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# Characterization of the Nutritive Value and Protein Fractions the Cornell Net Carbohydrate and Protein System in White and Red Grape (*Vitis vinifera* sp.) Pomace

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**Abstract:** The objective of this study investigate and compare the protein fraction of scheme CNCPS and chemical composition of seeds and pulps from White Grape Pomace (WGP) and Red Grape Pomace (RGP) origin as conventional feed in ruminants. The data was analyzed by using SAS 9.1 in completely randomized design. Comparison between the general compositions of both whole GPs, reveals important significant differences in ADF ($p<0.05$) and protein ($p<0.05$) values but chemical composition had non-significant difference (NDF, EE, OM, DM) in whole GPs ($p>0.01$). WGP seeds had higher CP ($p<0.05$) and ADF ($p<0.05$) than RGP seeds but RGP seeds had higher NDF ($p<0.01$) than RGP seeds but chemical composition had non-significant difference (EE, OM, DM) in whole GPs ($p<0.05$). Chemical analysis of GP pulp is remarkably similar with the notable exception of a lower level of ADF ($p<0.01$) and CP ($p<0.05$) in RGP pulp which had lower than WGP pulp. Nevertheless, chemical composition had non-significant difference (EE, OM, DM) in GPs pulp ($p<0.05$). Protein fractions A, B2 and B3 GPs had non-significant difference ($p<0.05$) but WGP had B1 lower ($p<0.05$) and B3 higher ($p<0.01$) than RGP (19.70, 13.40 and 28.06, 9.77 (CP%), respectively). WGP seeds had higher ($p<0.01$) C than RGP seeds (19.23 and 15.14 (CP%). Although, RGP seeds had higher ($p<0.01$) B2 than WGP seeds (10.70 and 4.33). WGP pulp had higher C ($p<0.05$) and B3 ($p<0.05$) than RGP pulp (19.34, 19.23 and 12.07, 15.14, respectively). Results showed that high proportion protein WGP and RGP is non-available for ruminant.

**Key words:** Nutritive value, protein fractions, cornell net carbohydrate and protein system, white grape, red grape, *Vitis viniferous*, pomace

## INTRODUCTION

Grape is one of the most important fruit crops in the world and its production in huge amounts in many parts of the world (Reynal *et al*., 2007), production of this by-product exceeds 50,000 tons year$^{-1}$ (Alipour and Rouzbehan, 2007) and Pacific Northwest region of the United States are the leading wine grape production states in the country in which Washington produced 156,000 tons in 2009 and Oregon 40,200 tons in 2009 (USDA-NASS, 2010a, b).

Grape pomace (*Vitis vinifera* sp.) is produced about 23% of the total grapes harvested are table grapes for fresh consumption while 86.6% of the crop is processed especially for wine making (Liu *et al*., 2006). Wine grapes are typically processed by crushing to extract variable amounts of juice, depending upon the wine being made, leaving a residue referred to as Grape Pomace (GP) that is mainly seeds, skin and pulp (18-20 kg/100 kg of grapes).

Thus, any useful production from these by-products could represent an interesting advance in the maintenance of the environmental equilibrium and economic revaluation of the raw material. On the other hand in the zones of wine production, great quantities of residues are generated, causing problems both in economical and ecological terms. In major wine growing regions such as France, Spain, Greece and the South of Australia, the seasonal utilization of GP in animal feeding is common because of its low price level if fed in a fresh state. Frreira *et al*. (1996) concluded that GP could partially replace alfalfa as a fiber source in diets for rabbits without adversely affecting growth.

Several investigations showed that dried or ensiled GP is very low in energy content but it could be used as a part of diets for ruminants fed close to maintenance level, especially for sheep. Nevertheless, traditional uses of GP have largely been restricted to land application due to be low nutritive value for ruminants (Alipour and

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Rouzbehan, 2007; Baumgartel *et al*., 2007; Bocque *et al*., 1984) and high moisture for stocking. Under these economic and environmental constraints, improving the efficiency of N utilization and reducing N excreted are very important to maintain the sustainability of dairy farms and nutrition models have become an effective farm management tool to accomplish the set asks (Fox *et al*., 2004; Wattiaux and Karg, 2004 ).

Reliable predictions of nutrient supply are critical for mathematical models to predict the effects of nutrients absorbed on milk composition and N efficiency because any intermediary metabolism model would rely on rumen models for their substrates (Tylutki *et al*., 2008; Dinn *et al*., 1998). The Cornell Net Carbohydrate and Protein System Model (CNCPS) was developed to predict requirements, feed utilization and nutrient excretion for ruminant in unique production settings. Feed protein fractionation systems have been integrated into nutrition models to account for differences in protein availability and utilization.

This model integrates knowledge of ruminant requirements as influenced by breed type and body size, production level and environment with the knowledge about feed composition, digestion and metabolism in supplying nutrients to meet requirements. The CP of all feedstuffs was partitioned into five fractions (A, B1-B3 and C) according to the CNCPS (Russell *et al*., 1992; Sniffen *et al*., 1992) using standardization and recommendations published by Licitra *et al*. (1996). Fraction A is Non-protein Nitrogen (NPN), B is true protein and C is unavailable true protein or bound protein. Fraction B is further divided into three fractions (B1-B3) that are believed to have different rates of ruminal degradation. Fractions A and B1 are soluble in borate phosphate buffer and are rapidly degraded in the rumen. Fraction B2 is fermented in the rumen at lower rates than buffer-soluble fractions and some fraction B2 escapes to the lower gut. Fraction B3 is believed to be more slowly degraded in the rumen than are fractions B1 and B2 because of its association with the cell wall; a larger proportion of B3 is thus believed to escape the rumen. Fraction C is the ADIP and is highly resistant to breakdown by microbial and mammalian enzymes and it is assumed unavailable for the animal. Fox *et al*. (2004) assessed the impact of feed carbohydrate and protein fractions and microbial composition on animal performance predictions (Goni *et al*., 2005) determined the inputs that routinely need to be analyzed to reduce risk of use of the CNCPS Model in field conditions. Limited information on the comparison nutritive value and protein fractions the CNCPS in White Grape Pomace (WGP) and Red Grape Pomace (RGP) previous studies involving seeds, grape or whole GP is unavailable. The objective of this study investigate and compare the protein fractionation of scheme CNCPS and chemical composition of seeds and pulps from WGP white and RGP origin as conventional feed in ruminants.

## MATERIALS AND METHODS

Grape pomaces obtained from winery production factories located in Urmia, Iran. The stalks are removed from the fresh material to avoid replacement tannins which particularly located in the green stalks. Pomace was weighed in portions of 1.2 kg immediately after receipt from the winery and frozen at -4°C. Sub-samples were taken, dried and ground to pass through a 1 mm screen and stored in a freezer until for chemical analyses. Feedstuffs Dry Matter (DM, Method ID 934.01), ash (Method ID 942.05), Ether Extract (EE, Method ID 920.30) and Crude Protein (CP, Method ID 984.13) were determined by procedures of AOAC (1997). The Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were determined according to the procedure of (Van Soest *et al*., 1991) without amylase.

The CP of all samples was partitioned into five fractions (A, B1-B3 and C) according to the CNCPS (Russell *et al*., 1992; Sniffen *et al*., 1992). To separate the True Protein (TP) and Non-protein Nitrogen (NPN) (Fraction A), Trichloroacetic Acid (TCA) was used according to the method described by Licitra *et al*. (1996). The TP was separated from the NPN by precipitation with TCA (final concentration 10%). Filtering was done by gravity and NPN was calculated as the difference between total sample N and the N content of the residue after filtration. Buffer soluble protein was defined as the true protein soluble in a borate-phosphate buffer at pH 6.7-6.8 (Krishnamoorthy *et al*., 1982). Samples were filtered by gravity through Whatman #541 filter papers for subsequent Kjeldahl nitrogen analysis.

The insoluble N fraction after filtration was defined as the buffer Insoluble Protein (IP) fraction. Soluble true protein (Fraction B1) was calculated as the difference between TP and IP. Neutral detergent soluble protein (Fraction B2) was estimated as the difference between IP and Protein Insoluble in Neutral Detergent (NDIP). The amount of soluble fibre-bound CP (Fraction B3) was calculated as CP in NDF minus acid detergent insoluble CP. Acid Detergent Fiber (ADF) was prepared according to (Van Soest *et al*., 1991) with filtering by gravity on 12.5 cm Whatman # 541 filter papers. Residual N×6.25 on the filter paper (Acid Detergent Insoluble Protein: ADIP) was classified as fraction C. The values of CP in all the fractions including NPN were calculated as

g N×6.25 $kg^{-1}$ CP. All analyses of CP and CP fractions were carried out at least in duplicate. The data was analyzed by using SAS 9.1 (SAS Institute, 2006) in completely randomized design.

## RESULTS AND DISCUSSION

**General composition:** The chemical composition GPs and GP fractions were markedly different has shown in Table 1. Comparison between the general compositions of both whole GPs reveals important significant differences in ADF ($p<0.01$) and protein ($p<0.05$) values but chemical composition had non-significant difference (NDF, EE, OM, DM) in whole GPs ($p>0.01$). WGP seeds had higher CP ($p<0.01$) and ADF ($p<0.01$) than RGP seeds but RGP seeds had higher NDF (<0.01) than RGP seeds but chemical composition had non-significant difference (EE, OM, DM) in whole GPs ($p>0.01$).

Chemical analysis of GP pulp is remarkably similar with the notable exception of a lower level of ADF ($p<0.01$) and CP ($p<0.05$) in RGP pulp which had lower than WGP pulp. Nevertheless, chemical composition had non-significant difference (EE, OM, DM) in GPs pulp ($p>0.05$). While the WGP still contained a substantial concentration of ADF that show the content of fiber fractions was much higher than RGP (whole, seeds and pulps). WGP and RGP protein content in the study was similar to the values reported by Bravo and Saura-Calixto (1998) and WGP pulp protein content (Valiente *et al*., 1995). The CP, NDF and ADF contents of WGP in the study were higher than those reported by Zalikaranab *et al*. (2007). But EE content was lower than those reported by Zalikarnab *et al*. (2007). The EE content WGP and RGP and partition GPs had non-significance difference (Aghsaghali *et al*., 2011) reported that CP content in WGP was 17.27% and Crude Fiber (CF), NDF and ADF in WGP were 22.8, 59.5 and 52.5%, respectively. Besharati and Taghizadeh (2009) reported the CP, ADF, NDF, TT, TP and ash contents in DGB were 63.5, 255, 259, 52.3, 67 and 74 g $kg^{-1}$ DM, respectively. Also, Llobera and Canellas (2007) reported the Dietary Fibre (DF) was the main component of the pomace >70% of the dry matter.

Table 1: Analyzed chemical composition of the grape pomace under study (dry matter%)

| Grape pomace | White | Red |
|---|---|---|
| **Whole** | | |
| Dry matter (%) | 88.44±2.61$^{NS}$ | 80.6±11.61$^{NS}$ |
| Organic matter | 94.35±0.58$^{NS}$ | 94.58±1.81$^{NS}$ |
| Crude Protein (CP) | 15.48±1.44* | 13.97±0.07* |
| Ether extract | 11.31±0.82$^{NS}$ | 11.33±3.51$^{NS}$ |
| Neutral Detergent Fiber (NDF) | 28.23±9.46$^{NS}$ | 24.68±3.38$^{NS}$ |
| Acid Detergent Fiber (ADF) | 41.52±2.25** | 25.71±2.22** |
| **Seeds** | | |
| Dry matter (%) | 92.15±2.80$^{NS}$ | 92.45±2.51$^{NS}$ |
| Organic matter | 95.02±0.99$^{NS}$ | 95.99±1.70$^{NS}$ |
| Crude Protein (CP) | 25.57±1.27** | 21.10 ±1.21** |
| Ether extract | 11.25±3.42$^{NS}$ | 14.33±1.52$^{NS}$ |
| Neutral Detergent Fiber (NDF) | 12.48±0.86** | 14.49±0.33** |
| Acid Detergent Fiber (ADF) | 46.5±40.08** | 25.71±1.79** |
| **Pulp** | | |
| Dry matter (%) | 77.22±1.42$^{NS}$ | 87.13±4.39$^{NS}$ |
| Organic matter | 92.5±10.33$^{NS}$ | 92.25±2.86$^{NS}$ |
| Crude Protein (CP) | 18.41±0.61* | 16.84±1.51* |
| Ether extract | 9.75±3.26$^{NS}$ | 8.35±1.52$^{NS}$ |
| Neutral Detergent Fiber (NDF) | 8.85±8.73$^{NS}$ | 9.06±6.67$^{NS}$ |
| Acid Detergent Fiber (ADF) | 43.02±4.47** | 20.38±9.83** |

*Means in a column differ significantly ($p<0.05$); **($p<0.01$); $^{NS}$Means in a column differ non-significant

Some of investigator reported the seed oil has high unsaturated fatty acid levels, >80% linoleic acid being predominant (Cao and Ito, 2003; Kamel *et al*., 1985). But D'Urso and Nicolosi Asmundo reported the major limitation of use of GP as a ruminant feed is the presence of grape seeds which are high in lignified fiber and are often largely undigested in bovines because few of the seeds are broken open during eating or rumination thereby preventing the grape seed oil from being digested. Organic Matter (OM) content is lower than other values described for similar GP obtained from WGP and RGP (5.7-9.2%) (Bravo and Saura-Calixto, 1998; Valiente *et al*., 1995). Moreover, the skin and pulp fraction has a lower fiber with less lignin as well as generally lower levels of secondary compounds (Makris *et al*., 2007). The ADF content had more than other nutritive value in GPs. As this large value would be a good indicator of the considerable amounts of celluloses and hemicelluloses present in the pomace. Grape seeds are not accurately characterized as ruminant feeds by conventional chemical analyses such as OM, CP, fat, NDF and ADF. Indeed, based upon those values 40- 60 ash, 120-125 CP and 100-140 g $kg^{-1}$ ether extract and only about 500 g $kg^{-1}$ a NDF their feeding value might be considered similar to high oil corn silage (Baumgartel *et al*., 2007).

**Protein fractions:** Protein fractions for seed and pulp GPs had shown in Table 2. Protein fractions A, B2 and B3 GPs had non-significant difference ($p>0.01$) but WGP had B1 lower ($p<0.01$) and B3 higher ($p<0.01$) than RGP (19.70,

Table 2: Protein fraction in white and red grape pomace (CP%)

| Grape pomace | White | Red |
|---|---|---|
| **Whole** | | |
| A | 32.68±5.60$^{NS}$ | 29.93±3.03$^{NS}$ |
| B1 | 19.76±3.48** | 28.06±33.16** |
| B2 | 19.29±6.23$^{NS}$ | 16.36±1.68$^{NS}$ |
| B3 | 14.87±2.72$^{NS}$ | 15.88±1.97$^{NS}$ |
| C | 13.40±0.36** | 9.77±0.22** |
| **Seed** | | |
| A | 12.71±0.65$^{NS}$ | 14.35±0.21$^{NS}$ |
| B1 | 36.54±3.42$^{NS}$ | 37.34±3.34$^{NS}$ |
| B2 | 4.33±1.20* | 10.70±2.47* |
| B3 | 16.26±1.02$^{NS}$ | 22.60±3.27$^{NS}$ |
| C | 30.16±3.27** | 15.01±0.22** |
| **Pulp** | | |
| A | 22.06±4.70$^{NS}$ | 37.85±0.75$^{NS}$ |
| B1 | 28.30±7.66$^{NS}$ | 24.73±5.70$^{NS}$ |
| B2 | 11.07±0.56$^{NS}$ | 10.21±2.44$^{NS}$ |
| B3 | 19.34±3.05* | 12.07±1.68* |
| C | 19.23±0.51** | 15.14±3.05* |

*Means in a column differ significantly ($p<0.05$); **($p<0.01$); $^{NS}$Means in a column difference non-significant

13.40 and 28.06, 9.77 (CP %), respectively). WGP seeds had higher ($p<0.01$) C than RGP seeds (19.23 and 15.14 (CP %). Although, RGP seeds had higher ($p<0.01$) B2 than WGP seeds (10.70 and 4.33). WGP pulp had higher C ($p<0.01$) and B3 ($p<0.05$) than RGP pulp (19.34, 19.23 and 12.07 15.14, respectively). The amount of protein in each fraction that escapes ruminal degradation is calculated by the model based on relative rates of degradation and rates of passage.

For the protein that escapes ruminal degradation, the model assigns intestinal digestibility coefficients specific to each of the protein fractions. Intestinal digestibility coefficients of 100, 100, 80 and 0% are assigned to the undegraded B1-B3 and C protein fractions, respectively. The pool sizes of the fraction A and soluble true protein have been updated to reflect the presence of small peptides in what was previously considered the fraction A. The soluble proteins and peptides move with the liquid phase from the rumen to the small intestine and supply the cow with AA (Reynal *et al*., 2007; Volden *et al*., 2002) thus, to account for the AA profile of these peptides, researchers need to provide an AA profile for the soluble pool. This is currently being done by mathematical manipulation of the pools and rates but a more robust approach is needed to account for more variation in the predicted AA flow. Foodstuffs vary widely in NPN, rate and extent of ruminal protein degradation, intestinal digestibility and Essential Amino Acid (EAA) supply (Broderick *et al*., 1989).

Degradation rates for the B1 fraction were reduced to reflect available published data and integrated with liquid rather than particle passage rate as assumed in the CNCPS. The MP supply for both GPs and GP fractions was sensitive because the B1 fraction represented a as well as proportion of the total protein supply (WGP and RGP, 19.76 and 31.03 of the total CP). Passage rates predicted by the CNCPS passage rate small changes in extent of B1 degradation. However, these modifications resulted in an increase in the Lys and Met flows, especially for the high protein diet, because Lys and Met flows were more sensitive to the variation in B1 fraction than total RUP flows (Lanzas *et al*., 2007). In addition, Lanzas *et al*. (2007) reported that Lys and Met flows were very sensitive to intestinal digestibilities. According to the CNCPS some fraction B2 is fermented in the rumen with degradation rate of 50-150 g $kg^{-1}$ h and some escapes to the lower gut (Sniffen *et al*., 1992).

Metabolizable protein and AA flows were sensitive to the degradation rates of the B protein fraction in the NRC and the B2 fraction in the CNCPS and intestinal digestibilities (Lanzas *et al*., 2007). The collapse of the fractions B2 and B3 had a greater effect on the RUP flows for the low protein diet because the B3 fraction represents a greater proportion of the total protein. The low rates for the protein B3 fraction are not always supported by the data (NRC, 2001; Coblentz *et al*., 1999).

Despite the differences in the WGP and RGP protein schemes CNCPS predictions of MP supply were similar in sensitivity to variation in protein, fractions and their degradation rates because of the use of common principles such as the competition between digestion and passage to predict site of digestion and the first-limiting nutrient to estimate microbial growth. Fraction C contains proteins associated with lignin, tannin-protein complexes and Maillard products that are not degradable in the rumen and are indigestible in the intestine (Krishnamoorthy *et al*., 1982). The content fraction C between the all fractions WGP and RGP signification difference was observed. Also, Llobera and Canellas (2007) show that high contents of Condensed Tannins (CT) of the order of 10.3% of dry matter are found in the RGP. Based on these results and in agreement with Spanghero *et al*. (2009) recent researches for (ADICP) fraction C for pulp and seed grape pomaces. It can be noted that the percentage of the WGP Resistant Protein (RP) is higher than that found in the pomace sample in spite of the WGP presenting higher Condensed Tannins (CT) amounts thus, revealing a better ability of the RGP CT to form tannin protein complexes. Hagerman *et al*. (1992) reported that tannins reduced CP digestibility. Goni *et al*. (2005) reported the protein concentration in the indigestible residues was high (14%) indicating that about 88% of the protein content in grapes was not digested by the digestive enzymes and may reach the colon along with digestible fiber constituents in nonruminant. The considerable amount of fraction C (protein in ADF)

showed that the WGP seeds proteins were poorly solubilized and degraded by the endogenous enzymes and microorganism in rumen. This was probably caused by the high lignin content since tannins are known to inhibit proteolysis enzymes.

In addition to protein, Klason Lignin (KL) contains important amounts of condensed CT. In the GP sample, the highly condensed tannins present a value of 22.3% of dry (Aghsaghali *et al*., 2011) smaller than that described by Bravo and Saura-Calixto (1998) for skins of red grapes (27% of dm). These compounds CT are found mainly in the KL fraction thus, confirming that CT is the principal components of KL. The high CT contents justify the presence of large values of resistant protein and the low protein digestibility by the formation of insoluble tannin protein complexes which remain, basically in the KL residue.

Llobera and Canellas (2007) reported the uronic acids present in indigestible fiber of the GP are also very high but their contribution to the total content of indigestible fiber is in a smaller proportion (8%). KL of the GP content is described for RGP and WGP (Bravo and Saura-Calixto, 1998). Fraction C is considered to be completely undegradable in the rumen and completely indigestible in the ruminant therefore if feeds contain higher proportions of fraction C, the intestinal absorption of dietary protein will be predicted by the model to be lower than if feeds contain greater proportions of the other fractions.

## CONCLUSION

This fact reveals a low digestibility of the protein fraction as the high fraction C content in CP%. Researchers found resistant protein in WGP seed had more protein that is resistant in GPs. If the seeds could be separated from the GP, the latter may have potential as a ruminant feed while allowing the former to be used for other purposes. This inconsistency may be due to GP varieties, different methods of grape processing, fruit maturity, management after harvest and growing conditions (including geographic/climatic conditions). Variations in chemical composition can lead to different nutritive value because chemical composition is one of the most important indices of nutritive value of feeds. Presuming adequate hygiene status, GP can be included in diets for ruminants especially when fed near to maintenance or in situations when high rates of growth or milk production are not needed especially sheep or goat. This large value can be explained, not only in terms of the grape variety differences but also in terms of the different contribution of the pulp and seed residues contained in WGP and RGP. Further research is needed to evaluate alternative ways to overcome negative effects of tannins in GP on protein fraction as well as to assess their impacts on the animal performance.

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