## Formulating poultry diets based on their indigestible components<sup>1</sup>

S. L. Vieira,<sup>\*2</sup> C. Stefanello,<sup>\*</sup> and J. O. B. Sorbara<sup>†</sup>

\*Departamento de Zootecnia, Universidade Federal do Rio Grande do Sul, Avenida Bento Gonçalves, 7712, Porto Alegre, RS, Brazil 91540-000; and †DSM Nutritional Products, Animal Nutrition and Health, Av. Eng<sup>°</sup> Billings, 1729, São Paulo, Caixa Postal 3003, Brazil

**ABSTRACT** Since it started as an organized economic activity, poultry production has been undergoing an evolution toward the optimization of its feed formulation features. Notably, advances in the knowledge of birds' nutrient requirements have allowed recommendations that are increasingly closer to the needs of the birds. Over time, availability of nutrients and energy has been incorporated into those recommendations, especially to compensate for the variability in the digestibility of nutrients originating from variable feed ingredient sources. Instead of using the total energy and nutrient content, current tables of nutrient recommendations provide an estimate of the digestible fractions of the nutrients in ingredients. For instance, nonphytate P is preferred instead of total P to account for the unavailable phytate P, and digestible amino acids to account for the differences in digestibility of amino acids in different ingredients, whereas energy is usually expressed as a proportion that has been digested and metabolized (AME). With the increasing interest in the

use of exogenous enzymes in poultry feeds, special attention is directed to the feed substrates such that an added enzyme can match it, forming an enzyme-substrate complex that will be followed by a chemical reaction within the gastrointestinal tract. As a consequence of a degradation reaction, nutrients released can be absorbed and metabolized. In general, nutritional data banks used in linear feed formulation software have limited data on the proportions of fractions of ingredients that are indigestible. Therefore, estimations of the presence of many substrates in the feeds, and therefore the benefits of adding exogenous enzymes, are frequently limited because of the scarcity of adequate information. The objective of this review paper is to provide insights into the use of expanded nutrient databanks to include all the molecules considered potentially indigestible for poultry such that the inclusion of exogenous enzymes allows the estimation of the values of the product originated by their hydrolysis.

Key words: enzyme, feed formulation, indigestible fraction

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## INTRODUCTION

Major differences exist between nutritionists in their approach to provide energy and nutrients in poultry feeds that can affect their effective utilization by the bird. These differences frequently affect the expression of animal growth even though similar reference tables of nutrients are used.

Linear least cost feed formulation software is presently the most popular system to formulate feed for poultry. This formulation system utilizes knowledge of feed and ingredient costs, nutrient and ingredient minimum and maximum constraints, as well as digestibility/availability of nutrients within each feed ingredient. Generally, once a final formulation solution is reached, the feed is supposed to have minimum energy and nutrients to allow appropriate growth or egg production within a window of economic profitability. In that scenario, performance of birds fed the formulated feed is expected to match expectations as shown by previous experimental work or field trials.

Commercial feed formulation has been evolving with the use of data that estimate nutrient and energy availability to be delivered in amounts attempting to match birds' requirements as closely as possible. A great deal of diversity exists in terms of ingredients used to formulate feeds for poultry around the world. Local resources are frequently of low cost; however, many times they contain high levels of antinutrients or components that vary in their utilization by birds.

The existing nutrient requirement tables for poultry provide suggestions of levels to be used in commercial feeds that should allow bird performance compatible

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<sup>&</sup>lt;sup>2</sup>Corresponding author: slvieira@ufrgs.br

with adequate business productivity in most environments. However, variation between different ingredients, and sometimes within the same ingredient, can potentially affect the expected bird performance due to differences in digestibility, and therefore, in the absorption of the products of their degradation. Limitations in the estimation of the effect of these variations many times are not adequately considered when feed is formulated. The expression of nutrients and energy according to its availability for birds has been changing (Han and Parsons, 1990; Lemme et al., 2004). This is the case with P, usually expressed as available P or nonphytate P (**NPP**). Even though they have similar values, available P and NPP have different meanings with the first representing the proportion of P that is available, usually in comparison with a reference source, whereas NPP is the amount of P that is not bound in the phytate molecule. Energy availability is highly affected by its source, especially in the case of carbohydrates, and because of that, the ME system has been used for a long time (Anderson et al., 1958). More recently, values of amino acid (AA) digestibility values in ingredients have been published (Ravindran et al., 2005; Bandegan et al., 2011; Kim and Corzo, 2012; Frikha et al., 2012; Kim et al., 2012; Rochell et al., 2012; Kong and Adeola, 2013; Adedokun et al., 2014), which allow for the formulation of poultry diets taking into consideration AA digestibility differences between sources.

Because there are many gaps in the knowledge of the impact of the indigestible components or fractions present in many ingredients, those become limiting factors during feed formulation, making it more difficult to understand their impact on feed utilization. Several substrates can be degraded by the endogenous enzyme array secreted by poultry (starch, proteins, lipids); however, other substrates have no match from the endogenous secretion (cellulose) and therefore are not digested, or may also have antinutritive effects, such as  $\beta$ -glucans, pentosans, and phytate (Ravindran, 2013). Recognizing the existing differences in DM digestibility between ingredients allows an estimation of availability of nutrients in different ingredients (Janssen and Carré, 1985; Dale, 1996; Ravindran et al., 1999).

Studies evaluating the use of exogenous enzymes supplemented in poultry diets are not new; however, the commercial use of enzymes has gained economic importance with phytase, which is now present in the majority of poultry diets around the world (Cowieson and Ravindran, 2007). The increasing cost of feed ingredients and the competition of biofuels for plant feedstuffs, such as corn and soybeans, indicates a possible scenario of even higher costs. Starting in 2007, the prices of major grains increased dramatically in real terms, reaching a peak in 2008; prices then declined in 2009 and 2010 without going back to their previous levels, moving sharply upward again in 2011 (Rosegrant et al., 2012). An increased interest in nonphytase enzymes has paralleled the increase in the cost of energy and protein feed ingredients with the objective of retrieving more energy and nutrients from the same feed by the enzyme-supplemented birds.

## Using Indigestible Fractions in the Feeding Formulation Programs

The addition of exogenous enzymes in poultry feeds only makes sense if there are substrates present in these feeds for which the enzymes have specificity. The hydrolytic effects of enzymes on substrates should generate products that may be absorbed and eventually utilized by the bird. However, substrates present in commercial feeds have large variations in digestibility due to the limitation in activities of the endogenously secreted enzymes to react with all present substrates and completely hydrolyze them. Therefore, the complementary hydrolysis produced by the exogenous enzymes on substrates depends on the substrates passing through the gastrointestinal tract (**GIT**) that are left indigested.

As a proportion of the original molecules, most of the indigestible substrates in corn-soy or wheat-soy diets are nonstarch polysaccharides (**NSP**). However, the actual amounts of enzymatically hydrolyzed products vary with the total quantities of original substrates. For instance, it is well known that starch is the largest energy-contributing source in poultry diets, and along with protein, is of higher digestibility than NSP. Starch digestion in broiler chickens is also high, but still incomplete, for wheat, barley, and peas, as well as from other ingredients (Hesselman and Åman, 1986; Longstaff and McNab, 1987; Rogel et al., 1987; Yutste et al., 1991). Starch granules are composed of variable proportions of amylose and amylopectin molecules, but digestibility is expected to decrease as its amylose proportion increases (Moran, 1982). Starch coefficients of digestibility of 93.8% for wheat, 97.4% for corn, 95.4% for sorghum, and 97.3% for rice have been reported (Weurding et al., 2001). Protein also represents a large fraction of all nutrients entering the GIT of poultry when fed commercial feeds with substantial amounts of protein and AA found in excreta and in their ileal contents (Ravindran et al., 1999). Several factors affect the digestibility of protein in plant feedstuffs for poultry, such as species, genetic variability within an ingredient, as well as thermal processing having the most effect (Parsons et al., 1992; Douglas et al., 2000; de Coca-Sinova et al., 2008). Digestibility of protein in animal by-product ingredients also varies, in part because of the effects of thermal processing, but the type of carcass components is also important in the process (Parsons et al., 1997; Wang and Parsons, 1998). Estimation of indigested protein amounts that can be hydrolyzed by exogenous proteases is affected by the digestibility coefficients determined using different methodologies (Ravindran and Bryden, 1999; Garcia et al., 2007). Regardless of the method, variation in protein and AA digestibility in feedstuffs can affect the total amount of indigestible

substrates left available for a potential complementary degradation of exogenously added proteases (Lemme et al., 2004; Stein et al., 2007).

Indigestible amounts of NSP potentially can provide direct gains as they release monomers that can be absorbed by the bird. Improvements in substrate hydrolysis are also expected due to the increasing exposure of cell contents to a greater available enzyme array when exogenous enzymes are added (Carré et al., 1995; Jørgensen et al., 1996). This is the case of starch and protein, which may be partially encapsulated by a rigid protein matrix or by cell walls from the same feedstuff (sometimes referred to as cage effect) such that endogenously secreted enzymes by poultry are unable to access these nutrients (Theander et al., 1989; Bedford and Morgan, 1996; Wiseman et al., 2000). For instance, starch and protein that escape digestion reach the hindgut and undergo fermentation with a relatively low energy yield (Theander et al., 1989; Slominski et al., 1993; Noy and Sklan, 1995). Digestibility of NSP for poultry is variable and highly affected by species of plant feedstuffs. For instance, Meng and Slominski (2005) demonstrated that NSP digestibility was of 9.4, 7.6, and 4.5% in diets containing corn that were added with soybean meal, canola meal, or peas. The authors pointed out that those values corresponded well with the water-soluble NSP fractions of the diets.

An increase in gut viscosity produced by the watersoluble NSP has also been referred as one mechanism by which nutrient digestibility is reduced (Fengler and Marguardt, 1988; Choct and Annison, 1992). Soluble fibers, such as those present in barley, wheat, and rye, form viscous gels that can trap nutrients and slow down rates of digestion and affect feed rate passage through the gut (Englyst, 1989; Bedford et al., 1991; Veldman and Vahl, 1994). Limited mixing of nutrients with pancreatic enzymes and bile acids (Edwards et al., 1988) and a consequent reduced rate of nutrient absorption occurs with increased digesta viscosity (Fengler and Marquardt, 1988). Evidence has been presented suggesting that increased digesta viscosity affects gut microflora especially by enhancing bacterial fermentation (Annison, 1993) and thus negatively affecting micelle formation (Coates et al., 1981) due to increased bile acid deconjugation (Cole and Fuller, 1984).

In practical terms, many limitations arise when attempting to compile a list of indigestible compounds and estimate the proportion of substrates that would be available to form complexes to be hydrolyzed by exogenously added enzymes. First of all, coefficient of digestibility data on many substrates are not included in traditional tables such as NRC (1994), Institut National Recherche Agronomique (2002), Fundación Española Desarrollo Nutrición Animal (2003), as well as the Brazilian tables (Rostagno et al., 2011). On the other hand, the presence of many substrates not routinely analyzed in most laboratories dedicated to animal nutrition (e.g., NSP) can be highly variable in feed ingredients (Choct and Annison, 1990). The limited practical information on the molecular structures of substrates present in feedstuffs, for which poultry lack or have a limited supply of endogenously secreted enzymes, is a major reason why indigestible or low-digestible fractions in feed ingredients for poultry may not be widely used in commercial feed formulation. However, this is essential information if a wider use of exogenous enzymes is to become more effective.

Large variation exists in terms of molecular composition between plant ingredients that are of low digestibility. Some of them are presented as part of large groups, such as hemicellulose and pectins. These, however, are composed of diverse monomers and different side chains. Hemicellulose, which is sometimes used interchangeably with the term pentosan, is composed mainly by pentose-containing polysaccharides (Neukom et al., 1967). Pectin is a general term for pectic polysaccharides and is mostly related to galacturonans branched with other sugars (Choct, 1997).

Hemicellulose dominates in the cell walls with variations in their compositions between plant ingredients. Corn cell wall composition is similar to that of wheat, although branched arabinoxylans predominate in the endosperm cell walls; small amounts of mixed-linked  $\beta$ -glucan and cellulose are also present in wheat (Chesson, 2001). Corn hemicelluloses are mainly made up of insoluble arabinoxylans, whereas in rice bran, xyloglucans and arabinoxylans are the ones that are more prevalent (Henry, 1987; Evers et al., 1999). On the other hand, oats, barley, and rye have significant proportions of  $\beta$ -glucans, xyloglucans, and arabinoxylans (Chesson, 1993; Partridge, 2001).

Pectic polysaccharides have a general structure comprised by a main chain of rhamnogalacturonan (galacturonic acid and rhamnose) residues with side chains containing arabinose, galactose, and xylose, as well as highly branched arabinans, galactans, and arabinogalactans present as side chains or free neutral polysaccharides (Aspinall and Cottrell, 1971; Siddiqui and Wood, 1972; Daveby and Aman, 1993). In practical terms, soybean meal is the ingredient that mostly supplies pectins in poultry diets; however, other less commonly used protein sources, such as lupines, sunflower, and canola, are also sources of pectins (Bacic et al., 1988; Choct, 1997).

Once the molecular structure of the indigestible fractions is elucidated, it will be possible to give suggestions for the use of exogenous enzymes, which are compatible with these substrates, and therefore estimate the beneficial effect of their degradation (Ravindran, 2013). In Table 1, feed formulations are presented using ingredients having diverse indigestible composition to formulate starter broiler feeds (23% CP, 2,950 kcal of AME/kg). It is possible to observe that differences exist between concentrations of fractions in the final feeds that can be possible substrates for exogenous enzymes. The inclusion of meat and bone meal, because it partially replaced soybean meal that is lower in AME, led to an important improvement in the feed AME and

Table 1. Composition of diets formulated with different degrees of complexity in terms of ingredient composition<sup>1</sup>

Item	Corn/soy	Wheat	$MBM^2$	Rice bran	$DDGS^3$
Ingredient, %					
Corn	55.8	10.0	59.7	40.2	42.7
Soybean meal	35.6	32.0	31.4	34.2	21.9
Wheat		47.2		_	
Meat and bone meal			5.0	_	5.0
Rice bran				15.0	
DDGS				_	25.0
Others (synthetic AA, limestone, DCP, and so on)	8.6	10.8	3.9	10.6	5.4
Total	100	100	100	100	100
Substrate					
Gross energy (GE), kcal/kg	4,240	4,297	3,948	4,346	4,375
IE <sup>4</sup> /GE	0.24	0.24	0.18	0.25	0.26
CP	20.62	20.87	21.20	20.73	22.05
Indigestible CP/total CP	0.10	0.10	0.11	0.11	0.12
Total Lys	1.12	1.28	1.12	1.14	1.00
Indigestible Lys/total Lys	0.09	0.23	0.09	0.10	0.10
TSAA	0.84	0.73	0.84	0.86	0.76
Indigestible TSAA/TSAA	0.08	0.07	0.09	0.10	0.12
Total Thr	0.81	0.83	0.82	0.81	0.79
Indigestible Thr/total Thr	0.12	0.19	0.13	0.14	0.13
Cellulose	3.9	3.6	3.6	5.1	4.6
Hemicellulose	4.8	4.4	4.6	6.0	6.7
Arabinoxylans	2.3	4.1	2.5	2.4	4.6
Glucomannans	0.7	0.1	0.6	0.9	0.5
Xyloglucans	1.6	0.2	1.5	1.9	1.4
Starch	36.0	33.2	38.3	29.7	28.7
Amylose	27.0	24.9	28.8	22.2	21.5
Amylopectin	9.0	8.3	9.6	7.4	7.2
Resistant starch	3.6	3.3	3.8	3.0	2.9
Phytate P	0.23	0.23	0.22	0.41	0.21
Pectin	2.1	1.9	1.9	2.8	1.3
Xylogalacturonans	0.6	1.2	0.7	0.9	0.5
Rhamnogalacturonans	1.1	0.6	1.0	1.3	0.6
Oligosaccharides	6.9	5.6	6.3	5.5	6.2

<sup>1</sup>Based on data from Englyst (1989), NRC (1994), Bach Knudsen (1997), FEDNA (2003), and Rostagno et al. (2011). <sup>2</sup>Meat and bone meal.

-Meat and bone meal.

 $^{3}\mathrm{Dried}$  digestible grains with solubles from corn.

<sup>4</sup>IE (indigestible energy) = GE – ME.

therefore reduced the amount of gross energy available as substrate. Another important outcome of changing feed formulation was that the inclusion of rice bran resulted in almost double the amount of phytate P in the final diet. This increased the amount of substrates available for phytase degradation. As presented earlier, starch digestibility is high; however, because starch occurs as the largest energy component type in poultry diets, any starch left undigested presents an opportunity for exogenous  $\alpha$ -amylase utilization. Because pectins and xylans have different compositions depending on the original ingredient, their proportional impact in the feeds presented in Table 1 are expected to come from the diets containing wheat and rice bran. On the other hand, soybean is the ingredient source highest in oligosaccharides; therefore, their proportional impact is higher when soybean meal increases; this was the case for the corn-soy and distillers dried grain with solublessoy diets (Cromwell et al., 1993).

Linear feed formulation software can be used to gather the amount of indigestible fractions present in poultry feeds as long as the individual ingredients utilized for that purpose contain data on their proportional composition. Knowing the variations in molecular structures of the indigestible substrates will enable the selection of exogenous enzymes, among those commercially available, that can hydrolyze them. Considering that the enzyme added in the feed has no limitations to function in the GIT (pH, time, temperature, and so on), accurate estimations of the final contribution in bird performance through substrate hydrolysis can be done.

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