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ANIFEE-12610; No. of Pages 6
ARTICLE IN PRESS

Animal Feed Science and Technology xxx (2011) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Using the NRC chemical summary and biological approaches to predict energy values of new co-product from bio-ethanol production for dairy cows

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ARTICLE INFO

Article history:
Received 13 October 2010
Received in revised form 19 August 2011
Accepted 8 September 2011
Available online xxx

Keywords:
Bioethanol co-products
Energy values
Truly digestible nutrients
Biological and chemical approaches

ABSTRACT

The dramatic increase in bio-ethanol production in Canada has resulted in millions of tones of different types of new co-products: wheat dried distillers grains with solubles (DDGS), corn DDGS and blended DDGS (*e.g.* wheat:corn = 70:30). In the determination of energy values, NRC-2001 described a chemical approach in which the equations for truly digestible nutrients are developed based on the chemical composition of native feeds. It is questionable whether this chemical approach described by NRC 2001 accurately estimates the energy values of these co-products (DDGS) for dairy cows from bio-ethanol production for dairy cows. The objectives of this study were: (1) to determine the effect of DDGS type and bio-ethanol plant on energy values ($DE_{3\times}$, $ME_{3\times}$ $NE_{L3\times}$, NE_m, NE_g) using a biological approach (*in situ* assay), and (2) to investigate the relationship between the NRC chemical summary approach and the biological approach on prediction of energy values. The results showed significant effects of DDGS type on $TDN_{1\times}$ and energy values ($DE_{3\times}$, $ME_{3\times}$, $NE_{L3\times}$, NE_m, NE_g) with the highest in corn DDGS and the lowest in wheat DDGS. (2) The differences between the NRC chemical approach and the biological approach were significant for the predicted truly digestible nutrients (tdNDF, tdCP, tdFA and tdNFC). The greatest difference was found in tdNDF (−77.4 g/kg DM, P<0.001) followed by tdCP (+47.9 g/kg DM). Higher tdNDF was found when using the *in situ* assay. However, higher tdCP, tdFA and tdNFC were found when using the NRC 2001 chemical approach. (3) No differences between the two approaches (P>0.05) were detected in the $TDN_{1\times}$, and energy values ($DE_{3\times}$, $ME_{3\times}$, $NE_{L3\times}$, NE_m, NE_g); (4) Pearson correlation analysis between the chemical approach and the biological approach showed strong relationships (P<0.05) for truly digestible nutrients, $TDN_{1\times}$, and all energy values. Although the predicted energy values from the two approaches were similar, these results indicate that NRC 2001 chemical summary approach was different from the biological approach (*in situ* assay) in prediction of tdNDF and tdCP for bioethanol co-products, indicating that a refinement of the NRC 2001 formula to predict tdNDF and tdCP is required for these products.

Abbreviations: ADF, acid detergent fibre expressed inclusive of residual ash; ADICP, acid detergent insoluble crude protein; CHO, carbohydrate; CFat, crude fat; CP, crude protein; ISDCP, *in situ* degradability of crude protein; ISDFA, *in situ* degradability of fatty acid; ISDNDF, *in situ* degradability of neutral detergent fibre; ISDNFC, *in situ* degradability of non-fibre carbohydrate; $DE_{3\times}$, digestible energy at production level of intake (3×); FA, fatty acids; $ME_{3\times}$, metabolizable energy at production level of intake (3×); NDF, neutral detergent fibre with a heat stable amylase and sodium sulfite and expressed inclusive of residual ash; NDICP, neutral detergent insoluble crude protein; $NEL_{3\times}$, net energy for lactation at production level of intake (3×); NE_m, net energy for maintenance; NE_g, net energy for growth; NSC, non-structural carbohydrates; dCP, truly digestible crude protein; tdFA, truly digestible fatty acid; tdNDF, truly digestible neutral detergent fibre; tdNFC, truly digestible non-fibre carbohydrate; $TDN_{1\times}$, total digestible nutrient at one times maintenance.

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doi:10.1016/j.anifeedsci.2011.09.007

Table 1
Samples for the study.

Samples	No. of samples	Plants
Corn	3 batches	North Dakota (through Co-op)
Corn DDGS	3 batches	North Dakota (through Co-op)
Wheat (original feedstock)	3 batches	Sask bioethanol Plants 1 and 2
Wheat DDGS	2 batches	Sask bioethanol Plant 1
Wheat DDGS	3 batches	Sask bioethanol Plant 2
Wheat:corn (70:30) blend DDGS	3 batches	Sask bioethanol Plant 2

1. Introduction

Dried distillers grains with solubles (DDGS) are byproducts of fermentation during ethanol production. As a result of the government policies to stimulate the expansion and consumption of bio-fuels, the number of ethanol plants has increased in western Canada. Although the bio-ethanol industry in this part of the country is wheat based, the fluctuation in the price of wheat has forced ethanol companies to include corn in the feedstock for ethanol processing. Consequently not only pure wheat DDGS, but also various blended (wheat to corn at different ratios) DDGS is becoming more available.

While the nutritional value of corn DDGS has been extensively documented in ruminants, information on wheat DDGS and wheat/corn blended DDGS has only been reported in pigs (Widyaratne and Zijlstra, 2006). Recently, Nuez-Ortín and Yu (2009, 2010a, 2010b) reported variation in the nutrient content and availability of wheat DDGS, corn DDGS and blended DDGS from bio-ethanol plants.

In the determination of energy values, NRC (2001) described a chemical approach in which the equations for truly digestible nutrients were developed based on the chemical composition of native feeds (Weiss et al., 1992). Yet, energy values of a feedstuff are not chemical constituents. Thus, it is questionable whether this chemical approach described by NRC 2001 can accurately estimate energy values of these co-products (DDGS) from bio-ethanol production.

Biological approaches including *in vitro* and *in situ* incubation are considered superior predictors of truly digestible nutrients (Robinson et al., 2004). The method preferred by some researchers, and also suggested by NRC 2001, is a 48 h *in vitro* incubation. However, over-estimated results for feeds with a high content of soluble sugars have been reported (Seker, 2002). For this reason, and because it has greater similarity to *in vivo* measurements, an *in situ* assay (48 h incubation) may provide the best estimation of the total tract digestion, and consequently truly digestible nutrients and energy values (Tamminga et al., 1994; Yu, 2006). To date, there is no information known to the authors on the relationship of the NRC 2001 chemical approach, and an *in situ* assay – biological approach that clarifies the accuracy of the NRC 2001 – chemical approach on the prediction of energy values for DDGS.

The objectives of this study were: (1) to determine the effect of DDGS type and bioethanol plant on energy values using a biological approach and (2) to investigate the relationship between the energy values calculated according to NRC (2001) equations and the value determined using the biological approach for these co-products (DDGS) from bio-ethanol processing.

2. Materials and methods

2.1. *Wheat DDGS, corn DDGS, blended DDGS and original cereal grains (corn and wheat)*

During May–December in 2007, two different batches of wheat DDGS ($n = 2$ batches) from SK-Plant 1, three different batches of wheat DDGS ($n = 3$ batches) from SK-Plant 2, three different batches of blended DDGS (wheat:corn = 70:30; $n = 3$ batches) from SK-Plant 2 (Table 1) (SK-Plant 1 does not produce the blended DDGS). Wheat samples ($n = 3$ batches) were collected from the two different bio-ethanol plants (SK-Plant 1 and SK-Plant 2) located in western Canada. Both plants used the same local wheat feedstock for ethanol production. During the same time frame, corn DDGS ($n = 3$ batches) and corn samples ($n = 3$ batches) produced by a bio-ethanol plant in North Dakota were obtained through Co-op Feeds in Saskatoon, SK. Due to cold climate conditions, western Canada does not produce large amount of corn and corn DDGS. However, corn DDGS and corn samples were used as reference samples for comparison with wheat and wheat DDGS. The detailed chemical and nutrient profiles were reported previously by Nuez-Ortín and Yu (2009, 2010a,b).

2.2. *Ruminal in situ assay*

A ruminal *in situ* assay was performed to measure rumen degradability and estimate the truly digestible nutrients (tdNDF, tdCP, tdFA, tdNFC) of all 11 DDGS samples with a randomized complete block design (RCBD). Three Holstein dry cows fitted with a rumen cannula (Bar Diamond Inc., Parma, ID, USA) with an internal diameter of 10 cm were used in this study. The cows were individually fed twice daily at 0800 and 1600 receiving 14 kg (7 kg at each feeding time) of a totally mixed ration consisting of 568 g/kg barley silage, 102 g/kg alfalfa hay, 45 g/kg dehydrated alfalfa pellets, 216 g/kg standard dairy concentrate (containing barley, wheat, oats, dairy supplement pellets and molasses) and 68 g/kg cow concentrate (containing barley, oats, canola meal, soybean meal, wheat DDGS, corn gluten meal, molasses, dried fat supplement (golden flakes), canola oil, minerals and vitamins) according to the nutrient requirement of NRC (2001). The animals were cared for in accordance

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with the guidelines of the Canadian Council on Animal Care (CCAC, 1993). Ruminal degradability of dry matter, non-fibre carbohydrate, crude protein, neutral detergent fibre, crude fat (CFat) and neutral detergent insoluble crude protein at 48 h incubation, were determined by an *in situ* method (Yu et al., 2000) using nylon bags (Nitex 03-41/31 monofilament open mesh fabric, Screentec Corp., Mississagua, ON) measuring 10 cm × 20 cm with a pore size of 40 μm. A polyester mesh bag (45 cm × 45 cm with a 90 cm length of rope to be anchored to the cannula) was used to hold the bags in the rumen. The 48 h incubation times were based on the NRC-2001 suggestion for energy estimation (48 h). The multi-bags (8 bags) were used for each sample at 48-h incubation time. All treatments were incubated in two experimental runs and randomly allocated to all three non-lactating cows (we did not know which bags went to which cows). After incubation, the bags were removed from the rumen and rinsed under a cold stream of tap water to remove excess ruminal contents and then washed 5 times in groups of 10 with 2 L of cool water without detergent. The last rinse cycle of water was clear. The bags were washed with cool water without detergent and subsequently dried at 55 °C for 48 h. Dried samples were stored in a refrigerated room (4 °C) until analysis. The residues were pooled according to each run and each sample (pooled 8 bags residue together) and ground through a 1-mm screen (Retsch ZM-1, Brinkmann Instruments (Canada) Ltd., Ontario) for chemical analysis.

2.3. Chemical analysis

All samples for chemical analysis were ground through a 1 mm screen (Retsch ZM 100, Retsch Inc.). Dry matter (DM, AOAC 930.15), ash (AOAC 942.05), crude fat (CFat, AOAC 954.02) and crude protein (CP, AOAC 984.13; Kjeltec 2400) contents were analyzed according to procedures from the AOAC (1990). The acid detergent fibre (ADF, expressed inclusive of residual ash), neutral detergent fibre (NDF) and acid detergent lignin values were determined according to Van Soest et al. (1991) by Ankom filter bag method (Ankom A200 Filter bag technique, Ankom Technology, Fairport, NY). Neutral detergent fibre was determined with the inclusion of heat stable α-amylase and express inclusive of residual ash (Van Soest et al., 1991). Sodium sulfite was used prior to neutral detergent extraction. The NDFn was also adjusted by: NDF-NDICP. Acid detergent lignin was determined by solubilization of cellulose with sulphuric acid. The acid (ADIN) and neutral detergent insoluble N (NDIN) values were determined according to Licitra et al. (1996). Acid detergent insoluble protein (ADICP) and neutral detergent insoluble protein (NDICP) were calculated as ADICP = 6.25 × ADIN and NIDCP = 6.25 × NDIN, respectively. The non-structural carbohydrates (NSC) including starch, sugars, organic acids, and other reserve carbohydrates such as fructan were estimated by non-fibre carbohydrates and determined according to Grings et al. (1992). All samples were analyzed in duplicate and repeated if chemical analysis error was in excess of 5%.

2.4. Energy values

Estimated energy contents for truly digestible crude protein (tdCP), fatty acid (tdFA), neutral detergent fibre (tdNDF) and non-fibre carbohydrates (tdNFC) were calculated separately using the two different approaches as follows:

Using NRC 2001-chemical summary approach:

(a) tdNFC (g/kg DM) = 0.98(100 − [(NDF − NDICP) + CP + EE + Ash]) × PAF, where PAF (processing adjustment factor) = 1.00.tdCP (g/kg DM) = [1 − (0.4 × (ADICP/CP))] × CP,
(c) tdFA ((g/kg DM) = FA, where FA = CFat − 10 (Allen, 2000),
(d) tdNDF (g/kg DM) = 0.75 × (NDFn − ADL) × [1 − (ADL_{sa}/NDFn)$^{0.667}$], where NDFn = NDF − NDICP. ADL, acid detergent lignin.

Using an *in situ* assay-biological approach:

(a) tdNFC (g/kg DM) = (g/kg DM) × ISNFCD, where NFC = 100 − ([NDF − NDICP] + CP + EE + Ash) and ISNFCD was *in situ* coefficient of digestibility of NFC after 48 h incubation.
(b) tdCP (g/kg DM) = CP (g/kg DM) × ISCPD, where ISCPD was *in situ* coefficient of digestibility of CP after 48 h incubation.
(c) tdFA (g/kg DM) = FA (g/kg DM) × ISFAD, where FA = CFat − 10 (Allen, 2000) and ISFAD was *in situ* coefficient of digestibility of FA after 48 h incubation.
(d) tdNDF (g/kg DM) = NDFn (g/kg DM) × ISNDFnD, where ISNDFD was *in situ* coefficient of digestibility of NDF after 48 h incubation.

Based on the values of truly digestible nutrients, the energy contents of total digestible nutrients at maintenance ($TDN_{1\times}$), digestible energy at production level of intake ($DE_{3\times}$), metabolizable energy at production level of intake ($ME_{3\times}$) and net energy for lactation at production level of intake ($NE_{L3\times}$) were determined using a summative approach (Weiss et al., 1992) from NRC (2001), while net energy for maintenance (NE_m), and net energy for growth (NE_g) were determined using NRC (1996). Both NRC dairy and NRC beef used the same formula to estimate NE_g and NE_m.

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Table 2
Comparison of grain source (wheat, corn or a blend of wheat:corn = 70:30) and two bio-ethanol plants on truly digestible nutrients, total digestible nutrient content at maintenance level, and energy values of distillers dried grains and solubles using the *in situ* assay biological approach.

Items	Grain sources				Bio-ethanol plant (wheat source)		
	Wheat (n = 5)	Corn (n = 3)	Blend (n = 3)	SEM	SK-Plant 1 (n = 2)	SK-Plant 2 (n = 3)	SEM
Truly digestible nutrient[a]							
tdNFC (g/kg DM)	236.3a	63.5b	169.9a	21.24	184.9b	270.6a	17.82
tdCP (g/kg DM)	355.3a	226.9b	336.4a	13.49	373.7	342.9	20.51
tdFA (g/kg DM)	37.2c	150.6a	72.2b	4.77	49.6a	28.9b	3.22
tdNDF (g/kg DM)	172.6c	339.2a	228.4b	11.85	201.5a	153.4b	7.36
Total digestible nutrient at maintenance level[b]							
$TDN_{1\times}$ (g/kg DM)	777.7b	898.5a	817.3b	20.68	801.6	761.8	35.05
Predicted energy values[c]							
$DE_{3\times}$ (MJ/kg DM) (Dairy)	14.52b	15.86a	15.02ab	0.381	14.98	14.23	0.636
$ME_{3\times}$ (MJ/kg DM) (Dairy)	12.84b	14.39a	13.39ab	0.389	13.31	12.47	0.644
$NE_{L3\times}$ (MJ/kg DM) (Dairy)	8.28b	9.62a	8.74ab	0.280	8.66	7.99	8.335
NE_m (MJ/kg DM) (beef)	8.83b	9.79a	9.20ab	1.372	9.16	8.62	0.469
NE_g (MJ/kg DM) (beef)	6.02b	6.82a	6.32ab	0.238	6.32	5.82	0.402

SEM, standard error of mean. Means with different letters in the same row are significantly different (P<0.05).
[a] tdCP, truly digestible crude protein; tdFA, truly digestible fatty acid; tdNDF, truly digestible neutral detergent fibre; tdNFC, truly digestible non-fibre carbohydrate.
[b] $TDN_{1\times}$, total digestible nutrient at one times maintenance.
[c] $DE_{3\times}$, digestible energy at production level of intake (3×); $ME_{3\times}$, metabolizable energy at production level of intake (3×); $NEL_{3\times}$, net energy for lactation at production level of intake (3×); NE_m, net energy for maintenance; NE_g, net energy for growth.

2.5. *Statistical analysis*

Study on the effect of grain source on energy values of DDGS using the biological approach. Statistical analyses were performed using the MIXED procedure of SAS (SAS, 2005). The model used for the analysis was: $Y_{ijk} = \mu + F_i + C_j + e_{ijk}$, where Y_{ijk} was an observation of the dependent variable ijk; μ was the population mean for the variable; F_i was the effect of grain sources (i = 1,2,3 for wheat DDGS, corn DDGS, blend DDGS), as a fixed effect; C_j was the effect of *in situ* experimental run with three cows (j = 1,2, *in situ* run1 and run2), as a random effect, and co-products batches as replications (wheat DDGS, corn DDGS and blend DDGS had 5, 3 and 3 batches, respectively), and e_{ijk} was the random error associated with the observation ijk.

Study on the effect of bio-ethanol plant on energy values of wheat DDGS. Statistical analyses were performed using the MIXED procedure of SAS (SAS, 2005). The model used for the analysis was: $Y_{ijk} = \mu + P_i + C_j + e_{ijk}$, where Y_{ijk} was an observation of the dependent variable ij; μ was the population mean for the variable; P_i was the effect of bio-ethanol plant (i = 1,2 for SK-Plant 1, and SK-Plant 2), as a fixed effect; C_j was the effect of *in situ* experimental run with three cows (j = 1,2, *in situ* run1 and run2), as a random effect, and co-product batches as replications (SK-Plant 1 and SK-Plant 2 had 3 and 2 batches, respectively), and e_{ijk} was the random error associated with the observation ijk.

Comparison of NRC-2001 chemical summary approach with biological approach in the determination of digestible nutrients and energy values. The paired *t* test procedure of SAS (SAS, 2005) and Pearson correlation analysis were performed to establish the relationship between the NRC 2001 chemical approach and the *in situ* assay-biological approach. For all statistical analyses, significance was declared at P<0.05 and trends at P≤0.10. Treatment means were compared using the Fisher's Protected LSD method.

3. Results and discussion

3.1. *Effects of DDGS type and bio-ethanol plant on energy values as determined from the in situ assay (a biological approach)*

The NRC 2001 formula is one method to estimate energy values for feeds for dairy cattle. This method is a chemical approach that uses analytical results to estimate the values of truly digestible nutrients (tdNFC, tdCP, tdFA, tdNDF, TDN). The effects of DDGS type and bio-ethanol plant on energy content as determined using the NRC (1996, 2001) chemical approach have been reported recently (Nuez-Ortín and Yu, 2009).

Table 2 presents the effects of DDGS type and bio-ethanol plant on energy content using an *in situ* assay (a biological approach). Wheat DDGS was higher (P<0.05) than corn DDGS in tdNFC (236.3 vs. 63.5 g/kg DM) and tdCP (355.2 vs. 227.0 g/kg DM) but lower (P<0.05) in tdFA (37.2 vs. 150.6 g/kg DM) and tdNDF (172.6 vs. 339.2 g/kg DM). Compared to wheat DDGS and corn DDGS, blended DDGS was intermediate for tdNDF and tdFA, but did not differ (P>0.05) from wheat DDGS for tdNFC and tdCP. As a result, $TDN_{1\times}$ and energy values ($DE_{3\times}$, $ME_{3\times}$, $NE_{L3\times}$, NE_m and NE_g) were higher (P<0.05) in corn DDGS than wheat DDGS while blended DDGS was intermediate for $TDN_{1\times}$ but did not differ for the other energy values.

Regarding the plant effect, wheat DDGS from SK-Plant 1 was lower (P<0.05) in tdNFC (184.9 vs. 270.6 g/kg DM) but higher (P<0.05) in tdFA (49.6 vs. 28.9 g/kg DM) and tdNDF (201.5 vs. 153.4 g/kg DM) than wheat DDGS from SK-Plant 2. However, there was no plant effect on $TDN_{1\times}$ and energy values ($DE_{3\times}$, $ME_{3\times}$, $NE_{L3\times}$, NE_m and NE_g) (P>0.05).

Table 3
Comparison and correlation analysis between NRC-2001-chemical approach and *in situ* assay-biological approach in the determination of truly digestible nutrients, total digestible nutrient content at maintenance level, and energy values of bioethanol co-products.

Items	Comparison NRC-2001 chemical vs. biological approach					Correlation analysis NRC-2001 chemical vs. biological approach	
	Mean[chemical]	Mean[biological]	Difference	SED	P value	R	P value
Truly digestible nutrient[a]							
tdNDF (g/kg DM)	155.9	233.2	−77.4	8.34	<0.0001	0.98	<0.0001
tdCP (g/kg DM)	360.3	312.4	+47.9	9.01	0.0003	0.93	<0.0001
tdFA (g/kg DM)	81.0	77.7	+3.3	0.36	<0.0001	1.00	<0.0001
tdNFC (g/kg DM)	180.9	171.1	+9.8	2.57	0.0034	1.00	<0.0001
Total digestible nutrient at maintenance level[b]							
$TDN_{1\times}$ (g/kg DM)	809.2	821.5	−12.3	6.89	0.1054	0.93	<0.0001
Energy values for dairy and beef[c]							
$DE_{3\times}$ (MJ/kg DM) (Dairy)	14.98	15.02	−0.04	0.142	0.6797	0.84	0.0009
$ME_{3\times}$ (MJ/kg DM) (Dairy)	13.31	13.39	−0.08	0.142	0.6797	0.87	0.0004
$NE_{L3\times}$ (MJ/kg DM) (Dairy)	8.70	8.74	−0.04	0.100	0.6854	0.91	0.0001
NE_m (MJ/kg DM) (Beef)	9.16	9.20	−0.04	0.100	0.6870	0.85	0.0010
NE_g (MJ/kg DM) (Beef)	6.28	6.32	−0.04	0.088	0.6919	0.85	0.0010

SED, standard error of the difference; R, Pearson correlation coefficient.

[a] tdCP, truly digestible crude protein; tdFA, truly digestible fatty acid; tdNDF, truly digestible neutral detergent fibre; tdNFC, truly digestible non-fibre carbohydrate.

[b] $TDN_{1\times}$, total digestible nutrient at one times maintenance.

[c] $DE_{3\times}$, digestible energy at production level of intake (3×); $ME_{3\times}$, metabolizable energy at production level of intake (3×); $NEL_{3\times}$, net energy for lactation at production level of intake (3×); NE_m, net energy for maintenance; NE_g, net energy for growth.

3.2. Comparison of NRC 2001 chemical summary approach with biological approach in the determination of truly digestible nutrients and energy values

Both approaches: the chemical and biological, detected that DDGS type had effects (P<0.05) on truly digestible nutrients (tdNDF, tdCP, tdFA, tdNFC), $TDN_{1\times}$, and energy values ($DE_{3\times}$, $ME_{3\times,}$ $NEL_{3\times}$, NE_m and NE_g). The comparison and correlation analysis between the chemical and the biological approach are presented in Table 3. The difference between the two approaches was significant for the predicted truly digestible nutrients (tdNDF, tdCP, tdFA, tdNFC). The greatest difference was found in tdNDF (−77.4 g/kg DM, P<0.001) followed by tdCP (+47.9 g/kg DM, P<0.001). Higher predicted tdNDF was found when using the *in situ* assay. However, higher tdCP, tdFA and tdNFC were found when using the NRC 2001 chemical approach. No differences between the two approaches were detected in $TDN_{1\times}$ (809 vs. 822 g/kg DM, P=0.105) and energy values ($DE_{3\times}$, $ME_{3\times,}$ $NEL_{3\times}$, NE_m and NE_g; P>0.10).

These results are in agreement with a previous study (Yu, 2006), in which the greatest difference between chemical and biological approaches was found in tdNDF, being higher when the biological approach was used. NRC (2001) estimates tdNDF based on the acid detergent lignin content of the feed. Robinson et al. (2004) showed the poor relationship between acid detergent lignin content and NDF digestibility in different feedstuffs including distillers grains, and concluded that the formula was not an accurate predictor of tdNDF. In that study, metabolizable energy of distillers grains was 13% higher when tdNDF was predicted *in vitro*. Differences among different feeds in the lignin content as well as in the extent to which lignin is bonded to other components of cell wall might be the reason for the deviation in the NDF digestibility (Chesson and Murison, 1989; Robinson et al., 2004).

In terms of tdCP, the calculation according to NRC 2001 is based on the ADICP content. However, a negative correlation between ADICP and protein digestibility was detected only when ADICP levels were higher than 130 g/kg CP (Harty et al., 1998). As reported by Nuez-Ortín and Yu (2009), ADCIP levels in the current DDGS samples ranged from 11.7 to 64.4 g/kg CP.

Correlation analysis between the chemical and the biological approaches showed strong relationships for all truly digestible nutrients, $TDN_{1\times}$, and energy values (R = 0.84–1.0; P<0.001). Although the predicted energy values from the two methods were similar, these results indicate that NRC 2001 chemical method differed (P<0.05) from an *in situ* assay to predict tdNDF and tdCP. It is well known that the content and digestibility of fibrous carbohydrates is one of the factors determining the energy value of high fibrous feeds (Robinson et al., 2004). An independent comparison (data not shown) of the chemical and biological approaches within each type of DDGS shows that the difference in tdNDF between the two approaches increased as the ruminal fermentability of NDF increased (Nuez-Ortín, 2010), thus the quantity and digestibility of NDF will determine the accuracy of the NRC 2001-chemical approach when evaluating DDGS products.

4. Conclusions

It was concluded that when using the biological approach, wheat DDGS, corn DDGS and blended DDGS (wheat:corn = 70:30) differed in truly digestible nutrients (tdNDF, tdCP, tdFA, tdNFC) and energy values at production levels for dairy cattle ($DE_{3\times}$, $ME_{3\times}$, $NEL_{3\times}$, NE_m and NE_g). The energy values of corn DDGS were higher than wheat DDGS and blended DDGS, indicating that corn DDGS is a superior source of energy in dairy diets. These energy values were similar to those obtained by the *in situ* assay-biological approach. However, the prediction of tdNDF and tdCP differed.

Acknowledgement

The authors wish to thank Northwest Bioenergy, Husky Energy Inc., NorAmera Bioenergy Corp. bio-ethanol plants for providing samples during the two year collection; the researchers (Z. Niu, K. Thiessen, A. Walker, R. Heendeniya, D. Christensen, J. McKinnon, R. Zilstra, T. McAllister) for kind assistance; and the Ministry of Agriculture Strategic Feed Research Chair Program, BCRC, ABIP-FOBI, and ADF for financial support. The authors are grateful for Fundación Caja Madrid for providing a full graduate study fellowship to WNO.

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Please cite this article in press as: Nuez-Ortín, W.G., Yu, P., Using the NRC chemical summary and biological approaches to predict energy values of new co-product from bio-ethanol production for dairy cows. Anim. Feed Sci. Technol. (2011), doi:10.1016/j.anifeedsci.2011.09.007