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"Research Note"

Crude Protein Fractionation and Fiber Analysis of Commercial Non-Forage Fiber Sources for Ruminants in Central Iran

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ABSTRACT- A total of fourteen dairy farms across the central province of Isfahan were visited for sampling wheat bran (WB) and beet pulp (BP) as the main commercial non-forage fiber sources fed to ruminants. Fiber fractions were determined using approved analytical procedures. Crude protein (CP) was fractionated into NPN (A), quickly degradable true CP (B_1), moderately degradable CP (B_2), slowly degradable CP (B_3), and unavailable CP (C), based on the Cornell Net Carbohydrate and Protein Model. Descriptive statistics and correlation coefficients between fibers and CP fractions were calculated for all samples, and data for WB were analyzed in a completely randomized design. All fiber and CP fractions of WB except for C differed significantly across farms. The NPN, B_3, and NDF were greater but CP, unavailable CP and B2 were lower in the WB of this study as compared to that of CNCPS feed library. The greater CP and lower fibers of BP used across Isfahan as compared to that of other reports weretranslated into more soluble fiber and thus more ruminally available energy in the samples of this study. The NPN, B_1, and C in BP were significantly greater than the values found in the literature. Results indicated the necessity of a more dynamic evaluation of the nutrients in by-products if the diet formulation is to provide a more predictable animal response.

Keywords: Beet pulp, Crude protein fractions, Non-forage fiber, Wheat bran

INTRODUCTION

Maintaining efficient ruminal fermentation is a prerequisite for economical productivity of ruminants. The development of biological concepts such as physically effective fiber to characterize plant cell walls has been a subject of extensive research during recent years (4, 11). Such concepts are used to meet microbial requirements for a less-disturbed symbiosis, and to optimize the flow of nutrients to the small intestine of high-yielding ruminants.

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The processing of human foods provides the ruminant husbandry with by-products such as, cereal brans and beet pulp (BP) that are more accessible and cheaper as compared to high-quality forages (1, 2, 6, 9). Notably evident, farmers have been traditionally using a variety of by-products for feeding their livestock as their major source of income (9). It can thus be postulated that adding non-forage fiber sources (NFFS) such as, wheat bran (WB) to the rations containing low-quality forages (such as, straw) in traditional, local Iranian farming should have been a true acknowledgement of a scientific theory that was recently conceptualized by Grant (8). Based on this theory, the simultaneous dietary inclusion of NFFS (with high ruminal passage rate) and low-quality forages may favor the ruminal fermentation via a positive associative effect on nutrient utilization in the well-controlled environment of the rumen.

The Cornell Net Carbohydrate and Protein System (CNCPS) was modeled to fractionate the carbohydrate and crude protein (CP) feed ingredients based on the nutrient requirements of the microbial groups' degrading structural and nonstructural carbohydrates (7). Such fractionation of the nutrients seems necessary for a more accurate prediction of microbial mass flowing into the small intestine thus obtaining the on-farm productive responses (15).

Our objective was to provide essential information on fiber content and CP fractions of NFFS (wheat bran and beet pulp) included in commercial ruminant diets in Isfahan province where the ruminant industry considerably contributes to milk and beef production in Iran.

MATERIALS AND METHODS

Sample collection and analyses

In the summer of 2003**,** fourteen modern dairy farms across Isfahan province in central Iran were visited for feed sampling. The commercial nonforage fiber sources (NFFS) i.e., wheat bran (WB) and dehydrated pelleted beet pulp (BP) were sampled. The WB was sampled from three batches at each of the thirteen farms, and BP was obtained from one batch in all fourteen farms. Feed samples were immediately transported to the Nutrition Laboratory of the Isfahan University of Technology, and stored at 5°C until further analysis. After grinding all samples through a 1 mm screen (Wiley's pulverizer for laboratory, Ogaw Seiki Co., LTD, Tokyo, Japan) and drying at 60°C for 48 h, the samples were analyzed for NDF (16; using heat-resistant α-amylase with no sodium sulfite), ADF (3; ID. 973.18), CP (3; ID. 984.13), and ether extract, EE (3; ID. 920.39). The CP of all feed samples was fractionated into A, B_1, B_2, B_3, and C, respectively representing nonprotein nitrogen (NPN) (tungstic acid method), easily degradable true protein, moderately degradable true protein, slowly degradable or escape true protein, and unavailable protein classified by CNCPS (6) and described by Licitra *et al.* (9). It is noteworthy to mention that, because tungstic acid precipitates peptides down to 3 amino acids to form NPN, more peptides are expected to separate from amino acids, giving a lower value for NPN as compared to the use of trichloroacetic acid (9). Therefore, NPN values reported in our study do not include, at least to some extent, the peptide content of CP.

Statistical analyses

Data for CP fractions, cell wall fibers, and EE contents of WB were analyzed in a completely randomized design using the GLM procedure (14). The differences between means were established using Tukey's multiple range test (14). Data for nutrient composition of BP and WB were analyzed for descriptive statistics using the Univariate procedure (14). Pearson's Correlation Coefficients (14) were used to develop the intra- and inter-relationships within and between fibers and CP fractions, respectively. Student t-test was used to make comparisons between the chemical composition of WB and BP with the corresponding reports by NRC (13) and CNCPS (7). Significant differences between means were considered at $P < 0.05$.

RESULTS AND DISCUSSION

Wheat bran

The CP and EE content of WB were significantly different across farms ($P < 0.0001$, Table 1). However, the C fraction was not different among samples. About 20-25% of wheat kernel is essentially separated as WB after the milling process (6). Wheat bran is composed of seed coat including testa, pericarp and aleurone layer that are rich in protein and fiber, as well as germ or embryo that is rich in protein (12). The ease of access to cheap WB as compared to high-quality forages (e.g., alfalfa) in Iran has made WB an important non-forage fiber and protein source in commercial beef and dairy rations, particularly for mid-and late lactation cows. Therefore, its CP fractions and fibers (although not as effective as forage fiber) would contribute significantly to the ruminal availability of nutrients. The mean CP content of WB used in our study (161.9 g/kg) was lower ($P < 0.01$) than those reported by NRC (173 g/kg, 13) and CNCPS (171 g/kg, 7). The CP of WB in 30% of the farms was, however, above the values of NRC and CNCPS (Table 1). The lower CP of WB could be attributed to either the CP content of original wheat grain varieties indigenously grown in Iran or the manufacturing processes during milling.Assuming the greater importance of the latter, WB used in the current study were expected to contain the starchy endosperm that would stimulate the ruminal fermentability of the dietary energy. Such greater fermentability could be supported by the lower ($P < 0.01$) NDF content (424 vs. 510 g/kg) and lower($P < 0.01$) unavailable CP (6 vs. 10.9 g/kg) of the WB analyzed in this study as compared to that in the CNCPS library (7).

The NPN of WB was higher ($P < 0.01$) in the current study demonstrating a greater level of readily available CP than that reported by the CNCPS (31.4 vs. 22.6 g/kg). This high ruminal availability of CP in WB is thought to be even more pronounced because NPN was determined using tungstic acid in our study and thus does not include all peptides. Indeed, peptides can be easily taken up by some rumen bacteria (10). The B_3 was also greater in WB of our study than that of CNCPS library (31.5 vs. 26.5 g̸ kg). This may show a potential for efficient supply of both quickly degradable CP in the rumen and escape CP to the intestine. The main fraction of CP or B_2 typically found in cereal glutelin and moderately degraded in the rumen (15) was lower in WB analyzed in our study than that listed by CNCPS (64.2 vs. 83.4 g/kg). In addition to the strong positive correlation of CP content with B_2 (r = 0.47; $P < 0.01$) and NPN (r = 0.52; $P < 0.001$) this reveald that the greater CP content of WB reported by others is, at least partly, due to greater B_2.

Table 1. Least square means and descriptive statistics for crude protein and its fractions[1], cell wall fibers, and ether extract in wheat brans commercially used across Isfahan dairy farms (g/kg, DM basis, n = 39)

Dairy farm	CP	A	B_1	B_2	B_3	C	NDF	ADF	EE
Abbasi	176.9 ^{a}	34.8 ^{bcd}	27.0 ^{bcde}	78.9 ^{a}	25.9 ^{bc}	10.1	348.0 ^{g}	123.9 ^{bc}	45.1 ^{ab}
Haghighat nejad	163.7 ^{b}	37.0 ^{b}	20.1 ^{e}	66.5 ^{ab}	36.2 ^{ab}	3.7	388.0 ^{ef}	85.6 ^{d}	41.0 ^{ab}
Nilforooshan	174.3 ^{a}	28.0 ^{cdef}	35.5 ^{a}	68.1 ^{ab}	37.6 ^{a}	5.0	410.6 ^{ed}	108.9 ^{cd}	51.3 ^{a}
Milk & Beef	149.8 ^{e}	26.9 ^{def}	22.9 ^{ed}	59.6 ^{b}	35.6 ^{ab}	4.7	428.6 ^{cd}	114.9 ^{bc}	43.0 ^{ab}
Sepahan	156.8 ^{cd}	26.5 ^{def}	25.6 ^{cde}	66.0 ^{ab}	34.0 ^{ab}	4.6	456.6 ^{abc}	115.0 ^{bc}	44.8 ^{ab}
Goldasht	173.0 ^{a}	35.5 ^{bc}	29.5 ^{abcd}	69.5 ^{ab}	34.7 ^{ab}	3.6	378.0 ^{fg}	103.6 ^{cd}	39.5 ^{b}
IUT [2]	160.8 ^{bc}	34.8 ^{bcd}	31.9 ^{abc}	56.4 ^{b}	32.9 ^{ab}	4.5	460.0 ^{abc}	132.3 ^{bc}	43.8 ^{ab}
Rafieyan	158.8 ^{bc}	22.4 ^{f}	35.7 ^{a}	56.8 ^{b}	36.9 ^{ab}	6.8	484.6 ^{a}	127.1 ^{bc}	42.3 ^{ab}
Taheri	159.7 ^{bc}	24.7 ^{ef}	34.5 ^{ab}	61.5 ^{b}	31.2 ^{ab}	7.6	439.3 ^{cd}	127.6 ^{bc}	45.3 ^{ab}
Dehdashti	176.4 ^{a}	47.6 ^{a}	27.7 ^{abcde}	62.3 ^{b}	30.9 ^{ab}	7.7	446.6 ^{bc}	142.7 ^{ab}	51.5 ^{a}
Saber ali	154.9 ^{cde}	31.7 ^{bcde}	25.1 ^{cde}	60.1 ^{b}	28.7 ^{ab}	9.2	474 ^{ab}	170.0 ^{a}	45.0 ^{ab}
Jamali	151.1 ^{de}	34.5 ^{bcd}	23.6 ^{ed}	59.1 ^{b}	29.1 ^{ab}	4.6	436 ^{cd}	120.0 ^{bc}	45.1 ^{ab}
Namfar	149.0 ^{e}	23.9 ^{ef}	33.9 ^{ab}	69.7 ^{ab}	15.6 ^{c}	6.0	367.3 ^{fg}	117.7 ^{bc}	43.1 ^{ab}
P	<.0001	<.0001	<.0001	.0003	<.0001	0.15	<.0001	<.0001	0.02
Mean across farms	161.9	31.4	28.7	64.2	31.5	6.0	424.5	122.3	44.7
SEM [3]	1.3	1.6	1.5	2.8	2.1	1.6	6.1	5.5.0	2.1
Maximum	179.1	51.9	39.2	81.0	39.6	19.1	492.0	177.0	58.5
Minimum	148.2	21.4	18.9	51.1	9.9	2.7	334.0	77.0	37.5
Quant. 75	173.2	34.8	32.7	69.7	35.4	7.4	456.0	130.0	47.5
Quant. 25	153.6	26.5	23.8	58.7	294	4.0	380.0	109.9	41.5

[1]A, non-protein N; B_1, easily degradable CP; B_2, moderately degradable CP; B_3, slowly degradable CP; C, unavailable CP (10, 15). n = total number of observations.
[2] IUT, Isfahan University of Technology's research dairy farm.
[3] SEM, standard error of the mean; Quant. 25, 25% of observations are lower than or equal to this value; Quant. 75, 75% of observations are lower than or equal to this value.

Overall, other dietary factors seem to be important in optimizing the ruminal efficiency of CP utilization when WB is included in commercial rations. The negative correlation of CP and NDF (r = -0.33; $P < 0.05$) in WB could be explained by the greater NDF and lower CP in the seed coat (testa and pericarp) than in the germ. The C fraction was positively correlated with ADF levels (r = 0.54; $P < 0.01$). The C fraction is essentially linked with indigestible fibers and is unavailable in both the rumen and small intestine (15).

Beet pulp

Substitution of BP for part of dietary starch in rations of high-producing dairy cows has been recommended by Woods *et al.* (17). The NDF values for BP provided by NRC (13) and CNCPS (7) are 458 and 446 g/kg; both higher ($P < 0.05$) than the 420.4 g/kg obtained in this study (Table 2). The NRC (13) values for ADF of BP ($P < 0.01$) are greater than the values determined in our study (231 vs. 198.5 g/kg). The discrepancies in fiber contents of BP could be partly explained by the greater CP (108.6 vs. 100and 98 g/kg; $P < 0.05$) at the expense of cell wall fibers in the BP commercially used across Isfahan than those reported by NRC (13) and CNCPS (7). In an Irish study, Woods *et al.* (17) reported similar CP (104.6 vs. 108.6g/kg), and greater NDF (528 vs. 420.4 g/kg) and ADF (276 vs. 198.5 g/kg) for BP as compared to that in our study. In contrast, in a Californian study, Arosemena *et al.* (2) reported a lower ADF (188.1g /kg) for BP as compared to our finding.

According to CNCPS (7), the main CP fraction of BP is B_3 or slowly degradable CP in the rumen (41.9% of CP), while in the samples of the present study, NPN had the greatest (38.7% of CP) level (Table 2).

Table 2. Crude protein and its fractions[1], cell wall fibers, and ether extract of beet pulp commercially used across Isfahan dairy farms (g/ kg, DM basis, n=14)

Item	CP	A	B_1	B_2	B_3	C	NDF	ADF	EE
Mean[2]	108.6	38.7	3.6	11.4	37.3	17.6	420.4	198.5	5.1
SD[2]	8.4	7.8	4.3	4.9	8.3	4.8	362.0	27.2	1.6
Maximum	126.0	46.6	15.7	18.0	49.4	27.9	487.3	254.8	10.0
Minimum	100.0	16.3	1.0	2.2	17.7	12.6	364.0	161.0	3.0
Quant. 75	110.7	43.3	3.9	15.2	43.0	18.7	546.0	392.0	5.7
Quant 25	101.7	37.0	1.0	8.8	34.0	15.0	294.5	191.6	4.0

[1] A, non-protein N; B_1, easily degradable CP; B_2, moderately degradable CP, and C, slowly degradable CP (10, 15). ADF, acid detergent fiber; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber. n = total number of observations.
[2] Mean across farms; SD, standard deviation; Quant. 25, 25% of observations are lower than or equal to this value; Quant. 75, 75% of observations are lower than or equal to this value.

Moreover, the more quickly degradable CP fraction (B_1) of BP was greater in our study (3.6 vs. 1.1 g/kg), whilst the moderately degradable fraction (B_2) was greater ($P < 0.01$) in CNCPS library (19.6 vs. 11.4 g/kg). Despite lower ADF, the unavailable CP was greater ($P < 0.01$) in the current study than that of CNCPS (7) (17.6 vs. 10.8 g/kg). Hence, the two extremes in the degradation rate of CP were both greater in the BP commercially used in central Iran than that reported by the CNCPS (7). This means that a more dynamic evaluation of ruminal nutrients released from other dietary ingredients is necessary to synchronously supplement those of BP. It can be speculated that greater NPN and unavailable CP may lead to a lower maximal allowance for the inclusion of BP in the rations of high-yielding ruminants exposed to greater risk from the fermentation asynchrony.

The fraction C significantly increased ($P < 0.001$) as the level of ADF in BP increased. This is because fraction C represents the CP associated with ADF either as lignin-surrounded, tannin-bound, or post-browning CHO-linked CP (15). The negative relationship ($r = - 0.56$; $P < 0.05$) between NDF and NPN could support the greater CP and NPN and the lower NDF content of BP in the present study as compared to those of other reports.

CONCLUSIONS

Results of the present study revealed the significant variability in CP fractions and fibers of the WB commercially used across Isfahan. The fiber content and remarkably high NPN, and B_1 of BP used by dairy producers across Isfahan could provide the rumen with more non-fiber carbohydrates (e.g., pectins) and degradable N required for ample microbial growth. Further evaluation of other nutrients, preferably in larger scales, is needed for a more predictable on-farm animal response.

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