Ingredient Classification According to the Digestible Amino Acid Profile: An Exploratory Analysis

This study aimed: 1) to classify ingredients according to the digestible amino acid (AA) profile; 2) to determine ingredients with AA profile closer to the ideal for broiler chickens; and 3) to compare digestible AA profiles from simulated diets with the ideal protein profile. The digestible AA levels of 30 ingredients were compiled from the literature and presented as percentages of lysine according to the ideal protein concept. Cluster and principal component analyses (exploratory analyses) were used to compose and describe groups of ingredients according to AA profiles. Four ingredient groups were identified by cluster analysis, and the classification of the ingredients within each of these groups was obtained from a principal component analysis, showing 11 classes of ingredients with similar digestible AA profiles. The ingredients with AA profiles closer to the ideal protein were meat and bone meal 45, fish meal 60 and wheat germ meal, all of them constituting Class 1; the ingredients from the other classes gradually diverged from the ideal protein. Soybean meal, which is the main protein source for poultry, showed good AA balance since it was included in Class 3. On the contrary, corn, which is the main energy source in poultry diets, was classified in Class 8. Dietary AA profiles were improved when corn and/or soybean meal were partially or totally replaced in the simulations by ingredients with better AA balance.

INTRODUCTION

Protein and amino acids (AA) are important determinants of diet production costs, and also affect animal production (Bregendahl et al., 2002) and carcass characteristics of broiler chickens (Kerr & Kidd, 1999). The ideal protein concept has emerged from this context, and means the use of essential AA to supply the exact requirements of the animals in terms of maintenance and protein accretion (neither exceeding nor lacking amounts) (Emmert & Baker, 1997). For practical purposes, the concept of ideal protein refers to the establishment of digestible essential AA requirements as percentages of digestible lysine (Baker & Han, 1994; Baker et al., 2002). However, diets formulated with ingredients commonly used for birds have an excess of non-limiting essential AA. Thus, the classification of ingredients according to the digestible AA patterns may indicate which ingredients would compose diets with the best AA balance.

For this proposal, exploratory statistical analyses might be useful. The multivariate techniques of cluster and principal component analyses allow the evaluation of sample similarities according to determined variables (Moita Neto & Moita, 1998). For instance, the ingredients (samples) can be studied considering all the essential AA together (variables).
variables so that homogeneity within groups and heterogeneity among groups are obtained (Sneath & Sokal, 1973). The principal component analysis linearly transforms the original variables, and thus compose a new set of independent variables (components) (Jackson, 1991). The principal components that retain a significant amount of information about the original data set can be used to identify the important variables that explain sample grouping. Hence, clustering analysis can be complemented by the principal component analysis to explain the group structures.

In this context, this present study aimed:

- To classify ingredients according to the digestible AA profile.
- To determine ingredients with AA profile close to the ideal for broiler chicken.
- To compare the digestible AA profiles of diet simulations with the ideal protein profile.

MATERIAL AND METHODS

Data collection

Data on crude protein (CP), nitrogen-corrected apparent metabolizable energy (ME), and digestible AA (as-fed basis) of 30 ingredients suitable for poultry diets were obtained from Rostagno et al. (2000) and Lesson et al. (2000). The levels of digestible methionine +cystine, tryptophan, threonine, arginine, isoleucine, valine, leucine, histidine and phenylalanine of the ingredients were expressed as percentages of digestible lysine (Table 1). In this data matrix, a vector representing the ideal AA profile for broiler chickens at the initial phase (from 1 to 21 days) was inserted, presenting the following composition (percentage in relation to lysine): 72% methionine+cystine, 105% arginine, 109% leucine, 32% histidine and 65% phenylalanine according to Baker & Han (1994); and 16.6% tryptophan, 55.7% threonine, 61.4% isoleucine and 77.5% valine as suggested by Baker et al. (2002). Lysine was not added because it is the reference AA (100%).

Cluster Analysis

For cluster analyses, the matrix of the digestible AA of the ingredients listed in Table 1 was used in addition to the vector of the ideal protein profile. The clustering process involves three steps: data standardization; assessment of a dissimilarity measure among samples; and the use of a grouping technique. In the present study, data standardization was not necessary because the AA were expressed in a same unit (% lysine). The Euclidean distance was used as dissimilarity distance (Sneath & Sokal, 1973). Ward’s method was used as the grouping technique (Ward, 1963).

Principal Component Analysis

Principal component analyses were used within the groups determined previously. Both matrix data related to the composition of digestible AA (% lysine) of the ingredients within each group and the ideal protein profile vector were used. The principal components were obtained by computing the eigenvalues and eigenvectors of data correlation matrix (Jackson, 1991). A bi-dimensional representation of this m-multidimensional set was made for the principal components that accumulated a significant percentage of original information. The principal components with significant contribution were those with eigenvalues greater than 1 (StatSoft, 2001). The correlation between each AA and the principal component was calculated. This procedure allowed the identification of the role of each AA in the classification. Only correlations with module equal or greater than 0.6 were discussed.

Simulations

Eight starter diets for broiler chickens were formulated based on the results of cluster and principal component analyses. The diets had the following nutritional levels: 3000 kcal/kg ME; 0.96% calcium; 0.45% available phosphorus; 0.22% sodium; 21.5% crude protein; 1.14% digestible lysine (Rostagno et al., 2000). The digestible AA levels were at least as suggested by Baker & Han (1994) and Baker et al. (2002). For presentation purposes the diets are shown in the Results after definition and characterization of the ingredient groups. The matrix of dietary digestible AA profile (% lysine) and the vector of the ideal protein profile were used for cluster and principal component analyses, as described above. Because methionine+cystine levels were similar among diets (72% of lysine), these AA were not used in the analysis.

Statistics

Cluster and principal component analyses were run in the software Statistica 6.0® (StatSoft, 2001).

RESULTS

Cluster Analysis

The results of cluster analyses are represented in a dendogram constructed from the matrix of ingredient
dissimilarities. Considering in the dendrogram a break point at the linkage distance around 600 (Figure 1), four ingredient groups with similar digestible AA profiles were identified. The AA profile of ingredients in group A was closer to ideal protein. In groups B, C and D, the AA balance of the ingredients gradually decreased.

**Principal component analysis**

The interpretations of the results obtained by principal component analysis were based on some presuppositions. Firstly, the principal component 1 was always represented on the abscissa, whereas comparisons to components 2 and 3 were made horizontally, whereas comparisons to components 2 and 3 were made only vertically. Another statement concerned the plus or minus signs of the coefficients of correlation, i.e., direct or inverse relationship between variables, respectively (Bussad & Morettin, 1987). Therefore, a positive correlation between an AA and component 1 means that all the ingredients plotted in the graph areas with positive values on the abscissa (horizontal comparison) would present higher amounts of that AA, while the ingredients in the negative graph areas would have lesser amounts of this AA. Similarly, a negative correlation between the AA and the principal component 1 means that the ingredients situated in the negative areas (horizontal comparison) present larger amounts of this AA, while those in the positive areas have low AA contents. It is important to stress that the principal component 1 always contained the largest set of information about the original values of digestible AA, and thus this component accounts for the most relevant results.

**Group A**

The principal component analysis within group A showed that the eigenvalues of the three first components were greater than 1 and accounted for 87.7% of the variability of the original values of digestible AA (Table 2). In this group, methionine +cystine was correlated positively with the principal

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### Table 1 – Crude protein (CP, %), nitrogen-corrected apparent metabolizable energy (ME; kcal/kg), digestible lysine (%) and AA (as % of lysine) for 30 ingredients (as-fed basis).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>CP</th>
<th>ME</th>
<th>Lys</th>
<th>Met+Cys</th>
<th>Trp</th>
<th>Thr</th>
<th>Arg</th>
<th>Ile</th>
<th>Val</th>
<th>Leu</th>
<th>His</th>
<th>Phe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cereals and subproducts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broken Rice*</td>
<td>7.3</td>
<td>2679</td>
<td>0.17</td>
<td>88.2</td>
<td>41.2</td>
<td>152.9</td>
<td>294.1</td>
<td>152.9</td>
<td>241.2</td>
<td>329.4</td>
<td>100.0</td>
<td>164.7</td>
</tr>
<tr>
<td>oat*</td>
<td>12.0</td>
<td>2756</td>
<td>0.37</td>
<td>97.3</td>
<td>48.6</td>
<td>91.9</td>
<td>173</td>
<td>121.6</td>
<td>135.1</td>
<td>218.9</td>
<td>48.6</td>
<td>148.6</td>
</tr>
<tr>
<td>Barley*</td>
<td>11.5</td>
<td>2795</td>
<td>0.31</td>
<td>103.2</td>
<td>51.6</td>
<td>96.8</td>
<td>132.3</td>
<td>132.3</td>
<td>154.8</td>
<td>235.5</td>
<td>83.9</td>
<td>171.0</td>
</tr>
<tr>
<td>Wheat meal</td>
<td>16.5</td>
<td>1888</td>
<td>0.48</td>
<td>81.3</td>
<td>33.3</td>
<td>75.0</td>
<td>212.5</td>
<td>89.6</td>
<td>108.3</td>
<td>152.1</td>
<td>70.8</td>
<td>106.3</td>
</tr>
<tr>
<td>Pearl millet</td>
<td>12.1</td>
<td>2910</td>
<td>0.32</td>
<td>128.1</td>
<td>44.4</td>
<td>125.0</td>
<td>143.8</td>
<td>140.6</td>
<td>181.3</td>
<td>356.3</td>
<td>78.1</td>
<td>175.0</td>
</tr>
<tr>
<td>Corn</td>
<td>8.6</td>
<td>3371</td>
<td>0.20</td>
<td>160.1</td>
<td>25.6</td>
<td>134.0</td>
<td>177.8</td>
<td>124.6</td>
<td>170.9</td>
<td>476.8</td>
<td>119.2</td>
<td>181.3</td>
</tr>
</tbody>
</table>

1 - Ingredients marked with an asterisk* were obtained from Lesson et al. (2000); the other ingredients were obtained from Rostagno et al. (2000).
component 1, but tryptophan, valine, leucine, histidine and phenylalanine were correlated negatively with this component (Table 2). Figure 2a shows that the principal component 1 (horizontal comparison) distinguished 3 classes of ingredients (1, 2 and 3). Class 1 contained ingredients with AA profile close to the ideal: meat and bone meal 45, fish meal 60 and wheat germ meal. Class 2 was composed of full fat canola, canola meal, poultry by-product meal and soybean derivatives. Blood meal was categorized into Class 3. The cluster analysis dendogram (Figure 1) shows the tendency toward formation of sub-groups within Group A. Class 2 and especially class 3 were characterized by containing ingredients with greater quantities of AA that were correlated negatively with the principal component 1. Blood meal presented the lowest concentration of methionine+cystine.

Threonine, arginine and isoleucine were correlated negatively with the principal component 2 (Table 2). Figure 2a shows an excess of these AA in the ingredients from Class 2 as compared with Class 1 (vertical comparison). Blood meal (Class 3) was deficient in these AA compared to both Class 1 and 2.
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Figure 2b shows the classification obtained from the correlation between the AA and the principal components 1 and 3. Methionine+cystine were negatively correlated with the principal component 3, and the values of these AA in full-fat canola, canola meal and poultry by-product meal were greater than those of soybean derivatives from Class 2 (Figure 2a). Because methionine+cystine are limiting elements in most poultry diets, the ingredients with greater values of these AA composed Class 2 (Figure 2b). Therefore, soybean derives were included into Class 3 and blood meal into Class 4.

**Group B**

For Group B, the two first principal components showed eigenvalues greater than 1 and accounted for 73.4% of the variability of the original data on digestible AA (Table 2). All the essential AA were correlated negatively with the principal component 1. However, a positive correlation was found between arginine and the principal component 2 (Table 2). Figure 3 shows that, considering the principal component 1 (horizontal comparison), wheat meal, linseed and sunflower meal were the closest to the ideal protein profile, which agrees with the cluster analysis for Group B (Figure 1). These ingredients constituted Class 5, and the others composed Class 6 (Figure 3). The principal component 2 (vertical comparison) was also important to differentiate the ingredients in terms of digestible arginine, and those ingredients positioned in the upper graph area presented the greater arginine contents while arginine levels decreased in ingredients located in the lower graph area. Thus, Class 5 had arginine in excess compared to the ideal protein pattern.

**Group C**

The three first principal components showed eigenvalues greater than 1 and accounted for 87.7% from the original information on digestible AA values (Table 2). Figure 4a shows three classes (7, 8 and 9) within Group C. Class 7 was composed of cotton seed meal, peanut meal, feather and by-products meal and broken rice. Pearl millet, wheat, corn gluten meal 22 and corn constituted Class 8. Feather meal was isolated in Class 9. This sub-structure was similar to the dendogram obtained from cluster analysis (Figure 1).
Considering the principal component 1 (horizontal comparison), Class 7 ingredients were closest to the ideal protein, while Class 8 and 9 ingredients gradually diverged from that. The AA that most influenced distribution of ingredients in relation to the principal component 1 (methionine+cystine, threonine, isoleucine, valine, leucine and phenylalanine; Table 2) were those correlated negatively with this component. Principal component 2 (vertical comparison) allowed ingredient differentiation in relation to methionine+cystine and histidine (negative correlation) and to arginine (positive correlation). Thus, ingredients located in the lower graph area tended to present higher values of methionine+cystine and histidine and lower levels of arginine. An inverse response was found for ingredients in the upper graph area (Figure 4a).

Principal component 3 differentiated the ingredients mainly as a function of the positive correlation with tryptophan. Ingredients in the upper graph area tended to show higher levels of this AA (Figure 4b).

**Group D**

The two first principal components showed eigenvalues higher than 1 and accounted for 99% of the original information on digestible AA content (Table 2). Considering the principal component 1 (horizontal comparison), low-tannin sorghum was closer to the ideal protein and thus presented a better AA profile compared to corn gluten meal 60. However, these two ingredients were far from the ideal protein. Even so, sorghum was classified in Class 10 and corn gluten meal 60 in Class 11. All the AA studied influenced the distribution of the ingredients in relation to the principal component 1 (negative correlation). Principal component 2 was important to distinguish the ingredients in relation to tryptophan and arginine levels, with sorghum showing stronger relation with these AA (upper graph area in Figure 5).

**Simulations**

Eight diets were formulated based on the ingredient classification obtained from cluster and principal component analyses. The first was a conventional corn-soybean diet (Corn-Soy). In two diets, corn was replaced by either high-lysine corn (Corn-Lys) or wheat middling, both of them with better AA profiles. Since sorghum was the cereal with the worst AA profile, a diet based on this ingredient was formulated to investigate its impact on diet AA profile. Afterwards,
two diets were formulated, one including meat and bone meal 45 (MBM45) and the other with fish meal 60 (FM60). Two other diets containing the ingredients with profiles closer to that of the ideal protein (Class 1) were formulated. These diets contained MBM45, FM60 and either 20% or 30% wheat germ meal (Diets 1a and 1b, respectively). All diets are shown in Table 4. Principal component analysis was applied to the matrix of the values of digestible AA (% lysine) in these diets and the vector of ideal AA profile.

The results showed that the eigenvalues of the two first principal components were greater than 1 and accounted for 83.8% of the original information on digestible AA content (Table 2). Threonine, arginine, isoleucine, leucine, histidine and phenylalanine were the AA that determined diet distribution in relation to the principal component 1 (negative correlation). The principal component 2 allowed the differentiation of the diets in relation to tryptophan (negative correlation) and valine (positive correlation). Thus, diets in the upper graph area have greater valine levels, whereas those in the lower area show greater tryptophan contents. Considering the principal component 1, the AA profiles of Diets 1a, 1b, MBM45 and wheat middling were closer to the ideal AA profile.

**DISCUSSION**

The structure obtained from cluster (Groups) and principal component (Classes) analyses allowed the determination of 11 classes of ingredients with similar digestible AA profiles (Table 3). Class 1 ingredients presented AA profiles closer to the ideal, and balance quality decreased gradually in the other classes.

According to the classification presented, soybean meal, which is the main protein source for poultry, has a good AA balance since it was included in Class 3. However, its partial replacement by Class 1 ingredients (MBM45, FM60 and wheat germ meal) improved the diet profile of AA, as evidenced from the simulations. Class 2 ingredients (Table 3) differed from Class 3 ingredients especially because they presented a greater digestible methionine + cystine profile. This is a desired characteristic, since these limiting AA are usually added in synthetic form to poultry diets and increase production cost.

Corn, the main energy source in poultry diets, was included in Class 8, i.e., one of the ingredients with the worst AA balance. Although energetic ingredients have low protein and AA levels, they are added to the diets in high amounts (about 60%), and thus may bring AA imbalance. The cereals with better AA balance than corn were broken rice (Class 7), high-lysine corn, wheat middling, oat and barley (Class 6). However, the replacement of corn by high-lysine corn did not improve the diet profile of AA (Figure 6) because the only difference in AA between these ingredients was related to lysine. Thus, supplementation of corn diet with synthetic lysine (Table 4) abolished these differences. The addition of wheat middling in the diet improved AA balance in relation to corn, except for tryptophan which was in excess.

**CONCLUSION**

The ingredients might be classified into 11 classes with similar digestible AA profiles. Those ingredients with AA profiles close to the ideal protein are meat and bone meal 45, fish meal 60 and wheat germ meal. In the simulations, dietary AA profiles were improved when corn and/or soybean meal were partially or totally replaced by ingredients with a better AA balance.
**Table 4** – Simulations of starter diets for broiler chickens.

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corn-soy</td>
</tr>
<tr>
<td>Corn</td>
<td>56.77</td>
</tr>
<tr>
<td>High-lysine corn</td>
<td>-</td>
</tr>
<tr>
<td>Wheat middling</td>
<td>-</td>
</tr>
<tr>
<td>Low-tannin sorghum</td>
<td>-</td>
</tr>
<tr>
<td>Soybean meal 45</td>
<td>36.31</td>
</tr>
<tr>
<td>Wheat germ meal</td>
<td>-</td>
</tr>
<tr>
<td>Fish meal 60</td>
<td>-</td>
</tr>
<tr>
<td>Meat and bone meal 45</td>
<td>-</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>3.00</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.81</td>
</tr>
<tr>
<td>Calciotic limestone</td>
<td>0.99</td>
</tr>
<tr>
<td>Salt</td>
<td>0.45</td>
</tr>
<tr>
<td>DL-methionine  98</td>
<td>0.23</td>
</tr>
<tr>
<td>L-lysine 78</td>
<td>0.14</td>
</tr>
<tr>
<td>Supplement (3kg/ton)</td>
<td>0.30</td>
</tr>
<tr>
<td>TOTAL</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Digestible AA (% of digestible lysine)**

- Lysine 100.0
- Methionine + cystine 72.0
- Tryptophan 59.6
- Threonine 63.1
- Arginine 115.3
- Isoleucine 72.1
- Valine 75.8
- Leucine 124.1
- Histidine 42.5
- Phenylalanine 79.0

1 - The AA profile was used for principal component analysis, except for lysine and methionine + cystine, which were equal among diets.

**REFERENCES**


