao Zhao, and S. J. Rochell. 2023. Dietary soy galactooligosaccharides affect the performance, intestinal function, and gut microbiota composition of growing chicks. *Poult. Sci.* 102:102440.
- Thakur, M., and C. Hurburgh. 2007. Quality of US soybean meal compared to the quality of soybean meal from other origins. *J. Am. Oil Chem. Soc.* 84:835-843.
- van Eys, J. E., and N. Ruiz. 2021. *Quality Manual and Analysis for Soybean Products in the*. 3er ed. USSEC, Chesterfield, MO, USA.
- van Soest, P. J., J. B. Robertson, and A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583-3597.
- Veluri, S., and O. A. Olukosi. 2020. Metabolizable energy of soybean meal and canola meals as influence by the reference diet used and assay method. *Animals* 10:2132.
- Wijewardanaa, C., K. R. Reddya, and N. Bellaloui. 2019. Soybean seed physiology, quality, and chemical composition under soil moisture stress. *Food Chem.* 278:92-100.
- Waldroup, P.W., B. E. Ransey, H. M. Hellwig, and N. K. Smith. 1985. Optimum processing for soybean meal used in broiler diets. *Poult. Sci.* 64:2314-2320.
- West, S. P. 2018. *Optimizing Methods to Determine Metabolizable Energy Values of Feed Ingredients for Broilers*. PhD Thesis. University of Arkansas, Fayetteville, AR, USA.
- Westgate, M. E., E. Piper, W. D. Batchelort, and C. Hurburgh. 2000. Effects of cultural environmental conditions during soybean growth on nutritive value of soy products.

- Pages 75-89 in Soy Animal Nutrition. Federation of Animal Science Society. J. K. Drackley, ed. Savoy, IL, USA.
- Wilcox, J. R., and R. M. Shibbles. 2001. Interrelationships among seed quality attributes in soybean. *Crop Sci.* 41:11-14.
- Whittle, E., and M. Araba. 1992. Sources of variability in the protein solubility assay for soybean meal. *J. Appl. Poult. Res.* 1:221-225.
- Wolf, R., J. Cavins, R. Kleiman, and L. Black. 1982. Effect of temperature on soybean seed constituent oil, protein, moisture, fatty acids, amino acids and sugars. *J. Am. Oil Chem. Soc.* 59:230-232.
- Woyengo T. A., R. Jha, E. Beltranena, and R. T. Zijlstra. 2015. In vitro digestion and fermentation characteristics of canola co-products simulate their digestion in the pig intestine. *Animal* 10:911-918.
- WPSA (World's Poultry Science Association). 1989. European Table of Energy Values for Poultry Feedstuffs. 3rd ed. Subcommittee Energy of the Working Group no 2. Nutrition of the European Federation of Branches of the World's Poultry Science Association, Spelderholt Centre for Poultry Research, Beekbergen, The Netherlands.
- Yazdi-Samadi, B., R. W. Rinne, and R. D. Seif. 1977. Components of developing soybean seeds: oil, protein, sugars, starch, organic acids, and amino acids. *Agron. J.* 69:481-486.
- Zaefarian, F., A. J. Cowieson, K. Pontoppidan, M. R. Abdollahi, and V. Ravindran. 2021. Trends in feed evaluation for poultry with emphasis on in vitro techniques. *Anim. Nutr.* 7:268-281.