ECRONICON OPEN ACCESS

EC NUTRITION
Research Article

Nutrient Absorption in Rats Fed with Diets Containing Grape Pomace of Different Particle Sizes

Jianmei Yu[1]*, Ivy N Smith[1] and Steven L Hurley[2]

[1]Department of Family and Consumer Sciences, North Carolina Agricultural and Technical State University, USA

[2]Department of Animal Science, North Carolina Agricultural and Technical State University, USA

***Corresponding Author**: Jianmei Yu, Department of Family and Consumer Sciences, North Carolina Agricultural and Technical State University, USA.

Received: August 11, 2017; **Published:** September 12, 2017

Abstract

This study investigated the effect of particle size of polyphenol and fiber rich grape pomace (GP) on feed intake, body weight and nutrient absorption using a rat model. Twenty adult Sprague Dawley female rats of 15-week old were randomly assigned to one of the four treatment groups fed diet containing 0% and 7.71% GP of three different particle sizes for 8 weeks. The group fed with 0% GP diet was used as control. Feed consumption and feces weight of each group were recorded every other day, body weight was measured weekly. The proximate composition including moisture, ash (minerals), crude protein and crude fat of fecal samples were determined experimentally, total carbohydrate of fecal material was obtained by difference. Data show that feed consumption and body weight of each group was significantly affected by treatment time, but not by diet GP content and particle size. The quantities of crude protein and total carbohydrate of rat feces increased due to the addition of GP in the diet, while the crude fat and ash contents of rat feces were not affected by the presence of GP in the diet. The particle size of GP influenced the total amount of feces excreted by each rat and carbohydrate absorption, but did not affect the quantities of other nutrients in the feces. This study demonstrated adding GP in the diet had significant impact on the absorption protein and carbohydrate, but not fat and minerals.

Keywords: *Grape Pomace; Particle Size; Nutrient Absorption; Rat Model*

Abbreviations

GP: Grape Pomace; TDF: Total Dietary Fiber; TPC: Total Phenolic Concentration; GAE: Gallic Acid Equivalent; AOAC: Association of Official Analytical Chemists; IACUC: Institutional Animal Care and Use Committee; GI: Gastrointestinal

Introduction

Excessive intake of energy dense foods and lower intake of fiber rich foods such as whole grain and fresh produce are major contributing factors of the current overweight and obesity epidemic. Both epidemiologic and intervention studies support dietary fiber, particularly, insoluble dietary fiber, intake is inversely associated with body weight, body fat and body mass index. Consumption of dietary fiber offers benefits including lower risk for coronary diseases, diabetes, and certain gastrointestinal diseases [1], but the average fiber intake of adults in the United States is less than half of the recommended levels [2]. Packing sufficient amounts of fiber in Americans' favorite foods is one method to reduce total energy intake.

There are increasing interests in applying fruit processing wastes as functional food ingredients since they are rich source of dietary fiber and beneficial bioactive compounds. Grinding or milling is a common practice to reduce particle size of food ingredients for better acceptability of products. Particle size reduction also affects polyphenol extractability, digestibility, functional and physicochemical properties of polyphenol and dietary fiber rich food ingredients. It was reported that the reduction in the particle size from 1127 to 550 micrometer resulted in increased hydration properties of coconut fiber [3]; ultrafine grinding of wheat bran dietary fiber resulted in reduced hydration properties, but increased total extractable polyphenols, metal chelating activity and reducing power of the fiber [4].

Grape is one of the oldest, largest and economically important fruit crops domesticated by humans. The uses of grapes in wine making generate large quantity of grape pomace (GP), consisting mainly grape seed and skins, Which contains high phenolic compounds and dietary fiber [5,6]. Grape pomace (GP) is a good fiber source. The total dietary fiber of Manto Negro grape pomace was about 75% of dry mass [6]. Total polyphenol content in dry grape pomace is about 4.8 - 9.6% [7] depending on the variety of grape. Grape pomace polyphenols exhibited a wide range of beneficial biologic properties acting as antioxidants and anti-inflammatory [8]. Therefore, grape pomace has great potential to serve as a cheap source of antioxidant rich dietary fiber to improve the nutritional value of food products. Both *in vitro* and *in vivo* studies found that GP polyphenols significantly inhibit the activities of digestive enzymes such as α-amylase, alpha-glucosidase, lipase and protease [9,10] which could lead to reduced absorption of macronutrients and weight gain. This study demonstrated the effect of adding GP in the diet on the absorption of protein, lipid, carbohydrate and minerals using a rat model.

Materials and Methods

Particle size analysis of ground grape pomace

Grape pomace from Muscadine Noble was obtained from a North Carolina winery. Pomace was collected right after pressing from the winery then immediately stored in the refrigerator. The pomace was then dried at 80°C for 24 hours using an Isotope Vacuum Oven (Fisher Scientific, USA). After removing the free seeds (the seeds not wrapped in skins), the pomace was ground into powder using a coffee grinder. The powders were sieved to pass 40, 60 and 80 mesh sieves using a set of sieves and sieve shaker (Fisher Scientific, USA), and three fractions of GP powders were collected separately and defined as coarse, medium and fine particles respectively. The particle size distribution of each fraction was analyzed using a Laser Particle Size Analyzer (Microtrac Inc., PA, USA).

Diet formulation and proximate composition analysis of diets

AIN-93M supplemented per kg with 10g of cholesterol source was used as base diet (BioServe). Diets containing GP were formulated by replacing the total carbohydrate in base diet by 10% GP of different particle sizes. This resulted in diets containing 7.71% GP. Four treatment diets used in the study were control diet, diet containing 7.7% of fine GP, diet containing 7.7% of medium GP and diet containing 7.7% of coarse GP.

Proximate composition, polyphenol and total dietary fiber (TDF) analysis of rat diets

The moisture, ash, crude fat and crude protein content (%) of formulated diets were determined experimentally, and the total carbohydrate was obtained by difference. Polyphenol in the diet samples was extracted by 70% ethanol solution. The total phenolic concentrations (TPC) of the extracts were determined by Folin-Ciocalteu method [11] and expressed as gallic acid equivalent (GAE) (mg gallic acid/ml extract). The phenolic content of the reformulated diet powder was expressed mg GAE/per gram feed. The TDF of each diet was determined according to AOAC official method 991.43 [12] using a TDF kit purchased from Sigma-Aldrich (St. Louis, MO USA). The analysis of TPC and TDF for each formula were conducted in triplicate.

Animal Experiment

The animal experiment was conducted in accordance to an protocol approved by the IACUC Committee at North Carolina Agricultural and Technical State University (protocol number: 16-007.0). Twenty adult Sprague Dawley female rats of 15-week old were purchase from Envigo RMS Inc (Indianapolis, IN USA). After two weeks acclimation feeding period, rats were randomly assigned to one of the four treatment groups for 8 weeks. The group of rats fed with control diet (0% GP) was used as control. Each group had 5 rats. Each rat was placed in a suspended stainless steel cage and housed in an animal room with controlled temperature (23°C), humidity (40%) and light cycle (12 hours light and 12 hours dark). The animals were offered 100g of feed initially. Feed consumption was measured by the weight difference between feed offered and feed left. Feed consumption and fecal sample of each group were measured every other day, and expressed average feed consumption and feces excretion per rat per week. The body weight of each rat was recorded weekly.

Analysis of nutrients in fecal materials

Fecal samples were dried in a vacuum oven for 18 hours and weight loss (moistures) were recorded. The ash, crude fat and crude protein content (%) of fecal samples were determined experimentally using dry fecal samples. The ash was used as indicator of total mineral content of rat feces. Total carbohydrate of fecal material was obtained by difference as follows:

Carbohydrate (% dry base) = 100 - protein% - Fat% - Ash%

Total amount of each nutrient excreted into feces per week for each rat was calculated according to the weight of dry feces produced by each rat per week as follows:

Nutrient excreted (g/rat.week) = Weight of dry feces (g/rat.week) x Nutrient% (dry base)

Results

Particle sizes of different fractions of grape pomace

The average particle sizes of GP passed through 20, 40 and 80 mesh sieves were 486, 209 and 104 μm, respectively, as determined by a Laser Particle Size Analyzer. The three fractions of GP powder corresponding to the three particle sizes were defined as coarse, medium and fine particles.

Chemical Composition of rat diets

Table 1 shows that addition of 7.7% GP powder in the rat diet decreased the moisture contents of diet by about 0.4%, but increased ash and TDF contents of diet by 0.6% and 4.35%, respectively; while crude fat, crude protein and total carbohydrate contents were not changed. However, the total polyphenol content of the diet increased from 147 mg/kg to 1117 mg/kg.

GP (%)	0	7.71
Moisture (%)	4.27 ± 0.25	3.86 ± 0.12
Ash (%)	2.60 ± 0.03	3.20 ± 0.04
Crude Fat (%)	5.55 ± 0.30	5.57 ± 0.29
Crude Protein (%)	18.28 ± 0.18	18.28 ± 0.21
Total Carbohydrate (%)	68.30 ± 0.76	69.09 ± 0.66
TDF (%)	5.19 ± 0.25	9.50 ± 0.30
TP (mg/kg)	146.77 ± 0.62	1117.15 ± 29.15

***Table 1:** Proximate composition, dietary fiber and total polyphenol contents of rat diet.*

Feed consumption and body weight of rats in different treatment groups

Figure 1A shows that feed consumption gradually decreased with feeding time (treatment time) for all treatment groups, but there was no significant difference among treatment groups in the same feeding period at P = 0.05. Similarly, Figure 1B shows that the weights of rats in treatment groups were not statistically different from that of control group. The results suggest that 10% addition of GP in the diet and the particle size of GP did not affect the feed consumption and the weights of rats.

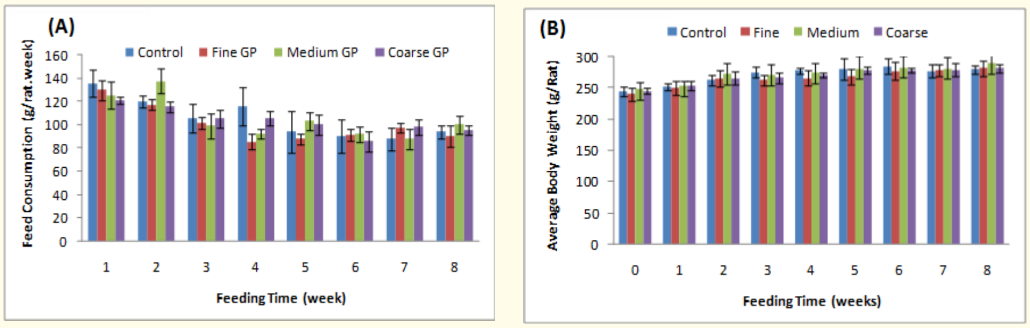

Figure 1: *Effects of GP Particle Size in the diet on (A) weekly feed consumption and (B) body weight of rats (n = 5).*

Effect of grape pomace on quantity of feces

Figure 2 illustrates that diet containing GP increased the quantity of feces excreted significantly (P < 0.05). In general, larger particle size of GP resulted in higher quantity of feces produced by each rat per week, but the quantities of feces produced by rats in medium and coarse GP diets were about the same.

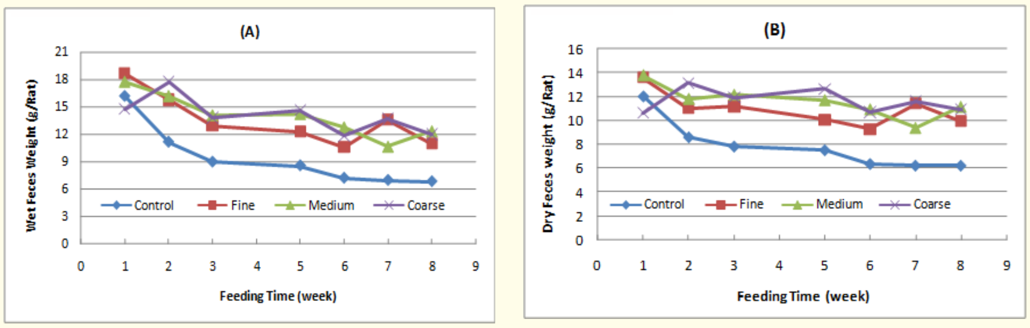

Figure 2: *Effect of GP and its particle size on the average quantity of feces produced by each rat weekly. (A)- Wet feces weight, (B) - dry feces weight.*

Effects of GP in the diet on nutrient absorption of rats

Protein residue of rat fecal samples

There were significant differences among the fecal protein concentrations of rats in different treatment groups (P < 0.05) (Figure 3A). More protein was excreted by the rats on GP diets than that by rats in the control diet (Figure 3B). However, the particle size of GP did influence on the quantity of fecal protein contents among GP diet groups (Figure 3B). The results suggest that the addition of GP in the diet reduced protein absorption in the gastrointestinal (GI) tract of rats.

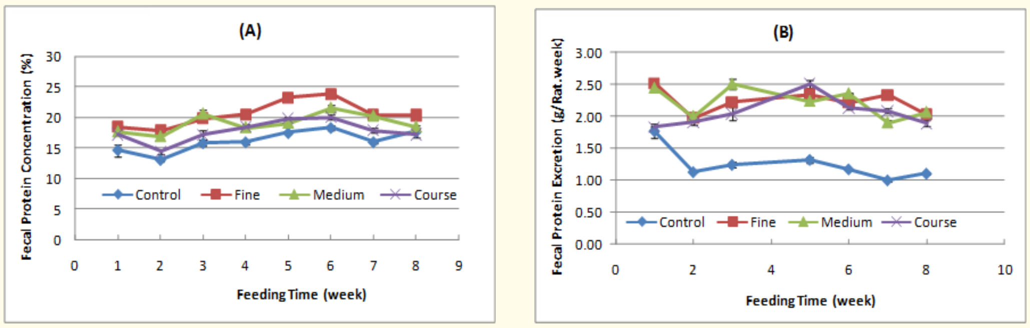

Figure 3: *Effect of diet GP particle size on fecal crude protein excretion of rats. (A)Fecal protein concentration, (B) quantity of protein excreted in feces per week for each rat.*

Fat residue of rat fecal samples

Figure 4 shows that although fat concentration in the feces of control group was significantly higher than those of treatment groups, there were no differences in the total fat quantities in the dry feces of different treatment groups. This suggests that addition of GP in the diet did not affect the absorption of fat.

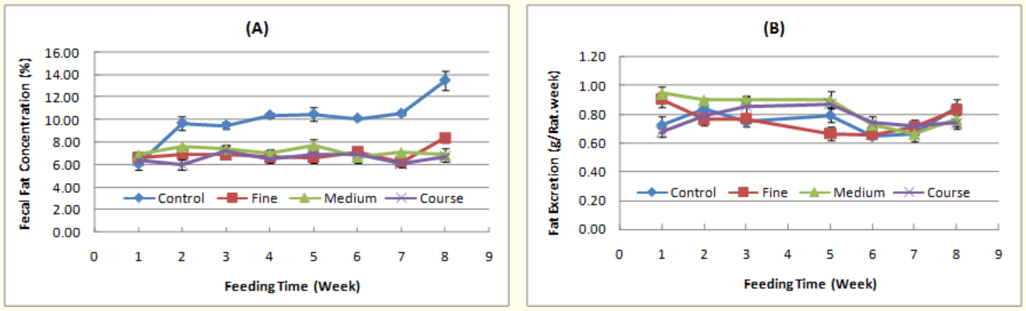

Figure 4: *Effects of particle size of GP on fat excretion in the feces of rats. (A) crude fat concentration of dry feces, (B) total amount of fat excreted per week per rat.*

Ash residue of rat fecal samples

As mentioned in section 2.5, fecal ash content was used to evaluate the impact of GP on the absorption of total mineral. Although the ash concentration of feces of control group was significantly higher than those of GP diet groups (Figure 5A), there were no difference in total ash excretions among treatment groups except week 1 ($P < 0.05$) (Figure 5B) due to the large quantity of feces produced by the rats in the GP diets as shown in figure 2B. These results indicate that the inclusion of GP in the rat diet did not affect the total mineral absorption.

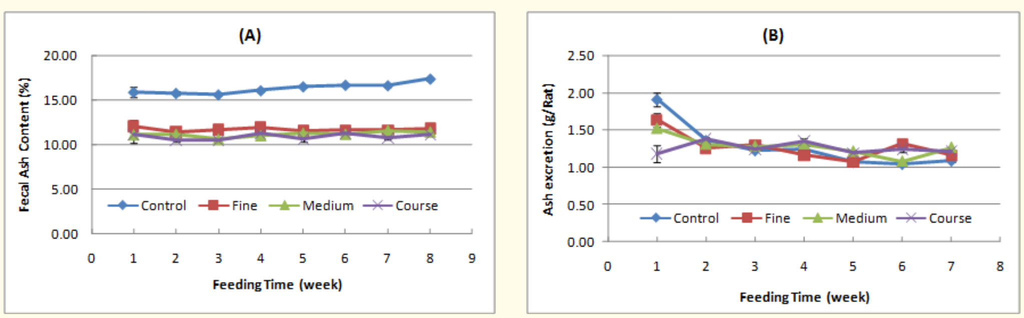

Figure 5: *Effects of particle size of GP on mineral excretion in the feces of rats. (A) Ash concentration of dry feces, (B) total amount of ash excreted per week per rat.*

Carbohydrate residue of rat fecal samples

In contrast with other nutrients, the total fecal carbohydrate concentrations of all treatment groups were similar (Figure 6A) but the total amount of carbohydrate excreted by the rats in the GP diet was noticeably higher than that in the control diet (Figure 6B) over the 8-week feeding period. Among GP diet groups, rats fed with diet containing medium and coarse GP excreted more carbohydrate than rats fed with diet containing fine GP except week 7. Results suggest that inclusion of GP in the diet decreased the carbohydrate absorption in the GI tract of rats.

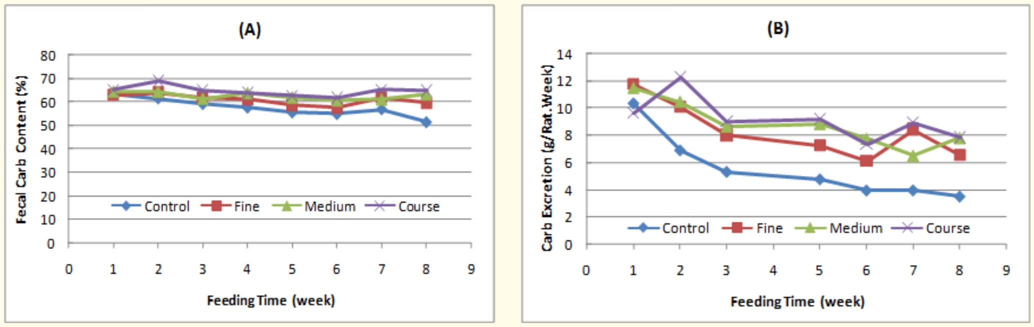

Figure 6: *Effects of particle size of GP on carbohydrate excretion in the feces of rats. (A) carbohydrate concentration of dry feces, (B) total amount of carbohydrate excreted per week per rat.*

Discussion

This study found that the feed consumption and body of rats fed with control diet and GP diets were not significantly different. These are similar to the findings of our previous study with female young rats [13]. The AIN-93 diets are purified diet for rodents and the only protein source of AIN-93M is casein; the carbohydrate source includes corn starch, maltodextrin, sucrose and cellulose [14]. Because grape pomace polyphenol inhibited the activities of digestive enzymes [9] and *in vitro* digestion of casein and starch [10], it is very likely that less protein and carbohydrate in GP diet were digested into small molecules which could be absorbed in the small intestine, thus resulting in the higher protein and carbohydrate contents in feces from rats fed with GP diet. In addition, the high dietary fiber content of GP diets also contributed to the higher carbohydrate content in the feces. Although some studies showed that most of polyphenols inhibit the activity of lipase [9,15], the enzyme responsible for the digestion of food lipid, this study did not find significant impact of GP on the fat absorption in rat model. This study shows GP in the diet did not influenced the total mineral absorption, but there is no evidence about the impact of GP on individual mineral absorption. Some *in vitro* studies showed that grape seed polyphenol extract significantly reduced iron and zinc absorption [16,17]. It has been well known that dietary fiber could have adverse effect on mineral bioavailability due to presence of phytate, and increased dietary fiber consumption reduced the absorption of many minerals by body [18]. However, such adverse effect varied greatly with the amount and type of fiber as well as many other agents in both food and digestive tract [19]. More studies are needed to illustrate the impact of polyphenol and fiber rich GP on the absorption of individual minerals.

Conclusion

This study shows that inclusion of GP in the diet did not affect the average feed consumptions and body weights but increased the fecal excretion of rats and affected nutrient absorption. Among macronutrients, the absorption of protein and carbohydrates were significantly reduced while the absorption of fat and mineral were not affected by the GP addition. Reducing the particle size of GP powder decreased the effects of GP diet on the total fecal excretion and carbohydrate absorption but did not influence the absorption of other nutrients. Due to the possible adverse effects of GP on the absorption of protein and minerals, care must be taken for some special populations, such as pregnant women, children, adolescents, the elderly, strict vegetarians or those with special dietary needs, to consume food product rich in both polyphenol and dietary fiber.

Acknowledgement

This study was supported by a project granted by National Institute of Food and Agriculture, US Department of Agriculture (Project Number NC.X 274-5-14-170-1) and Agricultural Research Station at North Carolina A&T State University.

Author Disclosure Statement

No competing financial interests exist.

Bibliography

1. Slavin JL. "Dietary fiber and body weight". *Nutrition* 21.3 (2005): 411-418.

2. Clemens R., *et al*. "Filling America's Fiber Intake Gap: Summary of a Roundtable to Probe Realistic Solutions with a Focus on Grain-Based Foods". *Journal of Nutrition* 142.7 (2012): 1390S-1401S.

3. Raghavendra S., *et al*. "Grinding characteristics and hydration properties of coconut residue: A source of dietary fiber". *Journal of Food Engineering* 72.3 (2006): 281-286.

4. Zhu KX., *et al*. "Effect of ultrafine grinding on hydration and antioxidant properties of wheat bran and dietary fiber". *Food Research International* 43.4 (2010): 943-948.

5. Deng Q., *et al.* "Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins". *Food Research International* 44.9 (2011): 2712-2720.

6. Llobera A., *et al*. "Dietary fiber content and antioxidant activity of manto negro red grape (Vitis vinifera), pomace and stem". *Food Chemistry* 101.2 (2007): 659-666.

7. Makris DP., *et al*. "Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts". *Journal of Food Composition Analysis* 20.2 (2007): 125-132.

8. Yu J., *et al*. "Functional components of grape pomace: their composition, biological properties and potential applications". *International Journal of Food Science and Technology* 48.2 (2013): 221-237.

9. Hogan., *et al*. "Antioxidant rich grape pomace extract suppresses postprandial hyperglycemia in diabetic mice by specifically inhibiting alpha-glucosidase". *Nutrition and Metabolism* 7 (2010): 71.

10. Yu J., *et al*. "In vitro evaluation of grape seed polyphenol extract on the digestibility of macronutrient". *Journal of Health Science* 4 (2016): 167-176.

11. Singleton VL., *et al*. "Analysis of total phenols and other oxidation substrates and their antioxidants by means of Folin-Ciocalteu reagent". *Methods of Enzymology* 299 (1999): 152-178.

12. AOAC, Association of Official Analytical Chemists International. "Total, Soluble, and Insoluble Dietary Fiber in Foods. AOAC official method 991.43". In Official Methods of Analysis, 16th edition. Washington, DC (1995).

13. Smith I., *et al*. "Impact of diet containing grape pomace on growth performance and blood lipid profile of young rats". *Journal of Medicinal Foods* 20.6 (2017): 550-556.

14. Reeves PG., *et al*. "AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet". *Journal of Nutrition* 123.11 (1993): 1939-1951.

15. He Q., *et al*. "Effects of tea polyphenols on the activities of α-amylase, pepsin, trypsin and lipase". *Food Chemistry* 101.3 (2006): 1178-1182.

16. Ma Q., *et al*. "Bioactive dietary polyphenols inhibit heme iron absorption in a dose-dependent manner in human intestinal Caco-2 cells". *Journal of Food Science* 76.5 (2011): H143-H150.

17. Kim EY., *et al*. "Bioactive dietary polyphenols on zinc transport across the intestinal Caco-s cell membrane". *Journal of Agricultural and Food Chemistry* 59.8 (2011): 3606-3612.

18. Harland BF. "Dietary fiber and mineral bioavailability". *Nutrition Research Reviews* 2.1 (1989): 133-147.

19. Torre M., *et al*. "Effects of dietary fiber and phytic acid on mineral availability". *Critical Review in Food Science and Nutrition* 30.1 (1991): 1-22.

Volume 10 Issue 6 September 2017