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Relationship of two *in vitro* assays in protein efficiency ratio determination on selected agricultural by-products

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Abstract

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Utilization of agricultural by-products as food and feed has been of increasing interest. Protein is a crucial nutrient obtained from those sources. However, *in vivo* method (a rat study) for protein efficiency ratio (PER) determination is time consuming and expensive. C-PER and DC-PER are *in vitro* assays, which involve mathematical calculations using amino acid information from the sample. These two methods have been proven suitable for predicting protein quality of various samples with high correlation to the *in vivo* assay. Rapid prediction with less cost is the advantage of these methods. Theoretically, C-PER and DC-PER of each sample should have high correlation as they are computed from the same amino acid information. However, the efficiency of the methods is probably based on a range of certain information, especially protein digestibility. This study was conducted to demonstrate one of the limitations of the *in vitro* assays as shown in selected agricultural by-products. Three categories of selected agricultural by-products were feed-grade egg product (FGEP; 8 samples), distillers' dried grain (DDG; 4 samples), and defatted wheat germ (DWG;

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8 samples). Protein contents, amino acid profiles, *in vitro* protein digestibility, C-PER and DC-PER were determined. Proteins in FGEP categories were significantly higher (P<0.05) than DWG and DDG, respectively. Both C-PER and DC-PER of all samples showed high correlation except in DWG-424. The low correlation in DWG-424 may be due to its low protein digestibility. It may also indicate the limitation of C-PER assay. The assay therefore may not be suitable for samples with low protein digestibility.

Key words : C-PER, DC-PER, by-product, wheat germ, distillers' grain

Reutilization of agricultural wastes or by-products from food and feed industries has been promoted due to world environmental concerns. Numbers of management systems, i.e. ISO 14000, green label, clean technology, etc. are introduced to efficiently control and reduce wastes from those sources. In order to reuse any wastes or by-products, objectives and possibility of the uses are certainly important. Most wastes from food and feed industries contain high organic materials. As a result, nutrients recovered from these wastes are important in order to be reused as human food or animal feed.

In wheat flour milling industry, wheat germ, a high fat portion, has to be removed to extend the flour shelf life. It has been used as a by-product since it contains significant amounts of protein, fiber, minerals and vitamins (Pomeranz *et al*., 1970). Defatted wheat germ (DWG) is consequently processed into wheat fiber. A number of research dealt with the utilization of wheat germ and defatted wheat germ as food ingredients, e.g. in bakery (Godunova *et al*., 1986; Zaitsev and Khomets, 1983) and meat products (Gnanasambandam and Zayas, 1992).

Distillers' dried grain (DDG) is a by-product from ethanol production, mostly from corn. After fermentation and alcohol distillation, residues, which contain mainly protein, fiber, and minerals, are discarded as feed. However, with certain manufacturing processes, the wastes can be treated as human food (GRAS, GRASP 5G0299; Rasco and McBurney, 1989). DDG can be used as food ingredients in a variety of products, e.g. bakery (Wampler and Gould, 1984; Tsen *et al*., 1983), spaghetti (Wu *et al*., 1987), canned meat (Reddy *et al*., 1986) and breading of onion rings (Connerton, 1983).

Besides cereal-based by-products, animal by-products are also important sources for nutrient recovery. Feed-grade egg product (FGEP) is a group of by-product from egg industry, i.e. hatcheries, graders and breakers. Although the FGEP manufacturing processes are not suitable for human food, the products are valuable for feed industry with their high protein and fat content.

Protein is an important nutrient recovered from these sources. However, for feed and food use, protein quality is as important as quantity. Various methods have been used to determine protein quality including chemical score, protein efficiency ratio (PER), protein digestibility-corrected amino acid scoring (PDCAAS), etc. The methods used are mostly *in vivo* of which animals (e.g. rats) are used. Although the results are quite reliable, they are time consuming and expensive. Researchers have been trying to correlate *in vivo* methods to *in vitro* methods in order to develop reliable methods for PER measurement. Two such methods are calculated protein efficiency ratio or C-PER (Satterlee *et al*., 1982) and discriminant computed protein efficiency ratio or DC-PER (Jewell *et al*., 1980). The C-PER is a PER prediction, calculated from essential amino acid information and *in vitro* protein digestibility, whereas the DC-PER is solely dependent on amino acid compositional data. This study demonstrated a correlation of the methods for the prediction of protein quality in both food and feed samples and revealed one of the limitations of the *in vitro* assays.

Materials and Methods

Sample preparation

Three categories of the samples were kindly provided by commercial firms. FGEP (8 samples)

were received as spray-dried products from hatcheries, hatchery blends, graders, and breakers in the US through the American Egg Board. Eight samples of DWG (Viobin®, Monticello, IL USA) were received as dried granule and pulverized products. Four small samples of DDG (with the cooperation of the Distillers Feed Research Council, Louisville University, Louisville, KY) were received as granular products. All samples were ground and divided into small portions and stored in PVC sample storage bags at ambient temperature until analyzed.

Protein determination

Total nitrogen content was analyzed using Kejdahl method (AOAC, 1990). Protein content was calculated using 6.25 as a conversion factor.

***In vitro* protein digestibility (enzymatic method)**

In vitro protein digestibility was determined on defatted samples by a pH-shift method (Satterlee *et al*., 1982). Three enzyme combinations, α-chymotrypsin from bovine pancreas (Sigma C-4129), peptidase from hog intestinal mucosa (Sigma P-7625) and trypsin from hog pancreas (Sigma T-0134) were added to the samples and stirred in 37ºC water bath for 10 min. A bacterial protease Type VI from *Streptomyces griseus* (Sigma P-5130) was then added to the samples and the solutions were immediately transferred to a 55ºC water bath. After 9 min at 55ºC, the solutions were returned to the 37ºC water bath and the pH were recorded after the total time of 20 min.

Amino acid compositions

Total amino acids in FGEP were determined as described by De Groot and Slump (1969). Samples were hydrolyzed with 6N HCl for 22 h at 110ºC. After HCl was neutralized, the residue was dissolved in 0.20N sodium citrate buffer and applied to an ion exchange column (a polystyrene sulfonic acid resin-sodium salt form). Amino acids in the elutant were detected using a Dionex 2000i ion chromatographic system equipped with a ninhydrin detection capability (Benson, 1976).

Sulfur amino acids (cystine and methionine) were determined by a pre-treatment of the samples with performic acid for 16 h (Moore, 1963) before the amino acid determination with the procedure described above.

Tryptophan was determined by alkaline hydrolysis (4.25N NaOH) as described by Hugli and Moore (1972). Samples were hydrolyzed for 22 h at 110ºC then diluted with 0.20N sodium citrate buffer before applying to an ion exchange system as described above.

C-PER and DC-PER calculations

Both C-PER and DC-PER were calculated from the amino acid profiles and protein digestibility. C-PER of each sample was calculated using the amino acid profile and data obtained from the *in vitro* protein digestibility procedure (enzyme method). The calculation was based on the equations described by AOAC (1997) and Satterlee *et al*. (1982). The procedure for the C-PER calculation may be summarized as follows:

Step 1 Determine the *in vitro* protein digestibility of the sample as described in Satterlee *et al*. (1982)

Step 2 Calculate essential amino acids (EAA) in the sample (g/100g protein) as well as in the ANRC casein

Step 3 Express each EAA as a percentage of the FAO/WHO standard using equation:

$$\%EAA_{FAO} = \frac{[\text{gEAA / 100g protein}] \times [\textit{in vitro} \text{ protein digestibility}]}{\text{FAO / WHO std. for that EAA}}$$

Step 4 Adjusting the $\%EAA_{FAO}$ as follows:

- If all $\%EAA_{FAO}$ are ≤ 100% of the FAO/WHO std., continue on to step 5.

- If any $\%EAA_{FAO}$ is > 100% of the FAO/WHO std., reduce to 100% before continuing on to step 5.

Step 5 Compute X and Y values as followed:

$X = \Sigma\ [1/((\%EAA_{FAO})\ (\text{associated weight}))]$

$Y = \Sigma\ [\text{associated weight}]$

%EAA$_{FAO}$	Associated Weight	%EAA$_{FAO}$	Associated Weight
100	1	51-60	8
91-99	2	41-50	11.31
81-90	2.83	31-40	16
71-80	4	21-30	22.63
61-70	5.66	11-20	32
		0-10	45.25

Step 6 Calculate EAA scores for the casein and samples as followed:

EAA score = Y / X

Step 7 Calculate sample's protein as a ratio of the casein (SPC)

SPC = EAA score of sample / EAA score of ref. casein

If $0.99 < SPC < 1.01$, then C-PER of sample is 2.5, otherwise goes to step 8.

Step 8 Calculate C-PER

C-PER = SPC x 2.5

Step 9 Compute 4 discriminant values to determine group for the samples using equations described by Satterlee *et al*. (1982). The largest group value is then chosen for the corresponding C-PER equation.

Protein digestibility used in the DC-PER calculation, however, was a predicted value from amino acid compositional data as described by Jewell *et al*. (1980) and Satterlee *et al*. (1982). The predicted protein digestibility and the amino acid compositional data of each sample were used in DC-PER calculations as described by AOAC (1997) and Jewell *et al*. (1980). Both calculations were compared to casein as a standard protein.

Experimental design and data analyses

A completely randomized design was used in this study with 3 replicates of each sample. The sample mean differentiations were analyzed using SPSS (v.11.0).

Results and Discussions

The studied agricultural by-products were classified into three groups, FGEP, DWG, and DDG. The first group, FGEP, was a spray-dried by-product from egg and egg processing industries. The latter two groups were in dry form from wheat milling industry and ethanol production industry, respectively. Egg is an important, nutrient-rich food for both human and animal. Wastes from hatcheries, breakers, and graders are routinely collected as by-products and treated for animal feed.

Protein contents in cereal by-products, DWG and DDG, however, were significantly lower than that in the FGEP ($p<0.05$). It was found to be in a range of 24-35% (wet basis, wb), whereas 48-59% (wb) was found in the FGEP (Table 1). C-PER of the samples was determined using essential amino acid information (data not shown) and *in vitro* protein digestibility, while the DC-PER was determined using the same amino acid data and predicted protein digestibility. The predicted protein digestibility of each sample was mathematically calculated from the amino acid compositional data. Therefore, quality of the proteins is dependent on essential amino acids presented in the samples as well as the ability of human or animal to digest and utilize these amino acids (Jansen, 1978). *In vitro* protein digestibility is a method for predicting the ability using enzyme combination to imitate human and animal digestive systems. Animal protein (FGEP) seemed to have higher protein digestibility compared to the cereal protein (Table 1). As a result, higher values of C-PER and DC-PER were shown. C-PER has been proven to have a significant correlation to the PER from the traditional method from a rat study (Hsu *et al*., 1978). DC-PER is another method which also correlates to the PER (Jewell *et al*., 1980). Both methods have the advantages in rapidly obtaining data with less expense as compared to the rat assay. They were proposed as suitable methods to predict PER for a variety of food protein and recommended for use in the food industry (Jewell *et al*., 1980; Satterlee *et al*., 1977).

However, there was a limitation on C-PER in which the PERs of the samples in studied model ranged from 0.67 to 3.22 (Hsu *et al*., 1978). In this study, samples with *in vitro* protein digestibility

of 75-93% seemed to have C-PER values that correlated well with those of DC-PER, as showed in Table 1 and Figure 1. However, when the *in vitro* protein digestibility is low, e.g. in DWG-424 where the value is 68.54%, the correlation between C-PER and DC-PER no longer exists (Table 1 and Figure 1). The C-PER and DC-PER values of the sample are 3.19 and 0.45, respectively, which appears to indicate that DC-PER may be more accurate in predicting PER values of samples with *in vitro* digestibility lower than 75%. This may be probably due to some limitations in the calculation procedure as well as the quantity of essential amino acids presented in the sample. For example, DWG-424 had lower lysine content (0.35mg/mg protein) than DWG-746 (0.45mg/mg protein). Comparing to the standard protein (casein), they were then weighted into different groups classified in the procedure. Consequently, differing discriminant function groups were chosen resulting in different

Table 1. Protein content, *in vitro* protein digestibility, C-PER, and DC-PER of selected agricultural by-products*.

Samples**	Protein (Nx6.25, %)	*In vitro* protein digestibility*** (%)	C-PER***	DC-PER***
FGEP				
- FGEP-412	48.40±0.09^{bc}	91.55±0.09^{ab}	2.60±0.00	2.17±0.01^{b}
- FGEP-102	47.70±1.14^{c}	93.24±1.54^{a}	2.61±0.02	2.16±0.02^{b}
- FGEP-554	49.80±0.31^{bc}	92.82±0.82^{ab}	2.61±0.01	2.19±0.00^{b}
- FGEP-741	48.67±1.56^{bc}	89.57±0.93^{b}	2.60±0.01	2.57±0.21^{a}
- FGEP-959	48.67±0.79^{bc}	94.16±0.59^{a}	2.62±0.02	2.18±0.00^{b}
- FGEP-623	58.45±2.22^{a}	91.25±0.92^{ab}	2.60±0.01	2.19±0.01^{b}
- FGEP-854	52.78±0.55^{b}	92.28±0.65^{ab}	2.61±0.01	2.25±0.07^{ab}
- FGEP-663	50.01±1.35^{bc}	92.96±0.45^{ab}	2.61±0.01	2.15±0.04^{b}
DWG				
- DWG-424	30.48±0.35^{bc}	68.54±0.47^{d}	3.19±0.06^{a}	0.45±0.00^{b}
- DWG-621	27.88±0.49^{de}	86.23±0.56^{a}	2.14±0.00^{b}	2.39±0.45^{a}
- DWG-858	31.22±0.99^{b}	82.92±0.98^{b}	2.14±0.00^{b}	2.07±0.01^{a}
- DWG-944	28.66±0.29^{cde}	86.43±1.48^{a}	2.40±0.37^{b}	2.39±0.45^{a}
- DWG-746	34.79±0.00^{a}	76.62±0.10^{c}	0.47±0.00^{c}	0.46±0.00^{b}
- DWG-214	29.74±0.32^{bcd}	82.65±0.52^{b}	2.37±0.32^{b}	2.50±0.61^{a}
- DWG-335	28.95±0.35^{cde}	75.60±0.28^{c}	0.49±0.00^{c}	0.49±0.01^{b}
- DWG-547	27.48±0.39^{e}	75.15±0.21^{c}	0.47±0.00^{c}	0.45±0.00^{b}
DDG				
- DDG-664	24.19±1.05	86.96±0.80^{b}	0.97±0.06^{b}	0.89±0.11^{b}
- DDG-947	25.15±0.39	81.88±0.00^{c}	1.40±0.08^{a}	1.99±0.23^{a}
- DDG-114	25.07±0.41	90.80±0.16^{a}	1.13±0.07^{b}	0.92±0.09^{b}
- DDG-107	26.34±0.38	77.48±0.80^{d}	0.68±0.02^{c}	0.75±0.02^{b}

*** Mean ± Standard error of mean (SEM).**

**** Sample categories: FGEP = feed grade egg products (8 samples), DWG = defatted wheat germ (8 samples), and DDG = distillers' dried grain (4 samples).**

***** Data based on an equal amount of protein. Casein was used as a standard protein with 96.10% protein content (Nx6.25), 94.79% *in vitro* protein digestibility and 2.50 C-PER. C-PER = calculated protein efficiency ratio, DC-PER = discriminant computed protein efficiency ratio.**

$^{a-e}$ Different letters in column of each sample category show significant differences among means ($p<0.05$).

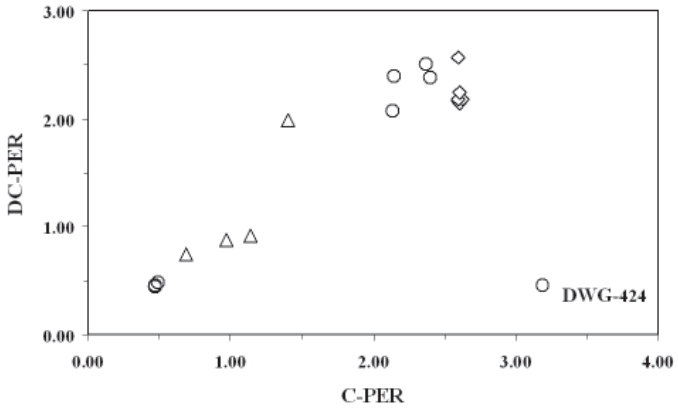

Figure 1. Relationship of calculated protein efficiency ratio (C-PER) and discriminant computed protein efficiency ratio (DC-PER) of feed-grade egg products (FGEP, ◇), defatted wheat germ (DWG, ○), and distillers' dried grain (DDG, △).

calculated C-PER of 3.19 for DWG-424 and 0.47 for DWG-746, respectively. In addition, enzymatic method (pH shift method) used in *in vitro* protein digestibility may not be suitable for samples which have high buffer capacity. Sample DWG-424 originally has low pH value (~4.2) resulting from the industrial process used. Thus, the pH value was probably shifted inaccurately and affected the digestibility. C-PER also has a limitation, especially on samples whose protein tissues are somewhat resistant to proteolytic enzyme used in the *in vitro* method, resulting in the underestimation of the protein quality (Jewell *et al*., 1980). However, in order to confirm this hypothesis, a rat study is needed.

If the DWG-424 data were omitted, C-PER and DC-PER assays showed high correlation to each other ($R^2 = 0.8985$). As mentioned above, the DC-PER uses only amino acid data in the calculation; it can be operated with less expense and is more rapid as compared to the C-PER. Therefore, the DC-PER may be more suitable than C-PER to predict the PER of food ingredients and processed food products.

Conclusion

C-PER and DC-PER are more rapid and less expensive methods for the prediction of PER than the traditional methods. However, C-PER seems to have limitations when used to predict PER of samples with low *in vitro* digestibility.

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