

PJN

ISSN 1680-5194

PAKISTAN JOURNAL OF **NUTRITION**

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

***In Vitro* and *in Vivo* Variability in the Nutritional Compositions of Wheat Varieties¹**

Sulhattin Yasar

Department of Animal sciences, Faculty of Agriculture,
Suleyman Demirel University, Isparta-Turkey
E-mail: sulhattiny@yahoo.com

Abstract: The present study involved in the determination of non-starch polysaccharides (NSP) of 15 wheat varieties and their *in vitro* and *in vivo* nutritional characteristics. The determination of *in vitro* viscosity, NSP, acid detergent fibre (ADF), neutral detergent fibre (NDF), crude protein and bushel weight were done for the samples of wheat varieties. There were significant differences between the samples of varieties in respect of these parameters. Especially, high correlations were estimated between the *in vitro* viscosity, NSP, ADF, NDF and bushel weight with regard to wheat varieties: For instance, the increased *in vitro* viscosity of 15 wheat varieties was associated with the increased ADF and NSP and the decreased bushel weight of wheat varieties. All the varieties were classified into 4 different groups according to their contents of NSP, bushel weight and *in vitro* viscosity. An animal digestibility trial was conducted with only one wheat variety from each of the four groups with broiler chickens from 14 d old to 42 d old. These varieties were Gun₉₅, Cakmak₇₉, Gerek₇₉ and Kundura₁₁₄₉. Animals fed with all diets based on wheat varieties consumed significantly higher feed than the animals fed with a control diet based on corn grain. Especially, Gun₉₅ and Cakmak₇₉ varieties caused to increased feed intake compared with the other two varieties. All wheat based diets lead to significantly low weight gain, worsened feed conversion ratio, longer digestive tract and heavier digestive tract of animals in comparison with their counterpart of control diet. In addition, all wheat-based diets caused increased amount of abdominal fat and reduced carcass yield. On the other hand, there were some significant differences between the diets of wheat varieties in some of the above measured parameters. For instance, the lowest carcass yield was obtained from the Kundura₁₁₄₉, Gerek₇₉, Cakmak₇₉ and Gun₉₅, respectively. Unlikely, the lowest abdominal fat was obtained from Gun₉₅, Cakmak₇₉, Gerek₇₉ and Kundura₁₁₄₉, respectively. There were no significant differences in the digestibility of dry matter and crude protein between the wheat varieties. The NSP digestibility at both 21d old age and 42 d old age were numerically found to be higher for Gun₉₅ and Cakmak₇₉ varieties only. Some wheat varieties have a greater nutritional potential in both animal and human nutrition.

Key words: Wheat, non-starch polysaccharides, nutrition

Introduction

Cereals and cereal by-products are the main dietary energy sources of the animal and human's diets. Cereal grains are also important dietary sources of cell-wall polysaccharides and cell storage polysaccharides. Van Soest (1982) suggested these polysaccharides as dietary fibre, in particular for ruminant feeds (e.g. forages, hay) with two fractions, soluble- (lipids, sugars, organic acids, starch, non-protein nitrogen and soluble protein) and insoluble-neutral detergent (soluble acid detergent fibre, hemicellulose, fibre-bound protein and insoluble acid detergent fibre, cellulose, lignin, silica). This definition of "dietary fibre" is lately not considered to be valid for the foods and feeds of human and monogastric animals, respectively. It was, then, defined complex carbohydrates as "starches" and "non-starch polysaccharides" (NSP) rather than dietary fibre by British Nutrition Foundation

(1990). In monogastric animals and humans, the use of "NSP" to define complex dietary polysaccharides seems to be widespread. The dietary constituents of NSPs are methodologically classified into soluble- and insoluble-NSP. The former commonly include pentosans (arabinan + xylan) and β -glucans of cell wall polysaccharides, and the latter cellulose, hemicellulose and lignin.

Johson and Gee (1981) reported that NSP components of cereals caused significantly lowered uptake of glucose and cholesterol from the intestines, suggesting a reduced risk for heart diseases. Even, insoluble fractions of NSP compounds were claimed to play role in the prevention of colon cancer (Boffa *et al.*, 1992). More detailed information on the health related nutritional effects of NSP components can be found in the review of Harris and Ferguson (1993). Thus, NSP components of cereals seemed to have significant health and nutrition effects in

¹This work (project number of TARP-2297) was financially supported by the Research Group of Food, agriculture and Forestry, Tubitak, Ataturk Bulvari, No. 221, Kavaklidere-Ankara, Turkey.

human nutrition. Therefore, the nutritional characteristics of various wheat varieties must be taken into consideration within the food industry.

On the other hand, animal digestibility studies have used ME (metabolizable energy) or AME (apparent metabolizable energy) values of cereal based-diets in order to evaluate the nutritive value of wheat grains for monogastric animals. According to Annison (1991), wheat grains contain pentosans, cell wall NSP compounds, at appreciable levels, 50-80 g/kg DM. He demonstrated a negative linearity between AME of wheat and soluble NSP content, suggesting that NSP is responsible for the low ME of certain wheat grains for poultry species. Recently, Bedford (1996) clearly indicated that there is a great variability in ME and FCE between 9 wheat varieties and 16 barley varieties whether hulled or hullless. Furthermore, Annison (1992, 1993) observed that the addition of wheat-isolated pentosans to a diet of broiler chicks caused to significant reduction in nutrient digestibility (particularly starch), weight gain and ME utilization by the animals. Some researchers took one step further to name these negative effects of NSP components as antinutritive for monogastric nutrition. The following effects were also reported in animal studies under the various inclusion rates of wheat NSP in the diets: increased digesta viscosity leading to reduced assimilation and absorption of nutrients (Bedford *et al.*, 1991; Bedford and Classen, 1992, 1993), reduced epithelial cell renewal rate (Johnson *et al.*, 1988; Yasar and Forbes, 1999, 2000), reduced digestibility of nutrient in the gut and significant changes in the size and histo-morphology of epithelial tissue of intestine (Silva and Simithard, 1996; Yasar and Forbes, 1999, 2000). These antinutritive effects of cereal NSP have been shown to be overcome by various dietary treatments, enzyme supplementation and/or water treatment (Yasar and Forbes, 1999, 2000 and 2001).

The objective of the present investigation was to examine the variability in the chemical composition of 15 wheat cultivars grown in Turkey, and to study how this variability affects weight gain and feed conversion in broiler chickens. Intestinal viscosity and NSP digestibility were determined in order to examine a possible negative influence of the NSP content and different fractions of NSP in the wheat. Furthermore, abdominal fat and the size of the gut were measured to study whether high inclusion of the different wheat cultivars in some way influenced abdominal fat and the size of the gut.

Materials and Methods

Wheat varieties, *in vitro* viscosity and analyses of chemical composition: All wheats used were autumn sown wheat varieties, and represented approximately 60% of the wheat types harvested in Turkey in terms of the total production. The officially approved names of wheat varieties used in the present experiment were Besoztaya₃₀ (B₃₀), Besoztaya₅ (B₅), Bome₉, Gun₉₅, Besoztaya₂₂ (B₂₂),

Cumhuriyet₁₂₅₂ (C₁₂₅₂), Cakmak₇₉, MBVD₁₈, BDMM₁₉, Gerek₇₉, Kutluk₉₄ and Besoztaya₃ (B₃), Kiziltan₉₁, Kundural₁₄₉ and Dagdas₉₄. The bushel weights of corresponding wheat varieties were previously measured during the sampling.

The samples of wheat varieties were fine ground (0.5 mm) through a hammer mill for *in vitro* viscosity measurement and various nutrient analyses. All analyses were performed in triplicate.

In vitro viscosity of ground grains was determined according to Silva *et al.* (1983) as follows:

One gram of sample was suspended in a 15 ml of 0.2 M HCl-KCl acidic buffer (pH, 1.5). Samples were then placed in an agitating water bath at 37 °C for 3 h at 200 stroke per minute. Samples with buffer solution were then centrifuged at 900 g for 10 minutes and viscosity of supernatants was measured in a digital, cone-plate viscometer (model LVTD-CP-40, Brookfield Engineering Laboratories, Inc. Massachusetts, USA), at 25 °C at 50 RPM.

Samples of wheat varieties were analyzed for the contents of dry matter, crude protein, ADF, NDF and NSP whereas the samples of animal droppings obtained from the animal digestibility trial were only chemically analyzed for the contents of dry matter, crude protein and total NSP.

Crude protein is usually expressed as nitrogen content of a sample of wheat in terms of its apparent protein content (Nx6.25), assuming that all nitrogen in the wheat grain is present in the form of protein, which contains about 160g/kg nitrogen. The nitrogen content of each sample was measured using the Kjeldahl technique. The weight of nitrogen collected was calculated and thus the proportion of nitrogen in the sample, and this was converted into the proportion of crude protein by multiplying by 6.25 (AOAC, 1984). All samples were dried at 105 °C overnight until to reach a constant weight to determine the dry matter contents.

The dietary fibre components of Acid Detergent Fibre (ADF) and Neutr Detergent Fibre (NDF) were determined according to the method of AOAC (1984). Total NSP and their total constituent sugars were determined by a spectrometrical method according to the procedure of Englyst *et al.* (1994). The method measures dietary fibre as NSP, using an enzymec-chemical method and evolved from the principles detailed in the work of Englyst *et al.* (1994). Starch is hydrolyzed enzymically; NSP is isolated by the precipitation in ethanol and then hydrolyzed by sulphuric acid. The constituent neutral sugars and uranic acids are measured by colorimetry. The method allows separation of the NSP fraction in soluble NSP (S-NSP) and insoluble NSP (I-NSP). Soluble NSP was calculated as:

$$\text{Soluble NSP} = \text{total NSP} - \text{insoluble NSP}$$

Performance and digestibility trial : Fifteen wheat varieties were classified into 4 group, each with similar

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Table 1: Composition of the experimental diets based on 4 representative wheat varieties and the control diet

| Ingredients, g/kg | Control | Gun ₉₅ | Cakmak ₇₉ | Gerek ₇₉ | Kundura ₁₁₄₉ |
|--|---------|-------------------|----------------------|---------------------|-------------------------|
| Wheat | ----- | 530 | 530 | 530 | 530 |
| Corn | 513.1 | ----- | ----- | ----- | ----- |
| Soybean meal | 342.3 | 299.6 | 299.6 | 299.6 | 299.6 |
| Sunflower meal | 35.7 | 60.2 | 60.2 | 60.2 | 60.2 |
| Fish meal | 34.2 | 34.1 | 34.1 | 34.1 | 34.1 |
| Vegetable oil | 40.0 | 42.3 | 42.3 | 42.3 | 42.3 |
| DCP | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| DL-methionine | 3.40 | 2.50 | 2.50 | 2.50 | 2.50 |
| L-lysine | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Vitamin premix ¹ | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Mineral premix ² | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Anti-Coccidants | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Salt | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Limestone | 13.3 | 13.3 | 13.3 | 13.3 | 13.3 |
| Total | 1000 | 1000 | 1000 | 1000 | 1000 |
| Analyzed nutrients | | | | | |
| Dry matter, g/kg | 918.4 | 920.0 | 918.2 | 921.0 | 919.2 |
| Crude protein, g/kg | 220.6 | 212.0 | 210.2 | 213.0 | 211.2 |
| Total NSP, g/kg | 22.76 | 35.63 | 39.88 | 47.41 | 57.00 |
| Total ash, g/kg | 56.20 | 58.0 | 59.10 | 56.70 | 57.80 |
| Total fat, g/kg | 53.60 | 55.2 | 54.80 | 61.50 | 60.90 |
| Total crude fibre, g/kg | 35.0 | 38.4 | 37.90 | 35.20 | 36.40 |
| Ca, g/kg (calculated) | 10.1 | 9.50 | 9.70 | 11.0 | 11.30 |
| Total P, g/kg (calculated) | 6.50 | 6.40 | 6.50 | 6.80 | 6.70 |
| Metabolizable Energy (calculated), (kcal/kg) | 3007 | 3038 | 3017 | 3175 | 3160 |

¹Vitamin Premix included in the following vitamins (in 1 kg feed): 4.800.000 IU Vit A, 600.000 IU Vit D₃, 12.000 mg Vit E, 2.000 mg Vit K₃, 1.200 mg Vit B₁, 2.400 mg Vit B₂, 2.000 mg Vit B₆, 12 mg Vit B₁₂, 16.000 mg Nicotinamide, 4.000 mg Calcium-D-Pantothenate, 300 mg Folic acid, 30 mg D-Biotin, 150.000 mg Choline chloride, 4.000 mg Antioxidant. ²Mineral Premix included in the following minerals (in 1 kg feed): 80.000 mg Mn, 80.000 mg Fe, 60.000 mg Zn, 8.000 mg Cu, 500 mg I, 200 mg Co, 150 mg Se.

Table 2: *In vitro* viscosity (cPs) of 15 wheat varieties

| Wheat varieties | Mean | Standard deviation |
|-------------------------|------|--------------------|
| B ₃₀ | 1.83 | 0.02 |
| B ₅ | 1.87 | 0.21 |
| Bome ₉ | 1.92 | 0.13 |
| Gun ₉₅ | 1.93 | 0.01 |
| B ₂₂ | 2.09 | 0.01 |
| C ₁₂₅₂ | 2.14 | 0.08 |
| Cakmak ₇₉ | 2.30 | 0.16 |
| MBVD ₁₈ | 2.30 | 0.11 |
| BDMM ₁₉ | 2.32 | 0.55 |
| Gerek ₇₉ | 2.36 | 0.62 |
| Kutluk ₉₄ | 2.40 | 0.08 |
| B ₃ | 2.50 | 0.17 |
| Kiziltan ₉₁ | 2.53 | 0.32 |
| Kundura ₁₁₄₉ | 2.62 | 0.26 |
| Dagdas ₉₄ | 2.72 | 0.14 |

chemical analyses of only *in vitro* viscosity, NSP content and ADF content: A representative wheat variety from each classified group was chosen to formulate experimental diets. The wheat varieties of first group

were B₃₀, B₅, Bome₉ and Gun₉₅, from which only Gun₉₅ was chosen; those of second group were B₂₂, C₁₂₅₂, Cakmak₇₉ and MBVD₁₈, from which only Cakmak₇₉ was chosen; those of third group were BDMM₁₉, Gerek₇₉, Kutluk₉₄ and B₃, from which only Gerek₇₉ was chosen and those of last group were Kiziltan₉₁, Kundura₁₁₄₉ and Dagdas₉₄, from which only Kundura₁₁₄₉ was chosen. The composition of the experimental diets based on the 4 representative wheat varieties and the composition of the control diet based on corn grain are given in Table 1. Experimental diets were formulated to provide the 4 wheat varieties at only one inclusion level of 530 g/kg. The diets differed only in the wheat cultivar included. Wheat was the only carbohydrate source in the 4 experimental diets, whereas in the control diet only ground corn grain was included as carbohydrate source. The diets were supplemented with essential amino acids, mineral and vitamin premixes to meet the requirements of broiler chickens. The all major dietary ingredients were milled to pass through a 3 mm screen in a hammer mill and mixed with the other ingredients in a small mixer. To avoid any cross-contamination of the different wheats, the hammer mill was cleaned after milling each

Table 3: Bushel weight, crude protein and dry matter content of wheat varieties (Means \pm standard deviation)

| Wheat varieties | Bushel weight kg/hl | Crude protein, % | Dry Matter, % |
|-------------------------|---------------------|------------------|-------------------|
| B ₃₀ | 81.95 \pm 0.15 | 11.45 \pm 0.22 | 88.88 \pm 0.02 |
| B ₅ | 78.33 \pm 0.19 | 13.96 \pm 0.13 | 85.27 \pm 0.01 |
| Bome ₉ | 77.78 \pm 1.12 | 14.11 \pm 0.33 | 85.21 \pm 0.03 |
| Gun ₉₅ | 74.79 \pm 0.05 | 10.72 \pm 0.27 | 87 \pm 0.01 |
| B ₂₂ | 75.43 \pm 0.34 | 11.58 \pm 0.19 | 88.55 \pm 0.01 |
| C ₁₂₅₂ | 74.56 \pm 0.54 | 10.08 \pm 0.06 | 88.34 \pm 0.02 |
| Cakmak ₇₉ | 75.59 \pm 0.12 | 7.84 \pm 0.48 | 87.74 \pm 0.01 |
| MBVD ₁₈ | 72.71 \pm 1.19 | 9.03 \pm 0.52 | 87.77 \pm 0.005 |
| BDMM ₁₉ | 71.24 \pm 0.45 | 11.41 \pm 0.26 | 87.23 \pm 0.001 |
| Gerek ₇₉ | 66.90 \pm 2.65 | 13.18 \pm 0.14 | 86.87 \pm 0.02 |
| Kutluk ₉₄ | 66.52 \pm 1.32 | 12.65 \pm 0.17 | 87.14 \pm 0.02 |
| B ₃ | 65.37 \pm 2.00 | 12.88 \pm 0.05 | 86.79 \pm 0.01 |
| Kiziltan ₉₁ | 61.58 \pm 1.78 | 12.32 \pm 0.05 | 86.83 \pm 0.01 |
| Kundura ₁₁₄₉ | 61.59 \pm 1.15 | 12.45 \pm 0.28 | 86.94 \pm 0.01 |
| Dagdas ₉₄ | 59.50 \pm 2.87 | 11.96 \pm 0.17 | 88.04 \pm 0.01 |

Table 4: ADF and NDF contents of 15 wheat varieties (Means \pm standard deviation)

| Wheat varieties | ADF, mg/g | NDF, mg/g |
|-------------------------|------------------|---------------------|
| B ₃₀ | 26.93 \pm 0.65 | 0.2005 \pm 0.0058 |
| B ₅ | 2412 \pm 2.16 | 0.1234 \pm 0.0072 |
| Bome ₉ | 29.79 \pm 2.14 | 0.1722 \pm 0.0313 |
| Gun ₉₅ | 30.84 \pm 1.37 | 0.1677 \pm 0.0148 |
| B ₂₂ | 35.46 \pm 0.42 | 0.2038 \pm 0.0083 |
| C ₁₂₅₂ | 28.00 \pm 0.80 | 0.1572 \pm 0.0039 |
| Cakmak ₇₉ | 31.06 \pm 1.18 | 0.1596 \pm 0.0062 |
| MBVD ₁₈ | 29.73 \pm 1.13 | 0.1548 \pm 0.0122 |
| BDMM ₁₉ | 26.99 \pm 0.10 | 0.1569 \pm 0.0135 |
| Gerek ₇₉ | 38.99 \pm 3.23 | 0.1541 \pm 0.0115 |
| Kutluk ₉₄ | 36.75 \pm 0.75 | 0.1618 \pm 0.0138 |
| B ₃ | 37.48 \pm 0.37 | 0.154 \pm 0.0120 |
| Kiziltan ₉₁ | 43.66 \pm 1.52 | 0.1684 \pm 0.0074 |
| Kundura ₁₁₄₉ | 42.88 \pm 1.41 | 0.1806 \pm 0.0084 |
| Dagdas ₉₄ | 43.66 \pm 1.52 | 0.1445 \pm 0.0047 |

cultivar. The diets were provided as mash and fed in the whole experimental period from 14 to 42 d of age. The birds were fed until 14 d of age with the control diet, then the experimental diets and the control diet were fed to birds from 14 to 42 d of age. Feed and water were available *ad libitum*.

Three-tier batteries with raised floors were used in the experiment. Pen dimensions were 30 \times 50 \times 50 cm (height \times length \times width). Each pen had a 1 cm² wire mesh bottom and, for the first 10 to 14 days, a plastic mat with 0.1 cm² mesh was placed on the wire bottom. Each pen was equipped with a feeding trough placed outside and two water cups inside the pen. All batteries were placed in the same house provided with forced ventilation. The temperature was controlled and gradually reduced from 33 to 22 °C until day 21, and light was continuous during the whole experimental period. A total of 990 male broiler chickens (Ross 208) were obtained from a local hatchery and were allotted at random to 90 pens in 6 batteries with

11 chickens housed per pen. Since each pen representing one replicate, totally 18 pens within each 11 birds included were randomly assigned for each of 5 diet groups. The chickens were wing-banded, weighed individually at 14-d-old and then each week until 42 d of age. The chickens were inspected daily. Feed intake per pen (corrected for feed wastage) was recorded at the same time and feed conversion was calculated on a pen weight basis. A fecal output collection period based on 24 hours was set for 4 consecutive days from 21 d to 25 d of age and from the 38 to 42 d of age. The droppings were only collected from the 5 representative pens for each treatment group. The samples were analyzed for dry matter, crude protein and total NSP. In fact, digestibility of nutrient can precisely be determined using an indigestive feed marker in the diet. However, we had no opportunity to carry out this. The apparent nutrient retentions or digestibility rather than the precise digestibility of dry matter, crude protein and total NSP was calculated as subtracting the daily output of nutrient in the faeces from the daily intake of each nutrient and dividing by the daily intake of each nutrient again, and finally multiplying by 100. At the end of 42 d of age, 5 birds from each pen were killed to determine the carcass yield, the size of whole digestive tract, the weight of abdominal fat and ileal digesta viscosity. The digesta sample from ileum of each bird was collected, and centrifuged at 9000 g. The supernatants were taken to the viscometer and the viscosity was measured at 41 °C (which is the deep body temperature of the animal) and at 50 RPM. The experiment complied with the guidelines of the Turkish Regulations with respect to animal experimentation and care of animals under study.

The statistical analyses of the data were carried out under the SPSS for Windows (1999). The analyses of variance was employed to establish the significance levels of treatment effects using a General Linear Model (GLM) where the differences between the treatment means were

Table 5: Soluble, insoluble and total NSP contents of 15 wheat varieties (Means \pm standard deviation)

| Wheat varieties | Soluble NSP | Insoluble NSP | Total NSP |
|-------------------------|-------------|---------------|-------------------|
| B ₃₀ | 12.38 | 43.88 | 56.26 \pm 0.83 |
| B ₅ | 12.64 | 42.30 | 54.93 \pm 1.01 |
| Bome ₉ | 14.52 | 43.56 | 58.08 \pm 0.72 |
| Gun ₉₅ | 15.01 | 47.54 | 62.55 \pm 0.55 |
| B ₂₂ | 14.80 | 55.65 | 70.45 \pm 0.57 |
| C ₁₂₅₂ | 16.08 | 48.25 | 64.33 \pm 0.42 |
| Cakmak ₇₉ | 18.39 | 45.04 | 63.43 \pm 0.48 |
| MBVD ₁₈ | 16.58 | 40.61 | 57.29 \pm 0.16 |
| BDM ₁₉ | 18.95 | 38.47 | 57.42 \pm 0.47 |
| Gerek ₇₉ | 28.00 | 56.85 | 84.85 \pm 1.55 |
| Kutluk ₉₄ | 20.72 | 48.35 | 69.07 \pm 0.38 |
| B ₃ | 29.05 | 67.77 | 96.82 \pm 0.79 |
| Kiziltan ₉₁ | 30.25 | 74.06 | 104.31 \pm 1.50 |
| Kundura ₁₁₄₉ | 32.13 | 71.52 | 103.65 \pm 0.47 |
| Dagdas ₉₄ | 28.13 | 76.07 | 104.20 \pm 0.66 |

Table 6: Mean values of feed intakes (g/ bird/period) of broiler chicken fed on 4 experimental and 1 control diets

| Treatments | 14 - 28 d | 28 - 42 d | 14 - 42 d |
|-------------------------|-------------------|----------------------|----------------------|
| Control | 1168 ^a | 2010.32 ^a | 3178.32 ^a |
| Gun ₉₅ | 1198 ^b | 2111.13 ^b | 3309.13 ^b |
| Cakmak ₇₉ | 1211 ^b | 2090.21 ^b | 3301.21 ^b |
| Gerek ₇₉ | 1226 ^c | 2043.45 ^c | 3269.45 ^c |
| Kundura ₁₁₄₉ | 1200 ^b | 2064.00 ^c | 3264.00 ^c |
| SEM | 7.00 | 8.01 | 8.45 |
| Significant level, P | 0.001 | 0.001 | 0.001 |

SEM, standard error of the differences between the means

^{ab}Different letters within each horizontal column indicated that the means of treatments significantly ($P < 0.05$) differed from each other.

separated by the Duncan's Multiple Comparison Test. The data were presented as the mean with SEM (a standart error of differences between the means). The significant level was set at 0.05.

Results

The *in vitro* viscosity data of the 15 wheat varieties were presented in Table 2, and the data on bushel weight, crude protein and dry matter of 15 wheat varieties were in Table 3.

Significant differences were observed between the wheat varieties in the parameters of *in vitro* viscosity, bushel weight, crude protein and dry matter. These differences in *in vitro* chemical composition of wheat varieties imply that wheat varieties can differ in NSP content. In order to examine the relationships between the above parameters, the correlation analysis was performed. No correlations were observed between bushel weight and nutrient compositions (crude protein and dry matter).

Significantly high negative correlation, -0.94, was observed between the bushel weight and *in vitro* viscosity values (Fig. 1). The varieties with high viscosity values, Dagdas₉₄, Kundura₁₁₄₉ and Kiziltan₉₁ and B₃, had a significantly lower bushel weight than that of varieties with low viscosity values, B₃₀, B₅, Bome₉ ve Gun₉₅.

The results of ADF and NFD analyses were given in Table 4, from which can be seen that there was a great variability in ADF and a little variability in NDF contents of wheat varieties. The correlation coefficients between the *in vitro* viscosity and ADF and between ADF and bushel weight were +0.82 and -0.89, respectively. In Table 5, soluble, insoluble and total NSP contents of the wheat varieties were presented. In cereal grains, a great proportion of ADF was constituted by NSP, suggesting that ADF can be an indicator for the NSP content of the examined varieties.

There were significantly high variabilities between the wheat varieties in total NSP contents, proportionally reflecting a similar positive or negative variability in *in vitro* viscosity, ADF and bushel weight. The correlation coefficients between total NSP and *in vitro* viscosity, between NSP and bushel weight and between total NSP and ADF values were +0.82, -0.90 and 0.93, respectively.

Since total NSP was positively correlated with *in vitro* viscosity and ADF, and negatively correlated with bushel weight total NSP content of wheat varieties can be estimated by these quick and fast *in vitro* measurements, particularly by *in vitro* viscosity and bushel weight parameters (Fig. 2). The regression equation of this relationship was as follows:

WheatNSP = 118.2 - 9.50 *in vitro* viscosity + 1.92 wheat ADF - 1.24 wheat bushel weight (Regression coefficient is 0.95).

As an overall, the examined varieties of wheat grains can be evaluated on the bases of *in vitro* chemical compositions within four groups, each with similar *in vitro* viscosity, bushel weight and the contents of NSP and ADF. As mentioned in the material and methods, one representative wheat variety was chosen from each group, and four broiler diets were based on these 4 wheat varieties and fed to broiler chickens. The data of performance were presented in Table 6 and 7. Broiler chicken fed with diets of 4 wheat varieties consumed significantly ($P < 0.05$) higher feed than the birds given a control diet based on corn grain. Within the wheat varieties, high values of feed intake were observed with the diets of Gun₉₅ and Cakmak₇₉ whereas low intakes were obtained from the diets of Gerek₇₉ and Kundura₁₁₄₉ (Table 6).

Wheat based-experimental diets significantly ($P < 0.05$) caused to reductions in live weight gain, compared to the effect of control diet (Table 7). Feed conversion ratios (g

Table 7: Mean values of live weight gain (g/ bird/period) and feed conversion ratios of broiler chicken fed on 4 experimental and 1 control diets

| Treatments | 14 - 28 d | 28 - 42 d | 14 - 42 d |
|-------------------------|---------------------|----------------------|----------------------|
| Live weight gain | | | |
| Control | 710.45 ^a | 1000.3 ^a | 1653.54 ^a |
| Gun ₉₅ | 650.23 ^b | 921.89 ^b | 1536.66 ^b |
| Cakmak ₇₉ | 645.65 ^b | 906.18 ^{bc} | 1609.8 ^c |
| Gerek ₇₉ | 630.85 ^c | 879.45 ^{cd} | 1505.05 ^b |
| Kundura ₁₁₄₉ | 665.56 ^d | 844.46 ^d | 1536.84 ^b |
| SEM | 5.45 | 17.50 | 22.50 |
| Significant level, P | 0.01 | 0.001 | 0.01 |
| Feed conversion ratios | | | |
| Control | 1.64 ^a | 2.01 ^a | 1.92 ^a |
| Gun ₉₅ | 1.84 ^b | 2.29 ^b | 2.15 ^{bd} |
| Cakmak ₇₉ | 1.87 ^{bd} | 2.30 ^b | 2.05 ^c |
| Gerek ₇₉ | 1.94 ^c | 2.32 ^b | 2.17 ^b |
| Kundura ₁₁₄₉ | 1.80 ^d | 2.44 ^c | 2.12 ^d |
| SEM | 0.02 | 0.03 | 0.02 |
| Significant level, P | 0.01 | 0.001 | 0.01 |

SEM, standard error of the differences between the means
^{ab}Different letters within each horizontal column indicated that the means of treatments significantly ($P < 0.05$) differed from each other.

Table 8: Mean values of carcass yield, total length and weight of whole digestive tract (DT), abdominal fat and *in vivo* ileal viscosity of the 4 experimental and 1 control diets

| Treatments | Carcass yield % | Total length of DT, cm | Total weight of DT, gr |
|-------------------------|---------------------|------------------------|------------------------|
| Control | 71.05 ^a | 226.65 ^a | 179.15 ^a |
| Gun ₉₅ | 71.51 ^a | 240.56 ^b | 201.71 ^b |
| Cakmak ₇₉ | 70.71 ^{ab} | 245.09 ^b | 205.15 ^b |
| Gerek ₇₉ | 69.62 ^b | 241.56 ^b | 205.25 ^b |
| Kundura ₁₁₄₉ | 68.60 ^b | 248.90 ^b | 205.09 ^b |
| SEM | 0.73 | 4.32 | 4.94 |
| Significant level, P | 0.463 | 0.183 | 0.525 |
| | Abdominal fat, gr | | Viscosity, cPs |
| Control | 33.00 ^a | | 4.52 ^a |
| Gun ₉₅ | 42.21 ^b | | 6.31 ^b |
| Cakmak ₇₉ | 47.46 ^c | | 6.24 ^b |
| Gerek ₇₉ | 49.78 ^c | | 7.81 ^c |
| Kundura ₁₁₄₉ | 48.82 ^c | | 8.97 ^c |
| SEM | 2.46 | | 0.62 |
| Significant level, P | 0.001 | | 0.05 |

SEM, standard error of the differences between the means
^{ab}Different letters within each horizontal column indicated that the means of treatments significantly ($P < 0.05$) differed from each other.

feed intake: g weight gain over a specified period) were also significantly worsened by wheat diets, compared to the control diet. During the period from 14 to 42 d of age, the diet of Cakmak₇₉ lead to significantly high live weight gain compared to the diets of other varieties whereas the

diets of Gun₉₅ and Kundura₁₁₄₉ between 14 to 28 d and the diet of Gun₉₅ and Cakmak₇₉ between 28 to 42 d caused significantly high weight gain, compared to other counterpart diets.

Similarly, there were high values of feed conversion ratios with the diets of Kundura₁₁₄₉ from 14 to 28 d, Gun₉₅ and Cakmak₇₉ from 28 to 42d, and only Cakmak₇₉ from 14 to 42 d in comparison to other counterpart diets.

In Table 7, total length and total weight of whole digestive tract were given, from which can be seen that the broiler chickens were observed to have a longer and heavier digestive tract with the experimental diets based on wheat varieties than the diet of control. Furthermore, the carcass yield was seen to be significantly reduced by wheat based-diets compared to the control diet whereas the abdominal fat significantly increased with the wheat diets. *In vivo* viscosity of the digesta of ileum was greatly increased under the influence of wheat diets compared to the control counterpart.

Kundura₁₁₄₉, Gerek₇₉, Cakmak₇₉ and Gun₉₅ produced the lowest carcass yield and the highest abdominal fat values, respectively. Amongst to the wheat varieties, Kundura₁₁₄₉ produced the highest *in vivo* viscosity, and this was followed by Gerek₇₉, Cakmak₇₉ and Gun₉₅, respectively (Table 8). However, the difference in *in vivo* viscosity was not proportionally reflected in the differences in feed intake and weight gain.

Apparent nutrient retentions of feed dry matter, crude protein and total NSP were significantly ($P < 0.05$) lower in the birds of wheat based-diets than the birds of control diet (Table 9). The birds of control diet retained similar amount of total NSP with the birds of wheat based-diets when the birds get older (42 d of age). No significant differences were observed in both dry matter and crude matter retention between the diets of different wheat varieties. Of the diets of wheat varieties, total NSP retentions were higher with the diets of Gun₉₅ and Cakmak₇₉ than the diets of Gerek₇₉ and Kundura₁₁₄₉.

Discussion

The studied wheat varieties can be classified into 4 groups according to their *in vitro* viscosity, ADF, bushel weight and total NSP contents in the present experiment (Table 10).

The reasons for this kind of classification were discussed as follows: In general, wheat varieties with < 64 kg/hl bushel weight is regarded as feed grade varieties with low nutritional quality, suggesting that the wheat grains with less bushel weight would have a lowered amount of crude protein (Leeson and Summers, 1997) (Table 3). However, this generalisation must not be considered to be so in the food industry since there were no positive or negative relationships between the grain bushel weight and crude protein level in the present study. Therefore, the apparent nutrient analyses of wheat grains such as Weende analyses are not sufficiently enough to provide precise information

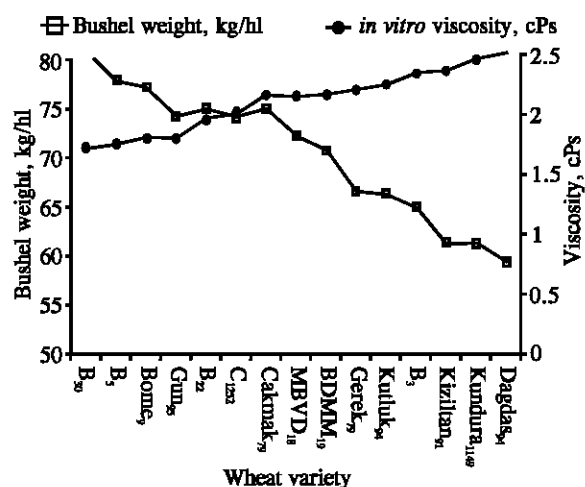


Fig. 1: Correlation (-0.94) between *in vitro* viscosity and bushel weight of wheat varieties.

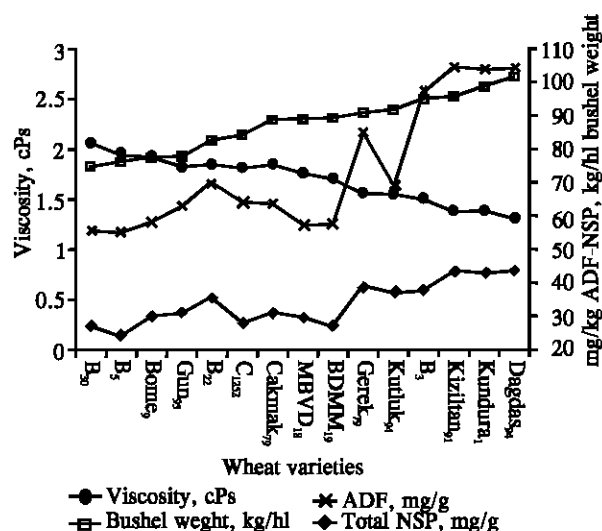


Fig. 2: The relationship between NSP and *in vitro* viscosity, ADF and bushel weight of 15 varieties

of grain quality. Regarding the other remaining chemical analyses there was a strong and meaningful association between the studied parameters of *in vitro* viscosity, bushel weight, ADF and total NSP (Fig. 1 and 2). In fact, there were high correlations between *in vitro* viscosity and NSP, ADF and NSP, and bushel weight and NSP. Since *in vitro* viscosity is a reliable and fast measurement, it can be a good indicator for the grain quality. Previously, Bedford (1993) and Yasar (1998) suggested that *in vitro* viscosity can provide more information on the total NSP contents of various varieties. In other words, the increased *in vitro* viscosity could indicate a high level of NSP of wheat grain, thereby implying low nutritional value for poultry (Bedford, 1993) since poultry species less utilise from the feeds of high

viscous. In humans, the effect of high viscous foods resulted from the dietary fibre only can be more beneficial for various health reasons, particularly on the reduction of blood cholesterol level and diminished uptakes of fatty acids and other nutrients (Johnson and Gee, 1981; Boffa *et al.*, 1992). In contrast to the study of Bedford (1993) in which only *in vitro* viscosity was evaluated as a good estimate for total NSP content of cereals, the regression analyses showed that *in vitro* viscosity, ADF and bushel weight are the good estimates for the total NSP contents of 15 wheat varieties in the present study.

The contents of total NSP determined in the present experiment were in a great agreement with the NSP values of wheat grains reported by Choct and Annison, 1990; Boros *et al.*, 1994; Aman and Graham, 1987; Henry, 1985; Henry, 1987).

The use of wheat varieties with high nutritive value is desirable in fast growing animals, especially in meat-type birds. Therefore, feeding quality wheat varieties with low bushel weight and high viscous have been long supplemented with NSP-degrading exogenous enzymes during the feed manufacturing, depending the rate of wheat inclusion (Yasar and Forbes, 1999, 2000). When baking quality wheat grains with low inclusion rates and high nutritive value (low in NSP and viscosity) are considered in animal production the enzyme treatment is not practised. We can, therefore, be classified the above 4 groups of wheat varieties according to the chemical analyses as follows: the groups of 1 and 2 as baking quality wheats and the groups of 3 and 4 as feeding quality wheats.

The data of performance and nutrient retention can support that the present classification of 15 wheat grains is appropriate. In comparison with the effects of the control diet in broiler chicken, there were significant reductions in weight gain and feed conversion ratio in the birds fed with the diets of 4 different wheat grains. This indicated that all the diets of wheat varieties must be supplemented with the exogenous NSP-degrading enzymes in order to overcome the negative effects of NSP from the cereal feeding. It was previously well established that enzyme supplementation and/or water treatment of cereal based-diets at high inclusions had beneficial effects in fast growing birds: reduced *in vitro* viscosity, improved weight and feed efficiency and increased ME intakes (Yasar and Forbes, 1999, 2000; Yasar *et al.*, 2000; Van Der Klis *et al.*, 1993a,b; Bedford, 1995). Of the wheat varieties groups, the decreased weight gain and worsened feed conversion ratio were less pronounced for Gun95 of the group 1 and Cakmak79 of the group 2 than Gerek79 of the group 3 and Kundura1149 of the group 4. This was also supported by the data of carcass yield and *in vivo* viscosities to a greater extent and by the data of nutrient retentions to a lesser extent. Similarly, the baking quality wheats (the groups of 1 and 2) had significantly higher

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Table 9: Dry matter, Crude protein and total NSP (%) digestibility of the diets of 4 wheat varieties and the control diet

| Treatments | 21 d of age | | | 42 d of age | | |
|-------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| | DM | CP | NSP | DM | CP | NSP |
| Control | 74.36 ^a | 67.56 ^a | 45.98 ^b | 77.87 ^a | 70.33 ^a | 41.23 ^a |
| Gun ₉₅ | 70.21 ^b | 62.73 ^b | 37.54 ^a | 72.33 ^b | 61.86 ^b | 42.56 ^a |
| Cakmak ₇₉ | 69.45 ^b | 63.00 ^b | 36.21 ^a | 73.21 ^b | 63.98 ^b | 43.18 ^a |
| Gerek ₇₉ | 65.03 ^b | 61.79 ^b | 36.59 ^a | 69.34 ^c | 63.23 ^b | 40.43 ^a |
| Kundura ₁₁₄₉ | 66.98 ^b | 63.39 ^b | 35.98 ^a | 64.00 ^c | 64.14 ^b | 40.30 ^a |
| SEM | 2.21 | 1.59 | 0.54 | 2.32 | 2.74 | 1.33 |
| Significant level, P | 0.01 | 0.051 | 0.233 | 0.01 | 0.812 | 0.123 |

SEM, standard error of the differences between the means. ^{ab}Different letters within each horizontal column indicated that the means of treatments significantly (P < 0.05) differed from each other.

Table 10: *In vitro* chemical compositions of 15 wheat varieties

| Groups | varieties | <i>in vitro</i> viscosity, cPs | ADF, mg/g | Bushel weight, kg/hl | Total NSP, mg/g |
|--------|-------------------------|--------------------------------|-----------|----------------------|-----------------|
| 1 | B ₃₀ | 1.83 | 26.93 | 81.90 | 57.31 |
| | B ₅ | 1.87 | 24.12 | 78.30 | 56.00 |
| | Bome ₉ | 1.92 | 29.79 | 77.70 | 58.00 |
| | Gun ₉₅ | 1.93 | 30.84 | 74.70 | 62.50 |
| 2 | B ₂₂ | 2.09 | 35.46 | 75.40 | 69.89 |
| | C ₁₂₅₂ | 2.14 | 28.00 | 74.50 | 64.89 |
| | Cakmak ₇₉ | 2.30 | 31.06 | 75.50 | 62.98 |
| | MBVD ₁₈ | 2.30 | 29.73 | 72.70 | 57.00 |
| 3 | BDM ₁₁₉ | 2.32 | 26.99 | 71.20 | 56.86 |
| | Gerek ₇₉ | 2.36 | 38.99 | 66.90 | 85.65 |
| | Kutluk ₉₄ | 2.40 | 36.75 | 66.50 | 69.40 |
| | B ₃ | 2.50 | 37.48 | 65.30 | 96.00 |
| 4 | Kiziltan ₉₁ | 2.53 | 43.66 | 61.50 | 106.00 |
| | Kundura ₁₁₄₉ | 2.62 | 42.88 | 61.50 | 103.00 |
| | Dagdas ₉₄ | 2.72 | 43.66 | 59.50 | 103.50 |

carcass yield and lower *in vivo* viscosity than the feeding quality wheats (the groups 3 and 4) whereas such differences were less observed in total NSP retentions between the wheat varieties. It can be therefore said that the wheat varieties of the groups 1 and 2 were higher in the nutritional value than the varieties of the groups 3 and 4 for poultry nutrition. In terms of human nutrition matters, no such differentiation can be made since the wheat varieties did not differ in the dry matter, crude protein and total NSP retentions although there were some numerical differences. Dietary fibre through human daily intake is supposed to be a beneficial nutrient for the maintenance of body weight and the mobility of the gut environments (Harris and Ferguson, 1993). The varieties studied in the present experiment can be said to have a potential in human nutrition although more nutritional measurements such as glucose and cholesterol uptake from the intestine must be taken under the different levels of wheat NSP.

In short, the followings can be concluded from the present experiments:

- 1 *In vitro* chemical characteristics of 15 Turkish wheat varieties were determined and the relationships between the studied parameters were established.
- 2 The nutritional value of the studied wheat varieties were determined in an animal experiment with the

some important physiological and digestibility parameters.

- 3 There were great variabilities in both *in vitro* and *in vivo* experimental parameters between the wheat varieties. This differentiation between the varieties is of great importance in both feed and food industry.

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Use of Grape Leaves in Canned Food

I. G. Sat, M. Sengul and F. Keles

Department of Food Engineering, Agricultural Faculty, Ataturk University,
TR-25240, Erzurum, Turkey
E-mail: igsat@atauni.edu.tr

Abstract: The objective of this research was to investigate suitability of fresh (unprocessed) and canned grape leaves to prepare Sarma, a traditional Turkish dish. Leaves from four different variety; Hacitesbihi, Agrazaki, Karaerik, and Kabuguyufka were blanched in two different solutions, and the leaves were placed into jars, then filled with tap water and brine containing 3.5 percent salt + 1 percent citric acid and stored for 7 months in ambient temperature. The results of chemical and physical analyses showed that quality of the Karaerik leaves was better than that of the other variety leaves. Also, sensory evaluation indicated that Karaerik and Kabuguyufka varieties were the most suitable grape leaves to produce Sarma dish. We conclude that Kabuguyufka and Karaerik leaves are convenient for Sarma dish as canned while Agrazaki and Hacitesbihi as fresh.

Key words: Grape leaf, brine, canned food, stuffed leaves

Introduction

A number of plants have been utilized for years by the human as a food source and therapeutical purposes. Currently, popularity of using different ingredients or raw materials to produce new food varieties has also been increased. As a result of this, consumers are facing variety of new types of food products, which provides more pleasant and healthier nutrition supply. For example grape leaf contains an abundant amount of vitamins and minerals as one of these kinds of foods. It has been reported that grape has important role in controlling of some liver diseases, high blood pressure and anemia. Also fibers and fruit acids in grape have vital role in cleaning blood functions of digestive system and kidney (Celik *et al.*, 1998). In addition, grape leaf is very good source of vitamin C. Baysal (1993) reported that grape leaf contained about 120 mg/100 g vitamin C. Grape leaf is used to make stuffed leaves (Sarma) and forcemeat (vegetable stuffed with forcemeat) which are very popular meals in middle east countries. Stuffing materials are surrounded with grape leaf and than boiled to prepare Sarma dish.

Grape leaves can be used as both in fresh and stored forms. Preservation methods for grape leaves are drying, dry salting, usage of different amount of salt, acids or starter cultures in brine and canned food. Consumers often preserve grape leaves with traditional methods. There is a little research about finding a new method for preservation of grape leaves or improve traditional methods in the literature. Commercially produced stuffed leaves can also be found in market, because there is an increasing demand from consumer to ready to eat foods. Some factors such as maturity and variety of grape, location of grape in shoot can considerably affect the

quality. Since old leaves are very hard, it is difficult to make a stuffed dish from these leaves. Therefore, young leaves (immature) are chosen for this purpose. Gokturk *et al.* (1997) reported that harvesting leaves at the beginning of vegetation could give thinner vines, while harvesting leaves late could decrease the quality of canned food. In addition, thick, hairy, with lobness leaves are usually rejected by consumers. Also the Narince and Sultani Cekirdeksiz leaves are reportedly preferred to use in canned foods.

In the study conducted by Dalgic and Akbulut (1988) some comparisons were made between the leaves harvested from fourth, fifth and sixth row from shoot, and they stored these leaves in brine containing different concentrations of salt and citric acid. The leaves from fifth row and 8 % salt concentration gave the best result in terms of the overall quality.

Basoglu *et al.* (1996) investigated brine preservation of Alfons, Erenkoy Beyazi and Sultani grape leaves. This study indicated that leaves harvested from fifth and sixth rows were suitable for brining, and Sultani leaves were the best for the stuffing. They suggested usage 5 % salt, 3% starter culture and 0.25 % lactic acid in brine preparation.

In the study conducted by Gokturk *et al.* (1997) Narince and Hamburg Misketi grapes, Kober 5 BB and 41 BM.G. grapevine rootstocks were used. Among different salt concentrations, 3.5% salt was given the best results for the brine. Furthermore, Narince and Hamburg Misketi leaves were determined to be the most convenient varieties for canned food. It was shown that 41 BM.G. could be used in canned leaf technology. While Kober 5 BB was not suitable for canning processes.

Ic and Denli (1997), preserved Sultani grape leaves in

brine containing 5% salt and 0.5% lactic acid, and this study concluded that natural fermentation made with the same amount of salt and lactic acid was also achieved in four weeks. Therefore, there is no need for longer fermentation time with controlling environmental conditions, and it can even be shorter.

The purpose of this experiment was to investigate usage of variety of grape leaves in canned food.

Materials and Methods

Materials: In this research, grape leaves from Hacitesbihi, Agrazaki, Kabuguyufka, and Karaerik varieties grown in Uzumlu, Erzincan were used as research materials. Grape leaves were harvested in June 2000 when their size reached to 2/3 of their full size. Wholesome and undamaged leaves with normal appearance were harvested for the research.

Canning Process: Canning processes of the grape leaves were as described in Fig. 1. First, the leaves were cleaned and classified according to their size suitability for processing and their petioles were shortened to 2 cm. Then the leaves were blanched in 0.5% citric acid + 1% NaCl solution and in plain tap water. After cooling to ambient temperature, 200 g leaves were put into 500 ml jars and filled with either of brine (3.5% NaCl + 0.5 % citric acid solution) or plain tap water as a control and the jars were exhausted. The sealing the jars were pasteurized with two different heat processes (75 °C 15 min and 90 °C 5 min), cooled under tap water. Then the jars were stored at ambient temperature (20 ± 2 °C) for 7 months.

Physical and Chemical Analyses: On fresh leaves (immediately after harvesting), some physical measurements were made: length of the petiole, dimensions of the leaves, hairiness, lobness, and the number of leaves in per 100 g sample. Both fresh and canned leaves were also subjected to following chemical analyses: moisture (%), ash (%), pH, titratable acidity, vitamin C (mg/100g), crude fiber (%), and salt content (%) by using the techniques outlined by Keles (1983); Anonymous (1983); Cemeroglu (1992). Color of the fresh and canned leaves were measured by a Minolta Colorimeter (Chroma Meter, CR-200, Japan) as described in Anonymous (1979).

Preparation of Sarma: In the second part of the research, a traditional Turkish dish called Sarma were made from both fresh and canned grape leaves containing following ingredients; rice, olive oil, salt and water. Slightly cooked rice was mixed with above ingredients, and due to the fact that only the outer part of the mix is grape leaf, the mix were placed onto a leaf lamina, and then the leaf rolled by hand to cover the mix, and the dish became Sarma after the cooking. The dish was served to the panel members as

cold (7-10 °C) due to eating habits. Traditional spices were not added to Sarma dish to evaluate sensory properties of the vine leaves only.

Sensory Evaluation: The Sarma dishes made from both leaf samples (fresh+canned) were subjected to sensory evaluation using 8-trained panelist with 3-4 years experience in food evaluation. The dish samples were scored 5 to 25 point scale (5 unacceptable, 25 very acceptable) for color, flavor, texture, and overall quality.

Results and Discussion

Fresh Leaves: The Results of the physical and chemical analyses of the fresh grape leaves were presented in Table 1 and Table 2.

For the leaf dimensions and number of leaves in 100 g sample, the varieties Hacitesbihi - Karaerik and Agrazaki - Kabuguyufka were similar. Also, Hacitesbihi and Karaerik varieties were large in size and thicker than that of Agrazaki and Kabuguyufka grape leaves (Table 1). As seen in the table, numbers of leaves in 100g samples were ranged from 24 to 41 for the 4 varieties studied. However, the average number reported by some researches in the 100g samples were 40 for Sultani (Ic and Denli, 1997), and ranged from 19.5 to 40.3 for Sultani, Alfons and Erenkoy grape varieties (Basoglu *et al.*, 1996). In terms of lobness, Agrazaki variety was unlobbed (Table 1), therefore, it might be most suitable vine leaf variety for the Sarma dish since unlobness is preferred by most of the cooks. Due to the fact that in the leaf with deep lobes, lamina becomes narrower and Sarma preparation gets difficult.

Karaerik variety had higher L, a and b values than that of the other varieties while these values of the Hacitesbihi, Agrazaki and Kabuguyufka varieties were similar (Table 1).

Percent dry matters of the fresh leaves were ranged from 21.41 to 24.72%. Hacitesbihi, Agrazai and Karaerik varieties seemed to be close with each other in terms of dry matter content (Table 2). Dry matter content was the lowest (21.41%) in the Kabuguyufka variety while it was the highest (24.72%) in Karaerik. However, the highest (2.11%) amounts of ash were in Hacitesbihi while the lowest (1.52%) in Karaerik.

pH of the all samples were similar while the titratable acidity varied among the leaf varieties, and the acidity values were 1.78, 1.78, 1.88 and 1.96% for Agrazaki, Karaerik, Hacitesbihi, and Kabuguyufka, respectively. Also, the amount of vitamin C varied significantly among the leaf samples (Table 2), and maximum vitamin C value was in Karaerik variety with 100.29 mg/100g concentration.

It has been known that fresh grape leaves are rich for vitamin C, therefore, the nutritive values of the leaves are usually considered to be high (Baysal, 1993).

Crude fiber content of the samples seemed to be high, and

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Table 1: The results of physical properties of fresh grape leaves

| Property | Variety | | | |
|-------------------------|-------------|----------|-------------|----------|
| | Hacitesbihi | Karaerik | Kabuguyufka | Agrazaki |
| Length cm | 17.75 | 18.20 | 15.00 | 13.76 |
| Width cm | 17.00 | 17.95 | 14.26 | 14.00 |
| Number of leaves /100 g | 28 | 24 | 35 | 41 |
| L | 43.99 | 48.03 | 42.62 | 42.60 |
| a | -14.92 | -18.54 | -13.88 | -14.06 |
| b | 19.60 | 26.93 | 16.73 | 17.48 |
| Hairiness | little | Little | little | Little |
| Lobness | 3 lobes | 5 lobes | 5 lobes | unlobes |

Table 2: The results of chemical properties of fresh grape leaves

| Property | Variety | | | |
|-----------------------|-------------|----------|-------------|----------|
| | Hacitesbihi | Karaerik | Kabuguyufka | Agrazaki |
| Dry matter % | 24.45 | 24.72 | 21.41 | 24.21 |
| Ash % | 2.11 | 1.52 | 1.86 | 1.93 |
| pH | 3.39 | 3.46 | 3.31 | 3.43 |
| Titrateable acidity % | 1.88 | 1.78 | 1.96 | 1.78 |
| Vitamin C (mg/100g) | 54.00 | 100.29 | 61.75 | 77.08 |
| Crude fiber % | 7.44 | 3.21 | 4.40 | 6.95 |
| Salt % | 0.16 | 0.19 | 0.22 | 0.25 |

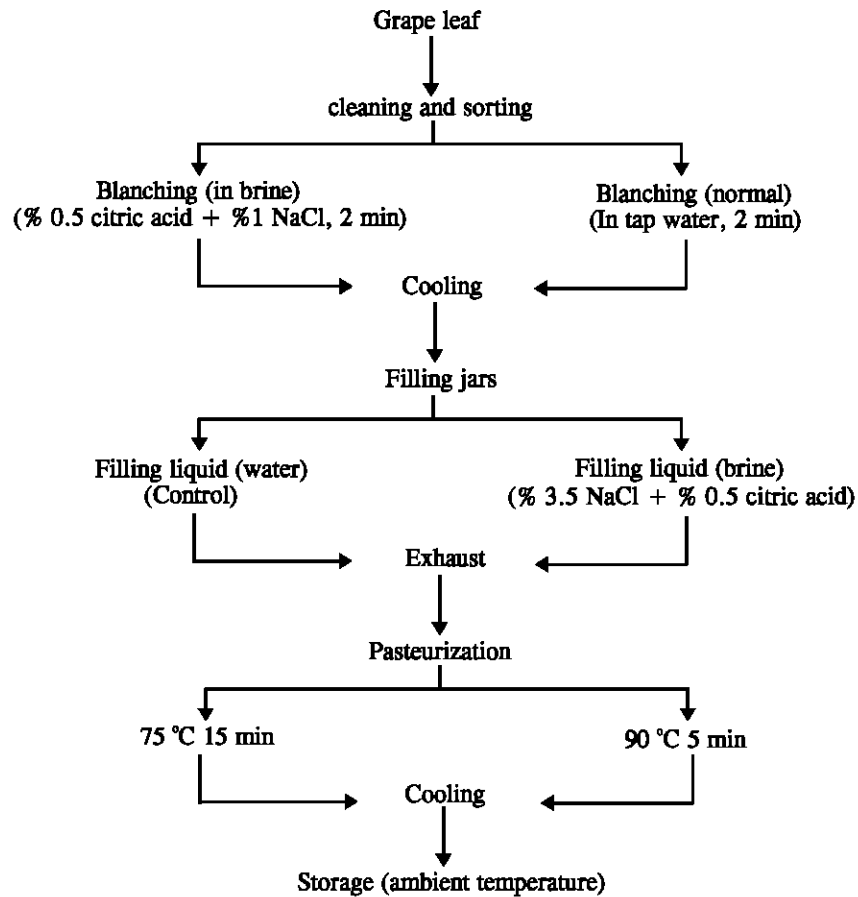


Fig. 1: Canning steps of grape leaves in this study

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Table 3. The results of color and some chemical properties of canned grape leaves

| Variety | | A* | B | C | D | E | F | G | H |
|--------------------|-------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Dry Matter | Hacitesbihi | 17.00 | 17.75 | 21.56 | 21.70 | 16.92 | 16.36 | 18.43 | 19.08 |
| | Agrazaki | 13.39 | 16.55 | 17.31 | 17.05 | 14.83 | 14.42 | 18.38 | 18.37 |
| | Kabuguyufka | 14.22 | 14.02 | 16.87 | 15.40 | 15.07 | 13.59 | 14.37 | 17.16 |
| | Karaerik | 15.19 | 13.74 | 18.10 | 16.53 | 15.64 | 17.74 | 17.21 | 16.87 |
| Ash | Hacitesbihi | 2.02 | 1.58 | 3.20 | 2.80 | 0.85 | 1.15 | 2.76 | 2.79 |
| | Agrazaki | 1.17 | 1.42 | 3.25 | 2.91 | 0.83 | 0.89 | 2.61 | 2.66 |
| | Kabuguyufka | 1.26 | 1.54 | 3.03 | 2.59 | 1.10 | 1.72 | 2.33 | 2.50 |
| | Karaerik | 1.13 | 1.23 | 2.91 | 3.02 | 1.13 | 0.69 | 2.55 | 2.55 |
| PH | Hacitesbihi | 3.64 | 3.65 | 3.28 | 3.27 | 3.59 | 3.59 | 3.26 | 3.17 |
| | Agrazaki | 3.73 | 3.67 | 3.43 | 3.35 | 3.62 | 3.70 | 3.33 | 3.27 |
| | Kabuguyufka | 3.58 | 3.62 | 3.30 | 3.32 | 3.59 | 3.52 | 3.26 | 3.26 |
| | Karaerik | 3.50 | 3.57 | 3.19 | 3.24 | 3.57 | 3.57 | 3.27 | 3.29 |
| Titratable Acidity | Hacitesbihi | 0.88 | 0.81 | 1.11 | 0.96 | 0.78 | 0.67 | 1.11 | 1.08 |
| | Agrazaki | 0.88 | 0.67 | 1.05 | 1.15 | 0.64 | 0.67 | 1.07 | 1.86 |
| | Kabuguyufka | 0.84 | 0.98 | 2.08 | 1.42 | 0.64 | 1.05 | 0.94 | 1.11 |
| | Karaerik | 0.91 | 0.86 | 1.16 | 1.15 | 0.74 | 0.78 | 0.89 | 0.82 |
| Vitamin C | Hacitesbihi | 5.16 | 6.85 | 5.16 | 6.38 | 5.16 | 6.85 | 5.16 | 5.16 |
| | Agrazaki | 10.28 | 10.29 | 3.38 | 6.87 | 6.90 | 6.40 | 3.42 | 6.89 |
| | Kabuguyufka | 13.73 | 24.04 | 12.03 | 15.47 | 6.87 | 22.32 | 6.87 | 10.29 |
| | Karaerik | 6.86 | 10.27 | 6.86 | 10.27 | 6.86 | 6.86 | 3.38 | 6.85 |
| Salt | Hacitesbihi | 0.09 | 0.14 | 2.12 | 2.23 | 0.11 | 0.12 | 1.96 | 2.29 |
| | Agrazaki | 0.12 | 0.14 | 2.99 | 2.10 | 0.18 | 0.19 | 2.73 | 2.16 |
| | Kabuguyufka | 0.16 | 0.18 | 2.77 | 2.56 | 0.17 | 0.20 | 2.37 | 2.42 |
| | Karaerik | 0.14 | 0.12 | 1.77 | 2.04 | 0.12 | 0.18 | 1.77 | 2.06 |
| Crude fiber | Hacitesbihi | 8.98 | 8.87 | 8.80 | 8.64 | 7.97 | 7.57 | 8.67 | 8.19 |
| | Agrazaki | 7.97 | 8.30 | 8.10 | 8.27 | 7.08 | 7.07 | 7.80 | 7.40 |
| | Kabuguyufka | 5.70 | 5.07 | 4.57 | 4.77 | 5.37 | 5.80 | 4.03 | 5.87 |
| | Karaerik | 3.20 | 3.42 | 3.12 | 3.03 | 3.53 | 3.20 | 3.30 | 3.37 |
| L | Hacitesbihi | 44.46 | 43.00 | 42.44 | 43.13 | 45.01 | 43.58 | 43.43 | 44.96 |
| | Agrazaki | 42.08 | 44.48 | 45.00 | 43.65 | 41.67 | 39.08 | 42.32 | 49.18 |
| | Kabuguyufka | 43.60 | 45.91 | 43.73 | 46.10 | 43.85 | 47.30 | 44.99 | 44.59 |
| | Karaerik | 43.17 | 47.34 | 47.51 | 45.85 | 49.28 | 51.36 | 48.41 | 44.16 |
| a | Hacitesbihi | -0.29 | 0.17 | -0.14 | -0.51 | -0.59 | 0.48 | -0.15 | 0.92 |
| | Agrazaki | -0.42 | 0.51 | -0.78 | -0.07 | -1.41 | 1.38 | 0.70 | -0.47 |
| | Kabuguyufka | 1.12 | 0.25 | 0.38 | -0.18 | 0.85 | -0.37 | -0.40 | 0.15 |
| | Karaerik | 1.37 | 0.27 | 0.69 | 0.14 | -0.41 | -0.87 | -0.16 | 1.37 |
| b | Hacitesbihi | 23.34 | 22.56 | 21.69 | 20.50 | 24.22 | 24.21 | 24.28 | 27.85 |
| | Agrazaki | 18.01 | 25.14 | 29.20 | 25.90 | 23.14 | 21.06 | 22.87 | 33.92 |
| | Kabuguyufka | 24.63 | 30.52 | 22.20 | 26.10 | 26.70 | 26.83 | 25.61 | 25.17 |
| | Karaerik | 24.76 | 24.86 | 24.26 | 23.53 | 29.80 | 30.23 | 29.82 | 21.24 |

A*: Blanched in brine (0.5%citric acid + 1%NaCl), heated at 75 °C for 15 min and the jars filled with tap water

B: Blanched in brine (0.5%citric acid + 1%NaCl), heated at 90 °C for 5 min and the jars filled with tap water

C: Blanched in brine (0.5%citric acid + 1%NaCl), heated at 75 °C for 15 min and the jars filled with the brine (3.5%NaCl+ 0.5%citric acid)

D: Blanched in brine (0.5%citric acid + 1%NaCl), heated at 90 °C for 5 min and the jars filled with the brine (3.5%NaCl + 0.5%citric acid)

E: Blanched tap water, heated at 75 °C for 15 min and the jars filled the tap water

F: Blanched tap water, heated at 90 °C for 5 min and the jars filled the tap water

G: Blanched tap water, heated at 75 °C for 15 min and the jars filled with the brine (3.5%NaCl + 0.5%citric acid)

H: Blanched tap water, heated at 90 °C for 5 min and the jars filled with the brine (3.5%NaCl + 0.5%citric acid)

Table 4: Results of sensory evaluation of the fresh and canned leaves

| Varieties | | Color | Flavor | Texture | Overall quality | Total |
|-------------|------------|-------|--------|---------|-----------------|-------|
| Hacitesbihi | Fresh leaf | 20.00 | 21.22 | 20.44 | 20.44 | 20.53 |
| | A | 14.33 | 18.05 | 11.94 | 13.89 | 14.55 |
| | B | 14.33 | 16.67 | 16.67 | 14.72 | 15.60 |
| | C | 20.36 | 16.11 | 17.58 | 17.78 | 17.96 |
| | D | 19.44 | 19.44 | 17.58 | 18.50 | 18.74 |
| | E | 15.28 | 14.81 | 12.78 | 14.17 | 14.26 |
| | F | 13.89 | 15.56 | 16.67 | 15.28 | 15.35 |
| | G | 19.44 | 14.81 | 16.19 | 16.19 | 16.66 |
| | H | 15.72 | 17.11 | 15.72 | 17.11 | 16.42 |
| Agrazaki | Fresh leaf | 22.22 | 21.53 | 21.16 | 21.53 | 21.61 |
| | A | 19.11 | 16.36 | 19.21 | 18.98 | 18.42 |
| | B | 19.02 | 15.05 | 19.02 | 19.22 | 18.08 |
| | C | 17.83 | 17.06 | 19.44 | 18.25 | 18.15 |
| | D | 16.86 | 18.25 | 15.47 | 16.06 | 16.66 |
| | E | 19.44 | 16.06 | 18.25 | 17.83 | 17.90 |
| | F | 19.03 | 15.86 | 17.06 | 18.06 | 17.50 |
| | G | 18.44 | 17.06 | 17.83 | 17.83 | 17.79 |
| | H | 17.83 | 19.83 | 19.64 | 19.03 | 19.08 |
| Kabuguyufka | Fresh leaf | 18.75 | 18.75 | 13.53 | 15.28 | 16.58 |
| | A | 16.25 | 14.66 | 15.05 | 13.89 | 14.96 |
| | B | 14.86 | 15.28 | 13.08 | 15.28 | 14.63 |
| | C | 23.61 | 22.22 | 23.19 | 23.61 | 23.16 |
| | D | 23.39 | 21.81 | 21.61 | 22.61 | 22.36 |
| | E | 13.89 | 14.17 | 12.50 | 17.78 | 14.59 |
| | F | 16.67 | 15.03 | 12.69 | 13.89 | 14.57 |
| | G | 17.06 | 23.39 | 18.64 | 19.44 | 19.63 |
| | H | 15.47 | 18.83 | 14.66 | 15.50 | 15.87 |
| Karaerik | Fresh leaf | 21.61 | 18.89 | 14.92 | 17.06 | 18.12 |
| | A | 14.28 | 17.06 | 16.67 | 17.84 | 16.46 |
| | B | 16.67 | 16.25 | 18.25 | 17.84 | 17.25 |
| | C | 19.44 | 21.03 | 20.84 | 20.53 | 20.46 |
| | D | 18.64 | 21.81 | 22.00 | 22.69 | 21.29 |
| | E | 17.06 | 12.69 | 18.64 | 15.86 | 16.06 |
| | F | 18.05 | 11.50 | 17.44 | 14.67 | 15.42 |
| | G | 22.22 | 21.22 | 21.42 | 21.81 | 21.67 |
| | H | 23.00 | 21.62 | 23.00 | 23.00 | 22.66 |

All samples were scored 25 point 25: very good 20: good 15: not bad 10: bad 5: very bad

Hacitesbihi and Agrazaki had similar amount of fiber while it was different in kabuguyufka and Karaerik (Table 2).

Canned Leaves: At the end of the 7-months of storage, the results of chemical analyses and color measurements of the canned leaves were summarized in Table 3. Dry matter contents of the processed leaves were lowered, that might be due to the increase in the moisture content of the leaves during the storage. In the meantime, dry matter content of the brine (filling liquid) was higher than that of the tap water groups. Additionally, the samples that blanched in brine and then canned had higher dry matter contents than that of the samples that blanched in tap water and then canned counterparts (Table 3). This result was probably due to the osmosis and salt content of the leaves.

As expected, the ash content decreased numerically in the

tap water leaves while increased in the brined samples. This is because of the brine that was also confirmed by the ash content of the samples, that was higher in brine-blanched leaves than tap water blanched samples (Table 3).

In comparison to fresh leaves, pH values of the tap water group of the canned samples were higher than that of the brined groups. This might be due to the acid content of the brine that had 0.5% citric acid. The titratable acidity of the preserved leaves was generally lower than that of the fresh leaves (Table 2 and 3), and at the moment, there is no explanation for this result.

Vitamin C levels of the all canned leaves were appreciably low when compared to their fresh counterparts. This decrease might be caused by the processing technique, and with the exception of Hacitesbihi variety, vitamin C contents of the blanched leaves seemed to be preserved better in brine compared

to samples that blanched in tap water (Table 2 and 3). This is due to the citric acid content in blanching liquid in which vitamin C is usually more stabile owing to acidic conditions (Lee and Kader, 2000). Additionally, citric acid makes chelats with Fe^{++} , Cu^{++} ions which catalyzes vitamin C loss (Cemeroglu, 1982). The results indicated that high processing temperatures (95 °C and 5 min) had further saving effect on vitamin C content as compared to the lower processing temperatures (75 °C 15 min).

As seen in Table 3, titratable salt content of the tap water groups was lower than that of the brined groups. That is, salt contents of the brined groups were naturally increased because of the osmotic activity between the tissue and the liquid. Compared to fresh leaves, crude fiber contents of canned leaves were high (Table 3), this might be due to the migration of water-soluble leaf compounds into the brine.

Natural green colors of the fresh leaves were decreased while yellow colors increased with both blanching and storage (Table 2 and 3). The color alteration might be due to the chemistry of the pigments like chlorophylls that is usually converted to pheophytine and some other compounds during the thermal processing and storage (Cemeroglu and Acar, 1986).

The Sensory Analysis: Results of the sensory evaluation of the leaves were presented in Table 4. Both fresh and canned leaves were subjected to sensory analysis since the grape leaves can be consumed in either fresh or processed. As the suitability of leaves to prepare Sarma dish determined, Agrazaki variety had the highest score among the fresh leaves, this is because of the leaf of this variety that unlobbed and can easily be rolled. Again the suitability of the leaves canned in brine for Sarma dish characteristics, that scored by the panelists Kabuguyufka and Karaerik variety were superior to the other leaves. Consequently, all the chemical and sensory evaluation results showed that Agrazaki varieties as a fresh, Kabuguyufka and Karaerik that canned in brine were determined to be most convenient grape leaves to produce Sarma dish in either cases.

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Mycotoxins Produced by Fungi Isolated from Wine Cork Stoppers

Centeno, S. and Calvo, M^a A.

Microbiology, Facultad de Veterinaria, Universitat Autònoma de Barcelona,
08193 Bellaterra (Barcelona) Spain
e-mail: MariAngels.Calvo@uab.es

Abstract: The research work was conducted to determine the production of mycotoxins from the fungi isolated from wine corks stoppers. When comparing the four fractions of *Alternaria alternata* obtained by the thin layer chromatography with the standards of tenuazonic acid, alter toxin I, altenuene, alternariol, alternariol monomethyl ether and ten toxin, it can be observed that *Alternaria alternata* only produces alter toxin I, altenuene and alternariol monomethyl ether. The results showed that the production of citrinin by *Penicillium citrinum* and of fumonisin B₁ by *Fusarium moniliforme*, due to the fact that the fractions isolated by TLC of these fungi coincide with the controls for such mycotoxins. *Fusarium solani* did not produce fumonisin B₁.

Key words: Mycotoxins, cork stopper, *Alternaria alternata*

Introduction

Mycotoxins are a group of secondary metabolites produced by filamentous fungi which may contaminate food, feeds or the raw materials used to produce them. They also produce mycotoxicoses in humans and animals (Moss, 1994).

The genera of mycotoxigenic fungi are mainly represented by *Aspergillus*, *Penicillium* and *Fusarium*, but *Trichoderma*, *Trichothecium* and *Alternaria* are also important as food contaminants or pathogens for plants, among others (Smith, 1983).

Among the diversity of mycotoxins described so far, we will only mention those directly related to the genera that are the objectives of this study.

Citrinin is produced by *Penicillium citrinum*, although it may also be produced by *Penicillium expansum* and *Penicillium verrucosum* and some species of *Aspergillus*. It is a quinone methide with a powerful antibacterial effect, but toxic to humans and animals. It is also a contaminant in cereals such as wheat, maize, barley and oats (Montani *et al.*, 1988; Franco *et al.*, 1996).

Fumonisin is another important group of mycotoxins produced by *Fusarium*, particularly by *Fusarium moniliforme*. The most abundant in nature is fumonisin B₁, which may be related to esophageal cancer in humans. Its hepatotoxic and hepatocarcinogenic properties have also been proven. They have also been shown to be nephrotoxic, immunodepressant and embryo toxic for experimental animals (Voss *et al.*, 1990; Nair, 1998; Sweeney and Dobson, 1999). These mycotoxins are contaminants of natural or processed maize (flour) used as animal or human food (Dombrink-Kurtzman and Dvorak, 1999).

Alternaria alternata produces mycotoxin in grain, rice and maize (Ramm *et al.*, 1994; Stack and Prival, 1986). Contamination with grain that has metabolites from the *Alternaria* species may be related to the occurrence of

esophageal cancer in some geographical regions (Davis and Stack, 1991). Mycotoxins produced by *Alternaria* and specifically by *Alternaria alternata* are numerous. Among them the most widely studied are: tenuazonic acid (TA), alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), alter toxin I and ten toxin (Griffin and Chu, 1983; Orvehed *et al.*, 1988).

The main goal of the present study is to determine the production of mycotoxins from the fungi previously mentioned, which have been isolated from wine corks.

Materials and Methods

Fungi isolated from wine corks stoppers, those which according to the corresponding literature were capable of elaborating and accumulating mycotoxins, used in this study. These fungi were: *Alternaria alternata*, *Penicillium citrinum*, *Fusarium moniliforme* and *Fusarium solani*.

The microorganisms already mentioned were cultivated in Petri dishes with 2% malt extract agar (MEA) at a temperature of 28 °C during 7 days. From these, discs of 6 mm diameter were obtained. These discs were placed on Merck® chromatoplates of silicagel at a distance of 2 cm from the lower edge for one minute. The chromatoplates had previously been activated at a temperature of 105 °C for 90 min.

In the same way, 10 µl of the specific mycotoxin standards for each microorganism, prepared in a concentration of 25 µl of the specific mycotoxin standards for each microorganism, prepared in a concentration of 25 µl/ml of chloroform, were placed next to each disc.

The mycotoxins assayed were: tenuazonic acid (Sigma®), ten toxin (Sigma®), Alternariol (Sigma®), altenuene (Sigma®), alternariol monomethyl ether (Sigma®), alter toxin I (Sigma®), citrinin (Sigma®) and fumonisin B₁ (Sigma®).

Thin layer chromatography (TLC) was carried out using of benzene/methanol/glacial (96:6:2, v:v:v) to make it

Table 1: Values of Rf of the mycotoxins produced by *Alternaria alternata*

| Mycotoxin | Rf | |
|------------------------------------|----------|-----------------------------|
| | Standard | <i>Alternaria alternata</i> |
| Tenuazonic acid (TA) | 0.06 | ND |
| Alter toxin I (ATX-I) | 0.26 | 0.26 |
| Altenuene (ALT) | 0.13 | 0.13 |
| Alternariol (AOH) | 0.18 | 0.18 |
| Alternariol monomethyl ether (AME) | 0.38 | 0.38 |
| Tentoxin (TEN) | 0.15 | ND |

Table 2: Values of the Rf of the mycotoxins produced by *Penicillium citrinum*, *Fusarium moniliforme* and *Fusarium solani*

| Microorganism | Mycotoxin | Rf | |
|-----------------------------|--------------------------|----------|---------------|
| | | Standard | Microorganism |
| <i>Penicillium citrinum</i> | Citrinin | 0.75 | 0.75 |
| <i>Fusarium moniliforme</i> | Fumonisin B ₁ | 0.71 | 0.71 |
| <i>Fusarium solani</i> | Fumonisin B ₁ | 0.71 | ND |

possible to detect the mycotoxins produced by *Alternaria alternata* and a mixture of chloroform/methanol (50:50, v:v) to detect the mycotoxins produced by *Penicillium citrinum*, *Fusarium moniliforme* and *Fusarium solani*. The chromatoplates removed after 90 minutes, or when the solvent reached 4 cm from the upper edge of the chromatoplates. They were dried mechanically. Reading the chromatoplates was carried out by observing the chromatographic spots from the discs containing the microorganisms and their standards was carried out under ultraviolet rays with λ of 254 and 356 nanometers.

Results and Discussion

Fig. 1, illustrates the results obtained from the study of the production of mycotoxins by *Alternaria alternata* after TLC. Fig. 2 shows the results of the production of citrinin by *Penicillium citrinum* and fumonisin B₁ by *Fusarium moniliforme* and *Fusarium solani*.

Table 1 shows the values of the Rf corresponding to the chromatographic spots of the mycotoxins produced by *Alternaria alternata*. Table 2 shows the values of the Rf corresponding to the chromatographic spots of the mycotoxins produced by *Penicillium citrinum*, *Fusarium moniliforme* and *Fusarium solani*.

When comparing the four fractions of *Alternaria alternata* obtained by the thin layer chromatography with the standards of tenuazonic acid, alter toxin I, altenuene, alternariol, alternariol monomethyl ether and ten toxin, it can be observed that *Alternaria alternata* only produces alter toxin I, altenuene and alternariol monomethyl ether. *Alternaria alternata* produce neither tenuazonic acid nor ten toxin under the experimental condition of this study.

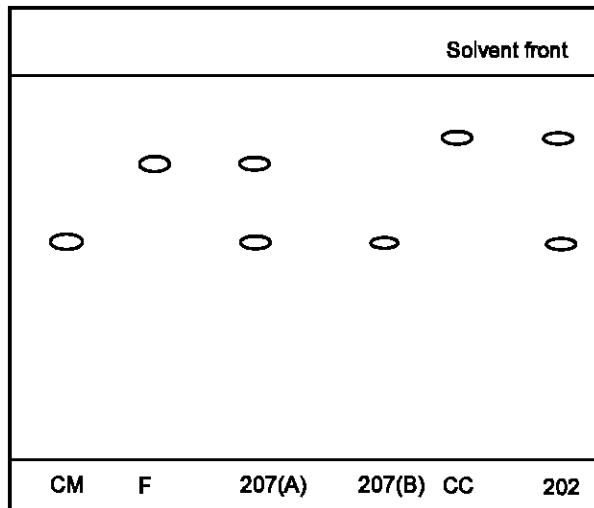


Fig. 1: TLC of the mycotoxins produced by *Alternaria alternata* 201: *Alternaria alternata*, TA: Tenuazonic acid, ATX-I: Alter toxin I, ALT: Altenuene, AOH: Alternariol, AME: Alternariol monomethyl ether, TEN, ten toxin

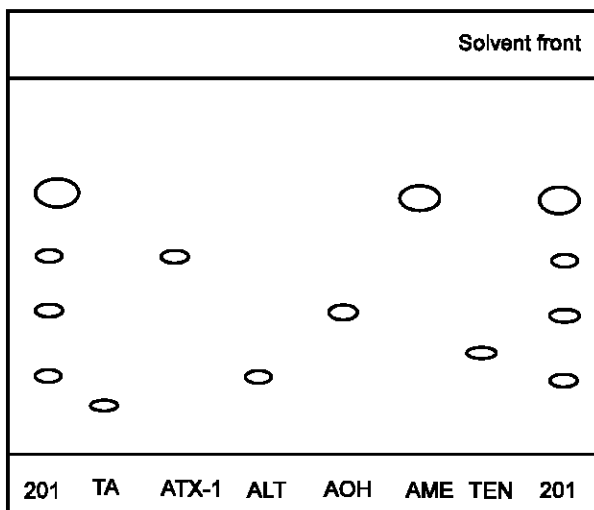


Fig. 2: TLC of the mycotoxins produced by *penicillium citrinum*, *Fusarium moniliforme* and *fusarium solani* CM: Control (2 % malt extract agar), F: Fumonisin, B₁: Control 207(A): *Fusarium moniliforme*, 207(B) : *Fusarium solani*, CC: Citrinin Control, 202: *Penicillium citrinum*

The production of ten toxin is conditioned by a limited quantity of phosphate in the cultivation environment (Ramm *et al.*, 1994), which may have affected the results. Tenuazonic acid is described by Stinson *et al.*, 1980 as a mycotoxin which may be produced by numerous species of *Alternaria*, in cultures isolated from different sources. It

is considered the most important *Alternaria alternata* toxic substance although its production is influenced by nitrogen concentration in a cultivation environment. However, isolated *Alternaria alternata* did not produce tenuazonic acid in this study.

Generally, the production of mycotoxins by *Alternaria alternata* is conditioned by high water activity (a_w), by the incubation temperature, by the pH substrate and by the type of substrate in which the microorganism grows (Burroughs *et al.*, 1976; Magan *et al.*, 1984).

The results obtained illustrate the production of citrinin by *Penicillium citrinum* and of fumonisin B₁ by *Fusarium moniliforme*, due to the fact that the fractions isolated by TLC of these fungi coincide with the controls for such mycotoxins. *Fusarium solani* did not produce fumosin B₁. As with the mycotoxins produced by *Alternaria alternata*, the production of citrinin and fumonisin B₁ depend on water activity (*Fusarium moniliforme* does not produce fumonisins below 0.87 water activity), on environmental, pH, on temperature and on the incubation period of *Penicillium citrinum* and *Fusarium moniliforme* (Montani *et al.*, 1988; Sweeney and Dobson, 1999; Alberts *et al.*, 1990; Wheeler *et al.*, 1991).

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Influences of Olive Oil and Ghee (samen balady) on Serum Cholesterol of Jordanians

Abdullah Y. A. Rawashdeh

Department of Nutrition and Food Technology, Faculty of Agriculture, Mu'tah University,
Mu'tah, Karak, P.O.Box 7, Jordan

Abstract: The effects of olive oil (OO) and ghee types (samen balady) on the serum lipid profile were studied in healthy volunteers (11 males, aged 36-44 year; 13 females aged 27-35 year). The 24 subjects ate their habitual diet with OO or ghee of cow milk fat (CMF), ghee of goat milk fat (GMF) or ghee of sheep milk fat (SMF) for 4 wk with 4 wk interval between the four diets. Compared with initial values, OO diet significantly ($P < 0.05$) reduced concentrations of serum total cholesterol (TC, -3.42%) and low density lipoprotein cholesterol (LDL-c, -4.31%). OO caused slight reduction in concentration of high density lipoprotein cholesterol (HDL-c, -2.86%), and ratios of TC/HDL-c and LDL-c/HDL-c. Whereas ghee types significantly ($P < 0.05$) increased these parameters and the SMF resulted in the highest rise of TC (+11.93%) and LDL-c (+16.16%). The highest rise in concentration of HDL-c (+8.81%) was shown with GMF. Ghee types slightly increased the ratios of TC/HDL-c and LDL-c/HDL-c compared with the initial values. SMF resulted in the highest rise of TC/HDL-c (+0.26) and LDL-c/HDL-c (+0.31). Serum triglycerides level increased (+3.38 %) after OO diet, whereas reduced (about -2.15%) after periods of ghee types. In general, the responses in serum lipids were greater in males than in females in all the four diets.

Key words: Olive oil, ghee, cholesterol, LDL-c, HDL-c, triglycerides

Introduction

Coronary heart disease (CHD), the common cause of heart attack, is one of the most frequent causes of death in the developed and developing countries, (AHA, 1989). Several inherited and lifestyle factors affecting the risk of heart diseases, among the latter are cigarette smoking, physical exercise, and diet habits (Elson, 1992; Macnair, 1994). Through a period of time many research workers showed a direct link between elevated level of blood cholesterol and the occurrence of CHD (John *et al.*, 1990). Cholesterol is the precursor of steroid hormones, bile acids and also required for normal cell functions. On the other hand, it is a major contributor to atherosclerosis plaques and most gallstones (Grundy, 1983). Low density lipoproteins (LDLs) and high density lipoproteins (HDLs) refer to the two types of lipoproteins, package of fat, cholesterol, and protein to transport it through the blood. LDLs are responsible for depositing cholesterol in the artery walls. These lipoproteins may be atherogenic. HDLs can acquire cholesterol from cells and transport it to the liver for reprocessing or bile acids formation. These lipoproteins are appearing to be antiatherogenic (Hui, 1992). The high blood LDL-cholesterol (LDL-c) concentration is associated with a higher risk of heart attack, whereas the high blood HDL-cholesterol (HDL-c) concentration may play a beneficial role (koo *et al.*, 1985). Among a number of possible causes related to blood cholesterol level, the one that is the type of fatty substances. Solid fats raise this level, whereas oils lower it (Macnair, 1994). There is a positive relationship between the dietary cholesterol intake and serum

cholesterol (Cohen *et al.*, 1994). Dairy products make appreciable contribution to saturated fat and cholesterol intake. Consumption these products may be correlated with high blood cholesterol level (Rossouw *et al.*, 1981). However, the height monounsaturated fat diet can be an alternative to the presently recommended 30% fat diet to reduce the risk of heart disease (Kris-Etherton, 1999). The effects of cholesterol, saturated fatty acids (SFA), mono unsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and total fat intakes on serum cholesterol have been studied extensively without clear cut relationship emerging (Thannoun, 1993).

Olive oil (OO) and Ghee (samen balady) are widely used in Jordanian foods. Ghee, which is rich in SFA, produce from butter of sheep milk fat (SMF) or goat milk fat (GMF) or cow milk fat (CMF). This study was interested in determining the effects of OO and ghee of mentioned three sources upon Jordanian blood lipid profile. So far none of the studies reported in Jordan to assess the effects of ghee types on serum lipid profile of human.

Materials and Methods

Subjects: Participants in the study were selected from different locations (Amman, Karak) in Jordan. Eleven males averaged 38 years old (ranged 36-44 yr), and thirteen females averaged 30 years old (ranged 27-35 yr) were involved in this study. Started from September 2000 to April 2001. Body weight and body mass index of males were 64 kg and 21.3, respectively, and of females were 57kg and 21.6, respectively. All subjects were healthy, normocholesterol level, normotensive, non diabetic, and

Table 1: Fatty acid profile of olive oil (OO), cow milk fat (CMF), goat milk fat (GMF) and sheep milk fat (SMF)

| Fatty acid | OO % | CMF % | GMF % | SMF % |
|------------|---------|----------|----------|----------|
| 4,6,8:0 | - | 6.7 | 8.5 | 9.7 |
| 10:0 | - | 3.5 | 9.0 | 7.0 |
| 12:0 | - | 3.8 | 5.0 | 5.0 |
| 14:0 | - | 8.2 | 10.4 | 12.2 |
| 14:1 | - | 0.8 | 1.2 | 1.0 |
| 14:2 | - | 0.4 | - | - |
| 15:0 | - | 0.2 | - | - |
| 16:0 | 14.8 | 25 | 25.2 | 26.4 |
| 16:1 | 0.8 | 2.1 | 2.4 | 3.3 |
| 16:2 | 0.2 | 0.2 | - | - |
| 17:0 | - | 0.5 | 0.6 | 1.5 |
| 18:0 | 5.3 | 10.5 | 8.1 | 11.0 |
| 18:1 | 66.4 | 32.5 | 26.6 | 21. |
| 18:2 | 11.9 | 4.2 | 2.3 | 1.4 |
| 18:3 | 0.6 | 1.2 | 0.7 | 0.5 |
| 20:0 | - | 0.2 | - | - |
| SFA% (s) * | 20.1 | 58.6 | 66.8 | 72.8 |
| MUFA% (m) | 67.2 | 35.4 | 30.2 | 25.3 |
| PUFA% (p) | 12.7 | 6.0 | 3.0 | 1.9 |
| P:S | 0.63 | 0.10 | 0.04 | 0.03 |
| m+p/s | 3.98 | 0.71 | 0.50 | 0.37 |
| TC(mg/dl) | - | 150 | 175 | 162 |

*SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TC, total cholesterol.

free from cardiac, renal, hepatic or bleeding disorders.

Diets: Bread (twice a day) and rice (four times a week) were contributing as the main source of carbohydrates. Chicken (twice a week) and eggs (three eggs a week) were contributing as the main source of protein. Milk and its products (three times a week). Meat was eaten only once a week. Vegetables were eaten four times a week, and fruits were eaten twice a week. This study depended on the habitual diets of subjects. The only variable was the type of oil and fat used in cooking or dipping. OO and ghee types were purchased from local producers and used in the preparation of participant diets. Fatty acid composition and cholesterol content of OO and ghee types used in this study are shown in Table 1. Repeated 24 hr-diet-recall for individual dietary intake was used in this study (Table 2). Macro nutrients and energy were calculated using food composition tables (Pellet and Shadarvian, 1970).

Study design: Before the beginning of the study the nutritional status of participants were studied, this work showed that the differences in food intake of individuals from week to another were negligible and they asked to maintain their traditional diets. The 24-subjects ate their

food with OO or CMF or GMF or SMF as the sole source of fat type for 4wk with 4wk interval between the four diets. However, the period 4wk was chosen as it had previously been shown that plasma lipid levels stabilized within 2-3 wk after initiating a change in dietary fat (Bonamon and Grundy, 1988). Fasting venous blood samples (10 ml) were collected at the entry and the end of each dietary period for next analysis.

Chemical analysis: Fatty acid (FA) compositions: samples of OO and ghee types were saponified and the free FAs were methylated following the procedure of Morrison and Smith (1962). FA methyl esters were separated by Hamlet Packard gas chromatography model 5710a equipped with flame ionization detectors and column of 10% DEGS on chromosorb WDMCS (Supelco Inc.). Carrier gas flow rate was 24 ml of N₂/min. Flow rate of detectors were 30 ml of H₂/min and 300ml/min of air. The initial column temperature was 90 °C then raised to 180 °C at a rate of 6 °C /min. The injector and detector temperatures were 200 °C and 250 °C, respectively. The identification of individual FA was made using FA-methyl ester standards to establish relative retention time. The relative content of each FA-methyl ester was reported as a percent area of total FA-methyl esters. Total cholesterol of ghee types was determined according to the method of Plummer (1978). Serum total cholesterol was determined using the enzymatic method from Arab Company for medical diagnostic, (Jordan). Triglycerides (TG) were determined by the enzymatic technique from Biocon, (Germany). HDL-c was analyzed by the precipitation technique using magnesium chloride and phosphotungstic acid from Biocon, (Germany). LDL-c was calculated using the formula of Friedewald *et al.* (1972).

Statistical analysis: The Completely Randomized Design (CRD) was used for each parameter. Differences between means were determined using Duncan's multiple range tests at $p < 0.05$ by SAS version (1986).

Results and Discussion

Food consumption: The fatty acid profiles and cholesterol content of OO and ghee types are presented in Table 1. SMF contained the higher SFA (72.8%), the lower MUFA (25.3%) and the lower PUFA (1.9%) than that in GMF, CMF, and OO. Whereas, OO contained the highest MUFA (67.2%) and PUFA (12.7%) and the lowest SFA (20.1%). The ratio of PUFA / SFA (p: s) and MUFA + PUFA (m+p)/s has been used by nutritionists to interpret the effect of dietary oil and fat on the level of blood cholesterol (Hodson *et al.*, 2001). The results showed that p: s and m+p/s ratios of OO, CMF, GMF, and SMF were (0.63, 3.98), (0.10, 0.71), (0.04, 0.50) and (0.03, 0.37), respectively. The results also indicated that OO was free cholesterol. CMF, GMF and SMF were contained 150, 175, and 162 mg cholesterol/dl, respectively.

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Table 2: Food consumption of subjects

| Dietary Fat Type* | Gender | Energy Cal | Carbohydrates | | Fats | | Proteins | |
|-------------------|---------|------------|---------------|----------|--------|----------|----------|----------|
| | | | g | % of Cal | g | % of Cal | g | % of Cal |
| OO | Males | 2026 ± 106 | 320 ± 27 | 63.2 | 62 ± 6 | 2.75 | 47 ± 5 | 9.3 |
| | Females | 1821 ± 124 | 296 ± 22 | 65.0 | 53 ± 6 | 26.2 | 40 ± 6 | 8.8 |
| | Total | 1915 | 307 | 64.1 | 57.1 | 26.8 | 43.2 | 9.1 |
| CMF | Male | 2004 ± 100 | 309 ± 32 | 61.7 | 64 ± 4 | 28.7 | 48 ± 9 | 9.6 |
| | Females | 1813 ± 131 | 288 ± 41 | 63.5 | 55 ± 8 | 27.3 | 42 ± 7 | 9.3 |
| | Total | 1901 | 297 | 62.6 | 59.1 | 28 | 44.8 | 9.4 |
| GMF | Males | 1947 ± 98 | 308 ± 33 | 63.3 | 59 ± 7 | 27.2 | 46 ± 6 | 9.5 |
| | Females | 1783 ± 119 | 292 ± 36 | 65.6 | 51 ± 6 | 25.7 | 39 ± 4 | 8.7 |
| | Total | 1858 | 300 | 64.5 | 54.7 | 26.5 | 42.2 | 9.1 |
| SMF | Males | 1964 ± 84 | 313 ± 36 | 63.7 | 60 ± 7 | 2.75 | 43 ± 5 | 8.8 |
| | Females | 1710 ± 132 | 278 ± 29 | 65.0 | 50 ± 4 | 26.3 | 37 ± 6 | 8.7 |
| | Total | 1826 | 294 | 64.4 | 54.6 | 26.9 | 39.8 | 8.7 |
| Means of Totals | | 1875 | 300 | 63.9 | 56.4 | 27.0 | 42.5 | 9.1 |

* OO, olive oil; CMF, cow milk fat; GMF, goat milk fat, SMF, sheep milk fat.

Table 3: Effects of dietary olive oil (OO), and ghee of cow milk fat (CMF), goat milk fat (GMF), and sheep milk fat (SMF) on concentrations of serum lipids of males and females

| Serum ⁽¹⁾ Lipids | O O | | CMF | | GMF | | SMF | |
|-----------------------------|------------|------------|------------|------------|------------|------------|------------|-------------|
| | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| TC (mg/dl) | 162 ± 14 | 155 ± 15* | 160 ± 10 | 170 ± 17* | 164 ± 7 | 177 ± 8* | 165 ± 12 | 186 ± 14* |
| Males | 151 ± 15 | 147 ± 18* | 154 ± 14 | 163 ± 11* | 161 ± 16 | 172 ± 18* | 160 ± 15 | 178 ± 19* |
| Females | 156 | 151 | 157 | 166 | 162 | 174 | 162 | 182 |
| LDL-c(mg/dl) | | | | | | | | |
| Male | 106 ± 13 | 100 ± 8 | 104 ± 9 | 112 ± 7* | 109 ± 11 | 119 ± 13* | 109 ± 15 | 128 ± 17* |
| Females | 95 ± 14 | 92 ± 15* | 99 ± 16 | 107 ± 12* | 104 ± 13 | 112 ± 16* | 106 ± 10 | 122 ± 17* |
| Total | 100 | 96 | 102 | 110 | 166 | 115 | 107 | 125 |
| HDL-c(mg/dl) | | | | | | | | |
| Males | 35 ± 7 | 34 ± 4 | 35 ± 6 | 37 ± 2 | 33 ± 5 | 36 ± 5 | 36 ± 4 | 38 ± 2 |
| Females | 35 ± 3 | 34 ± 4 | 32 ± 2 | 33 ± 3 | 35 ± 6 | 38 ± 7 | 33 ± 4 | 35 ± 3 |
| Total | 35 | 34 | 33.4 | 34.8 | 34.1 | 37.1 | 34.4 | 36.4 |
| TC/HDL-c | | | | | | | | |
| Males | 4.63 ± 0.4 | 4.56 ± 0.6 | 4.57 ± 0.5 | 4.59 ± 0.6 | 4.97 ± 0.3 | 4.92 ± 0.1 | 4.58 ± 0.3 | 4.89 ± 0.4 |
| Females | 4.31 ± 0.3 | 4.32 ± 0.4 | 4.81 ± 0.3 | 4.94 ± 0.5 | 4.60 ± 0.1 | 4.53 ± 0.7 | 4.85 ± 0.6 | 5.09 ± 0.8 |
| Total | 4.46 | 4.43 | 4.70 | 4.78 | 4.77 | 4.71 | 4.73 | 4.99 |
| LDL-c/HDL-c | | | | | | | | |
| Males | 3.03 ± 0.2 | 2.94 ± 0.1 | 2.97 ± 0.3 | 3.03 ± 0.2 | 3.30 ± 0.5 | 3.31 ± 0.1 | 3.03 ± 0.3 | 3.37 ± 0.4* |
| Females | 2.71 ± 0.2 | 2.71 ± 0.1 | 3.09 ± 0.3 | 3.24 ± 0.5 | 2.97 ± 0.1 | 2.75 ± 0.2 | 3.21 ± 0.3 | 3.49 ± 0.4* |
| Total | 2.86 | 2.81 | 3.09 | 3.19 | 3.12 | 3.11 | 3.12 | 3.43 |
| TG (mg/dl) | | | | | | | | |
| Males | 104 ± 7 | 107 ± 6 | 107 ± 8 | 105 ± 6 | 111 ± 7 | 108 ± 15 | 101 ± 14 | 99 ± 13 |
| Females | 105 ± 7 | 109 ± 6 | 115 ± 5 | 113 ± 7 | 110 ± 6 | 108 ± 3 | 107 ± 4 | 104 ± 5 |
| Total | 105 | 108 | 111 | 109 | 111 | 108 | 104 | 106 |

⁽¹⁾: TC, total cholesterol; LDL-c, low density lipoprotein- cholesterol; HDL-c, high density lipoprotein- cholesterol; TG, triglycerides. * Significantly different from initial value P < 0.05.

As seen in Table 2, the proximate analysis for mean daily intake of energy, carbohydrates, fats and proteins, of both sexes at the four diets, was 1875Kcal, 300g, 56.4g and 42.5g, respectively. Food intake study was noted that OO consumed by volunteers more than any other oil or fat. It was used at least twice a day; consistently for breakfast

and lunch, especially with chickpea and thyme, and many times used for cooking. The ghee made from SMF may be used more than other ghee types. The usage of ghee of CMF may be negligible. Food consumption of males was generally higher than that for females. Investigated oil and fats formed about 58% of the total dietary fat intake.

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Table 4: Serum lipid responses (mg/dl and %) from the base line of males and females fed dietary olive oil (OO), and ghee of cow milk fat (CMF), of goat milk fat (GMF), and of sheep milk fat (SMF)

| Dietary Fat Type | Gender | *TC | | LDL-C | | HDL-C | | Triglycerides | |
|------------------|---------|--------|--------|--------|--------|-------|-------|---------------|-------|
| | | mg/dl | % | mg/dl | % | mg/dl | % | mg/dl | % |
| OO | Males | -7 | -4.32 | -6 | -5.66 | -1 | -2.86 | +3 | +2.88 |
| | Females | -4 | -2.65 | -3 | -3.16 | -1 | -2.86 | +4 | +3.81 |
| | Total | -5.38 | -3.42 | -4.38 | -4.31 | -1 | -2.86 | +3.54 | +3.38 |
| CMF | Males | +10 | +6.25 | +8 | +7.41 | +2 | +5.71 | -2 | -1.87 |
| | Females | +9 | +5.84 | +8 | +8.08 | +1 | +3.13 | -2 | -1.74 |
| | Total | +9.46 | +6.03 | +8 | +7.77 | +1.88 | +4.31 | -2 | -1.80 |
| GMF | Males | +13 | +7.93 | +10 | +9.17 | +3 | +9.09 | -3 | -2.70 |
| | Females | +11 | +6.83 | +8 | +7.69 | +3 | +8.57 | -2 | -1.82 |
| | Total | +11.92 | +7.33 | +8.92 | +8.37 | +3 | +8.81 | -2.46 | -2.22 |
| SMF | Males | +21 | +12.73 | +19 | +17.43 | +2 | +5.56 | -2 | -1.98 |
| | Females | +18 | +11.25 | +16 | +15.09 | +2 | +6.06 | -3 | -2.80 |
| | Total | +19.38 | +11.93 | +17.38 | +16.16 | +2 | +5.83 | -2.54 | -2.42 |

* TC, total cholesterol; LDL-c, low density lipoprotein- cholesterol; HDL-c, high density lipoprotein-cholesterol.

Serum lipid concentrations of the participants at starting and final of the 4wk feeding of OO or ghee types are shown in Table 3, and the serum lipids responses of the subjects are shown in Table 4. OO consumption was significantly lowered levels of serum total cholesterol (-3.42%), LDL-c (-4.31%) and caused slight reduction in HDL-c (-2.86%) under the initial level. On the contrary, ghee types were significantly raised serum total cholesterol level (about +8.43%), LDL-c (about +10.8%), and caused an increase in HDL-c level (about +6.32%) above the initial levels. Among the ghee types, SMF caused the highest raise in serum total cholesterol level (+11.93%) and LDL-c level (+16.16%). The highest raise in HDL-c level (+8.81%) showed with GMF. When the responses in serum cholesterol in the males and females subjects were considered separately for the four diets (Table 4), males were generally found to exhibit greater than females. Table 3 also show that the ratios of serum total cholesterol (TC) /HDL-c and LDL-c /HDL were slightly decreased with OO, whereas slightly increased with ghee types, but the LDL-c/HDL-c ratio after GMT was sustained. SMF resulted in higher raising effect of TC/HDL-c ratio (+0.26) and LDL-c/HDL-c ratio (+0.31) as compared to the other ghee types. Serum TG level was increased in both sexes after OO period (+3.38%), whereas reduced in both sexes after periods of ghee types (about -2.15%).

In general, serum cholesterol levels of volunteers tended to be low (average value < 200mg/dl). Several contributing factor may be affected this level. Many nutrients other than dietary fat influence cholesterol concentrations such as low cholesterol concentration diet, dietary fiber, complex carbohydrate intake and protein source (Grundy, 1986; Cohen *et al*, 1994). The results showed that OO brought about a dramatic reduction in the concentrations of serum total cholesterol, LDL-c and HDL-c and these reductions may be due to high n-9

MUFA (66.4% oleic acid) content in OO.

There has been much interest regarding the components that contribute to the beneficial health effects of the Mediterranean diet. Olive oil is the fat of choice in the Mediterranean area. Polyphenolic compounds found in olive oil may be contribute to the lower incidence of coronary heart disease in this area. Recent findings demonstrate that olive oil phenolics inhibit oxidation of low-density lipoproteins (Visioli and Galli, 1999). Moreover, OO can be advised as an alternative to high-carbohydrate diets in diabetic and carbohydrate-sensitive patients (Garg, 1994), and for routine frying or cooking practices, that is it did not produce toxic aldehydes (Grootvelt, 1998). The results were in agreement with observations of Perez-Jimenez (1995) and Kris-Etherton (1999), whereas in disagreement with results of Spiller *et al.* (1998) who mentioned that OO diet resulted in no significant change in men and women plasma total cholesterol, LDL-c and HDL-c. The hypercholesterolemic effect of ghee types, especially SMF may be related to high SFA content and low p: s and m +p/s ratios (Lee *et al.*, 1989). Besides that the ghee types contained appreciable amount of short and medium chain FAs. These FAs may be work as an activator for hepatic hydroxyl-methyl-glutarate-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis. Short chain FAs with two other FAs namely, lauric and myristic acids were thought as hypercholesterolemic agents (Elson, 1992) and may be cause high rate of cholesterol absorption (Ide *et al.*, 1979). Myristic acid appeared to be the most potent cholesterol raising SFA in human (Hajri *et al.*, 1998). Other reason, but less effect, that is the cholesterol content of ghee types may be increased blood cholesterol concentration (Gurr, 1989). Among the ghee types, SMF came the first serum cholesterol raising fat and may be due to its relatively high myristic acid content (12.2%), low p:s ratio (0.03) and m +p/s ratio (0.37), (Table 1). The results were in agreement with those results showed in

human (Barr *et al.*, 1992), in rats (Thannoun, 1993), and hamster (Cohen *et al.*, 1994).

The effect of dietary fat type on serum TG was reversed its effect on serum cholesterol, that is the blood cholesterol lowering effect of OO accompanied with slight increase in serum TG compared with the vice versa effect of ghee types. These effects imply that the differences in FA compositions of OO and ghee types may be resulted in differences in their effects on secreting and degrading of serum TG. OO period resulted in slight increase in serum TG concentration of both sexes and may be due to the increased secretion of very low density lipoprotein (VLDL)-TG (Heimberg and Wilcox, 1972). Hence, the TG reducing effect showed in both sexes after periods of ghee types may be attributed to the decreased TG secretion (Jackson *et al.*, 1977). Potenger and Getz (1971) stated that the orotic acid blocked the final step of VLDL secretion by inhibiting the linkage of carbohydrate to the apoprotein. This observation indicates the role of orotate, which occurred in milk fats, in reducing the TG transportation from liver to the blood (Nair and Mann, 1977).

High blood cholesterol is the major risk factor for cerebrovascular disease (CVD), (NCEP, 1993). Several studies confirmed that the high blood cholesterol in young adults is a predict of CVD risk in later life (Myers *et al.*, 1995). Present results showed that the replacing ghee by OO lowered the predicted risk of CVD due to the decline in serum total cholesterol. The ratio of TC/HDL-c was mentioned as indicator of CVD risk (Kinoshian *et al.*, 1994). Results of this study showed a slight reduction in TC/HDL-c ratio after OO periods and slight raising in this ratio after periods of ghee types. The results also showed that the LDL-c/HDL-c ratio was in accordance with the former ratio.

Finally, ghee types showed raising effects of blood cholesterol. These effects may be due to their low p:s and m +p/s ratios. The differences between effects of ghee types on blood cholesterol level mainly attributed to these differences in their fatty acid compositions. However, the cholesterol content of ghee types had no effect on these differences. OO, which is high oleic acid, may be a good alternative of dietary fat for reducing blood cholesterol level.

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Taste Profiles That Correlate with Soy Consumption in Developing Countries

Brian Wansink and JaeHak Cheong
University of Illinois, Urbana-Champaign, Illinois 350 Wohlers Hall, USA
E-mail: Wansink@uiuc.edu

Abstract: While insufficient protein consumption is a concern to many demographic segments in developed countries, it is a greater concern in developing nations where the cost or availability of traditional forms of animal protein results in protein deficiencies. Soy is a low-cost, highly available protein source, yet it is largely overlooked because of its unfamiliar taste and texture. To determine how to best encourage soy consumption, a convenience sample of 132 Indians and Pakistanis living in the United States was examined for insights in to what characterizes someone who regularly eats soy for taste-related reasons. Three groups of consumers were analyzed, people who ate soy primarily for taste-related reasons, those who ate it primarily for health-related reasons, and those who did not eat it. People who ate soy primarily for taste-related reasons were found to be more likely to appreciate fine food, to live with a great cook, and to be more of an opinion leader than did those in either of the other two groups. These along with additional findings have implications for targeting soy-predisposed consumers, who will adopt soy for the long-term, and who can influence others because of their role as opinion-leaders within their peer or reference group.

Key words: Protein-deficiency, soy consumption, taste profiles

Introduction

For consumers in many countries, a key issue is not whether they consume enough calories, but whether they consume the appropriate mix of calories. Shortages of meat-related protein can cause nutritional deficiencies even though total calorie consumption is at an appropriate level (Harper, 1999). While insufficient protein consumption is a concern to many demographic segments in developed countries, it is a greater concern in developing nations where the cost or availability of traditional forms of animal protein results in under consumption (Roberfroid, 1999). Soy is a low-cost, highly available protein source (Barnes, 1998). The problem, however, lies in encouraging acceptance among those who are hesitant or resistant to consume it (Shork, 2000).

Although perceptions that a food is nutritious can influence changes in behavior, such changes can be short-lived if immediate results are not seen or if the food becomes tiring or inconvenient (Logue, 1991). In contrast, when new dietary patterns are changed because of taste-related reasons, they have longer-term consequences (Nestle *et al.*, 1998). While some part of the population will adopt new foods into their regular diet simply because they are a healthy alternative, a much larger percentage will do so only if the taste of the product is equal or more preferable to what is currently being eaten (Coletta, 1999).

In trying to encourage people in a developing country to adopt a healthy protein alternative such as soy, it is useful to profile the type of people from that country who have already adopted the product. Doing so will provide insights in to how individuals with a similar profile can be targeted and encouraged to consume the product (Wansink

and Park, 2000). Consider two segments of people who frequently consume soy: One segment consumes it primarily because of its health benefits, while a second segment consumes it primarily because of its taste. If we can understand why some people like the taste of soy, it might be possible to determine what could be done to encourage more people to consume soy.

The objective of this study is to determine what taste-related profiles and behaviors are most closely related to people who claim to consume soy primarily because of its taste. Understanding this will help us better understand how to encourage consumption in similar segments of people. Because of the growing nutritional concerns in over-populated countries, we will focus on a segment of consumers with Indian or Pakistani roots.

Materials and Methods

To determine what factors are associated with individuals who frequently consume soy, qualitative and quantitative phases were conducted. A qualitative study was first conducted with a non-representative sample of 33 people who had clear taste preferences for soy and who had been recruited through fliers placed in a health food store, a regular supermarket, a health food restaurant, and a college cafeteria. Two eight person focus groups were conducted, and in-depth laddering interviews were conducted with the remaining 17 participants.

The results of this qualitative phase of the project indicated that two major reasons people evolved from infrequent to frequent users of soy was because of either health-related reasons (lactose intolerant, heart disease concerns, blood pressure) or because they liked the taste and texture of soy. Since our interest was primarily in

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Table 1: Taste-related Profiles Associated with Indian and Pakistani Soy Consumers

| Beliefs About Soy | Non-soy Consuming Segment | Health- related Segment | Taste- related Segment | F-Value (d.f. 2,130) |
|--|---------------------------------|-------------------------------|------------------------------|-------------------------|
| I live with (or am) an above average cook | 3.9 | 4.8 | 5.5 | 6.6** |
| I live with (or am) a great cook | 2.8 | 5.6 | 7.1 | 9.8** |
| I am traditional | 5.6 | 5.7 | 4.5 | 5.3** |
| I appreciate fine food | 4.9 | 5.7 | 7.9 | 6.9** |
| In general, I am an adventurous person | 4.9 | 4.3 | 5.4 | 3.6* |
| I believe that I eat healthier than most | 4.5 | 7.9 | 5.8 | 14.4** |
| I am an opinion-leader among my peers | 3.2 | 5.6 | 6.8 | 9.4** |
| Number of evening meals eaten away from home during the average week | 0.8 | 1.1 | 1.9 | 7.1** |
| Number of evening meals which contain a meat during the average week | 6.3 | 5.8 | 6.0 | 0.7 |
| Number of evening meals with which you drink wine during the average week | 0.6 | 0.5 | 1.2 | 5.9* |
| Number of evening meals you eat a soy-related food during the average week | 0.6 | 3.7 | 2.7 | 15.4** |

* $p < 0.10$ ** $p < 0.05$

those who ate soy because of the taste, most of the qualitative work was focused on determining similarities among the "tasters". In addition to spending more time preparing food and enjoying fine dining, this segment claimed themselves to be more adventurous and more likely to be considered an opinion-leader among their peers.

To examine these notions, we next conducted a study with a convenience sample of 132 Indians and Pakistanis who were residing in the United States and who were either graduate students, faculty, or spouses at the University of Illinois in Urbana-Champaign. After obtaining these names from the local phone book, seven minute interviews were conducted either in person or over the phone. These individuals were asked a series of questions related to how frequently they ate soy products as well as other food-related habits and preferences that had been identified in the qualitative portion of the study.

After screening for whether the person ate meat, each person was asked the number of times he or she was involved in specific target behaviors in an average week, and they were asked questions requiring them to respond as to whether they disagreed or agreed with a series of questions asked on 9-point scales (1 = disagree; 9 = agree).

Results

In analyzing the data, consumers were grouped by whether they had indicated that they primarily ate soy for health reasons, taste reasons, or did not eat soy. This was determined by using both checked boxes (health, taste, neither), and by using 9-point Likert scales regarding why they ate soy (1 = strongly disagree; 9 = strongly agree). Of those 91 people who were frequent consumers of soy (2 or more times a week), 63 could be unambiguously categorized as eating soy primarily for health reasons

(69.2%) and 28 as eating soy primarily for taste reasons (30.8%). While some people ate soy for both taste reasons and health reasons, the more important or dominate reason was used to group these individuals. The taste-related segment consumed soy an average of 2.7 times each week, compared to the health-related segment who consumed it more frequently (3.7).

The taste profile of people who consumed soy for taste-related purposes was consistently different across many of the measured variables. To a large extent, this confirmed the findings of the qualitative portion of the study. This "taste-related" segment of consumers were more likely to believe they lived with a "great cook," than the health-related segment or the non-soy eating segment (7.1 vs. 5.6 and 2.8; $F_{1,130} = 6.6$; $p < .05$). In addition, compared to these other segments, they rated themselves as less traditional (4.5 vs. 5.7 and 5.6; $F_{1,130} = 9.8$; $p < .05$), more appreciative of fine food (7.9 vs. 5.7 and 4.9; $F_{1,130} = 6.9$; $p < .05$), marginally more adventurous (5.4 vs. 4.3 and 4.9; $F_{1,130} = 3.6$; $p < .10$), and more likely to be an opinion leader (6.8 vs. 5.6 and 3.2; $F_{1,130} = 9.4$; $p < .05$).

As Table 1 indicates, in addition to these personality variables, this segment of soy "tasters" was more likely to eat evening meals away from home than were the health-related segment and the non-soy eating segment (1.9 vs. 1.1 and 0.8; $F_{1,130} = 7.1$; $p < .05$), and they were more likely to enjoy wine with their meal (1.2 vs. 0.5 and 0.6; $F_{1,130} = 4.1$; $p < .05$).

Discussion

We often underestimate the power and importance that the meal preparer or gatekeeper can unknowingly plays in establishing and modifying family preferences toward unfamiliar foods (Nestle *et al.*, 1998). The findings reported here underscore that a taste-related preference

for soy can be a learned preference. That is, given the right circumstances - a great cook - the taste of soy can be one that people learn and grow to like.

While most efforts to change nutrition-related behaviors are focused on mass efforts to a general population (Nestle *et al.*, 1998), this study suggests two important considerations. First, there are some profiles of individuals or segments who are more predisposed to changing their consumption behavior in a desired direction than others. To focus nutritional education efforts on a general population will be much less effective than if these efforts are instead focused on a more targeted group. Second, in the case of soy, targeting a taste-oriented segment of consumers who prefer soy can seed potential opinion-leaders who may eventually filter down the influence of these dietary habits on other consumers.

Instead of focusing efforts on encouraging people to eat soy for health reasons, a more productive method may be to target the types of people who are more likely to prefer it for taste-related reasons. Past research suggests that people who eat foods for taste-related reasons are more likely to continue with these dietary changes than one who simply does so for health reasons (Wansink, 2002). Part of the importance that this "taste-related" segment claims to live with "great cooks" is that any exposure they have to soy is likely to be favorable. Repeated exposure is likely to develop preferences in a way that highly varied experiences will not.

How are these taste-predisposed segments located? Table 1 indicates that these people are likely to believe they live with good cooks are more likely to exhibit behaviors associated with food appreciation, such as dining out and wine consumption. While such variables may not have practical analogues in a developing country, they do suggest that people who eat soy for taste-related reasons exhibit evidence of being more appreciative of quality dining experiences compared to their peer group. When extending these results to developing countries, the

results should be taken as somewhat exploratory because of the sample. This study was conducted with a non-representative sample of people who had emigrated from India and Pakistan. They were wealthier, better educated, and more Westernized than what would be expected in their home countries. Still, these results suggest important directions to consider when trying to introduce a healthy and unfamiliar protein source in to these cultures.

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Effect of Different Sources of Protein on Growth and Reproductive Performances of Rabbits

J. Roy¹, N. Sultana¹, Z. Khondoker¹, A. Reza¹ and S. M. J. Hossain²

¹Department of Animal Nutrition, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

²Department of General Animal Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Abstract: Twenty-four New Zealand white crossbred growing rabbits (Aged 120 days) were used to study the effect of different protein sources on growth and reproductive performances of rabbit does. The animals were fed three diets containing til oil cake (A), soybean meal (B) and gram (C) along with *ad-libitum* green grasses. However daily live weight gain was higher in gram containing diet but not significant ($P > 0.05$) than those received til oil cake or soybean meal. Daily live weight gain of rabbits was 2.34, 2.33 and 3.14 g/day for til oil cake (A) Soybean meal (B) and gram (C) respectively. Feed conversion ratios were differed significantly ($P < 0.05$) among the three treatment groups. Age at first breeding, age at first kidling gestation period, litter size, number of litter alive, number of litter dead and percentage alive were not differed significantly ($P > 0.05$) among the treatment groups. Only kit mortality up to 7 days were differed significantly ($P < 0.05$). Lower kit mortality (12.51) in gram containing feed (C) and increased in group B (36.37) and group A diet (36.37) respectively. Based on the present research findings gram diet may be used as a protein supplement for raising rabbits in Bangladesh. But further research is needed using large number of rabbits in this aspect before final recommendation.

Key words: Rabbit, protein source, growth, reproduction

Introduction

Rabbits are herbivores and can be successfully raised on diets that are low in grains and high in roughage (Cheeke, 1986a). Rabbits have a number of specific characteristics such as small body size, short generation interval (28.32 days), rapid growth rate (Cheeke, 1986b), the poor and unbalanced quality of forage based diets, usually provided to rabbits in developing countries was described by Cheeke *et al.*, 1985; Deshmukh and Pathak, 1995. Rabbit meat is acknowledged as of high quality being high in protein and low in fat cholesterol (Jones, 1990; Handa *et al.*, 1995). It has been reported that growing rabbits can be maintained satisfactorily on diets consisting of 100-200g green roughage and 40-60g concentrate mixtures preferably in the form of pellet (Ranjhan, 1980) for optimum production and about 4 months are required to produce a 2 kg market rabbit under subsistence condition (NRC, 1991).

There is an increasing interest in the diversification of animal production system in Bangladesh to produce products which are not surplus nationally. Therefore, production of meat from rabbit is one such enterprise which has recently attracted attention for development. The climatic condition, commercial factors, legal environment, religious, social practices and technological aspects support the rabbit raising potential in Bangladesh (MIDAS, 1992). Nutrition is a major constraint for rabbit rearing in Bangladesh. Supplementation of soybean meal as a source of protein has been suggested on growth and reproductive performance of rabbit (Rahim *et al.*, 1997). Information regarding rearing rabbits on different source

Table 1: Ingredient and chemical composition of green grass and different concentrate mixture

| Ingredients | Dietary group | | |
|-------------------------------|-------------------|-------------------|-------------------|
| | A | B | C |
| Green grass | <i>Ad-libitum</i> | <i>Ad-libitum</i> | <i>Ad-libitum</i> |
| Wheat | 59.85 | 59.48 | 34.48 |
| Wheat bran | 25.00 | 30.00 | 30.00 |
| Til oil cake | 15.00 | - | - |
| Soybean meal | - | 10.00 | - |
| Gram | - | - | 35.00 |
| Embavit WS | 0.02 | 0.02 | 0.025 |
| Common salt | 0.50 | 0.50 | 0.50 |
| Chemical composition (%) | | | |
| Dry matter | 90.05 | 89.87 | 89.35 |
| Crude protein | 16.22 | 16.11 | 16.13 |
| Crude fibre | 4.57 | 4.46 | 4.67 |
| Ether extract | 4.32 | 4.76 | 4.14 |
| Nitrogen free extract | 69.05 | 69.18 | 69.94 |
| Ash | 5.82 | 5.09 | 4.9 |
| Calcium ¹ | 0.39 | 0.10 | 0.14 |
| Total phosphorus ² | 0.69 | 0.65 | 0.53 |
| ME kcal/100g DM ³ | 26.01 | 26.53 | 26.13 |

^{1,2,3}calculated from the manual of selected topics in Animal Nutrition by Close and Menke (1976)

of protein in Bangladesh is scanty. The present experiment was therefore, designed to compare the effect of supplementing til oil cake, soybean meal and gram on growth and reproductive performances of rabbits fed *ad-libitum* green grasses.

Table 2: Effect of feeding three different sources of protein on growth performance of rabbits

| Parameters | Dietary group H | | | Level of significance |
|---------------------------------|--------------------|-------------------|-------------------|-----------------------|
| | A | B | C | |
| Initial live weight (kg) | 1.77 ± 0.11 | 1.93 ± 0.9 | 1.86 ± 0.13 | - |
| Final live weight (kg) | 2.04 ± 0.17 | 2.25 ± 0.2 | 2.29 ± 0.14 | - |
| Daily live weight gain (g) | 2.30 | 2.30 | 3.09 | NS |
| Feed intake (DM/g) Green grass | 33.06 | 32.92 | 33.24 | NS |
| Concentrate mixture | 60.36 | 60.86 | 58.36 | NS |
| Total | 93.42 | 93.78 | 91.60 | NS |
| Feed conversion ration (DM/LWG) | 40.06 ^b | 40.7 ^b | 29.6 ^a | * |

^{ab} Means with difference superscript in the same row differ significantly. * P > 0.05)

Materials and Methods

A total of 18 female and 6 male New Zealand white crossbred growing rabbits, aged 120 days were used to study the effect of different sources of protein on growth and reproductive performance. Eighteen does and six bucks were randomly assigned to three groups, so that each group consisted of six does and two buck. The three dietary treatments consisted of three sources of protein such as til oil cake, soybean meal and gram (Table 1). The three diets were almost iso-nitrogenous and iso-energetic. The diets were compounded in mash form. Green grass and water were provided at *ad-libitum* basis. Experimental rabbits were housed individually in cages with arrangements for separate feeding, watering and left over collection. All animals were treated with a common anthelmintic drug and coccidiostat before starting the trial. Housing and other management practices were kept identical for all groups. Feeds offered and the left over were recorded regularly for individual rabbit. The live weight changes, feed intake and reproductive performance of rabbit does were recorded regularly till the end of the trial. The data on samples of feed offered, residues, live weight changes and reproductive performances were analyzed statistically in CRD following the method steel and Torrie (1980).

Results and Discussion

The average DM intake and live weight changes of rabbits are shown in Table 2. DM intake from green grass was almost similar among the three dietary group. The average DM intake from concentrate mixture was almost similar among the dietary group A and B but DM intake of dietary group C was lower than others two group. The total DM intake (g/d) were 93.42, 93.78 and 91.6 for dietary group A, B and C respectively. The lower dry matter intake was observed in group C having gram as protein source and lower compared with other groups (A and B). Highest daily growth rate was recorded for dietary group C (3.09 g, used gram as protein source) than those two group. Growth rate was similar for dietary group A & B (2.30g and 2.30g used til oil cake and soybean meal used respectively). Rahim *et al.* (1997) found a growth rate of 6.5 g/day with soybean meal and

6.40g/day with whole gram. However, Cheeke and Amberg (1972) found a growth rate of 34 g/day with soybean meal and 25g/day with cotton seed meal. However, Solarte (1989) reported a growth rate of 11.5g/day in New Zealand white rabbits when fed can juice and erythrina foliage and Farinu (1994) found 15.2g/day using compound diet containing 30 percent soybean meal. All findings are higher than the present study. The differences in growth rates of rabbits may be due to variations in their genotypes, feeding and management between the studies.

Feed conversion efficiency of the rabbits that received till oil cake (group A), soybean meal (group B) and gram (group C) were 40.06, 40.7 as 29.94 g/d respectively (Table 2). Feed conversion efficiency was similar for dietary group A and B that was significantly P > 0.05 different from received gram diet (group C) and persisted this trend throughout the study.

Reproductive characteristic of does are shown in Table 3. Age at first breeding that might be one of the most important measures for reproductive performance was 167.66 (A), 169 (B) and 164.5 day (C). The age at first kidling was recorded as 202.3 days for dietary group A, 202.0 for dietary group B and 201.0 days for dietary group C and was not differed significantly (P > 0.05) with each other. Rahim *et al.* (1997) was recorded that age at first kid ling was recorded as 198 days for soybean meal and 183 days for whole gram. The average gestation period was 42.0 (A), 31.7 (B) and 28.3 days for diets A, B and C respectively and was not differed significantly (P > 0.05). The litter size at birth was not differed significantly (P > 0.05) among different treatment (Table 3), litter size at birth mostly depends upon the ovulation rate (i.e the number of ova shed from ovary at a time). Herbert (1998) stated that litter size at birth and weaning was not affected by feeding diets with different sources of protein. Similar results were found in this study. Average number of litter alive was lower in dietary C (0.4) then the dietary group B (2.4) and A (2.3). Kit mortality (1 to 7 days) of dietary group A was significantly (P > 0.05) higher (87.5) compared with those given diet B (36.37) and C (12.5) (Table 3). It was shown from this study, the lowest kit mortality (12.5%) was in group C received

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Table 3 : Effect of different sources of protein on reproductive performance of rabbits

| Parameters! | Dietary group# | | | Level of significance |
|------------------------------|-------------------|--------------------|-------------------|-----------------------|
| | A | B | C | |
| Age at first breeding (days) | 167.66 | 169.0 | 164.0 | - |
| Age at first kidling (days) | 20.3 | 202.0 | 201.0 | - |
| Gestation period (days) | 42.0 | 31.7 | 28.3 | NS |
| Litter size (number) | 2.7 | 3.7 | 2.7 | NS |
| Number of litter alive | 0.4 | 2.4 | 2.3 | NS |
| Kit mortality (1 to 7 days) | 87.5 ^c | 36.37 ^b | 12.5 ^a | * |

^{abc} Means with different superscript in the same row differ significantly, * P > 0.05

gram as protein sources. Rahim *et al.* (1997) found that kit mortality (50 vs 33.5%) in fed whole gram compared soybean meal that result was higher gram containing feed. In case of soybean meal, present result partially support Rahim *et al.* (1997).

It is observed that feeding of gram to rabbits resulted in a higher growth rate, better feed conversion efficiency, lower gestation period and lower kit mortality than those of fed diet containing til oil cake and soybean meal as protein sources. Therefore, supplementation of gram as protein source may be used for production of rabbit fed *ad-libitum* green grass. However, further studies with a greater number of animals are required to develop rabbit production systems using the three protein supplement

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Consumption Pattern of Milk and Milk Products among Different Income Levels in Some Selected Areas of Bangladesh

B. K. Roy, K. S. Huque, M. R. Islam, M. Hasanuzzaman and M. M. Rahman
Animal Production Research Division, Bangladesh Livestock Research Institute,
Savar, Dhaka-1341, Bangladesh
E-mail: aprdblri@accessstel.net

Abstract: The present study examines the consumption pattern of milk and milk products among different income groups in some selected areas in Bangladesh. Family budget data were collected through household survey during September' 01 to May 2002 for 179 selected households from the three different areas of Bangladesh, comprising 44 from Rural, 61 from Municipality town and 74 from Metropolitan city households formed the basis. The sample households were post classified into 5 income groups based on monthly household income as follows: (I) < Tk. 3000; (II) Tk. 3001-6000; (III) Tk. 6001-9000; (IV) Tk. 9001-12000 and (V) > Tk. 12000. Among milk and milk products, the major allocation of expenditure was devoted to liquid milk followed by sweetmeats and powder milk and 'other milk products'. The municipality town households consume more milk, sweetmeats and dahi than Rural and Metropolitan city. On the other hand, Metropolitan households consume more powder milk, condensed milk, ghee and ice cream. Milk and some milk products consumption and expenditure on it increased substantially, with the increase of income in all the areas.

Key words: Consumption pattern, milk, milk products, income level

Introduction

Consumption plays the key role in guiding an economy to the production of goods and service that they demand. Per capita fresh milk and milk products consumption is one of the most important point of basis to measure the living standard of a nation. The situation of milk production and consumption in Bangladesh is far below the normal level when compared to the requirement. It is estimated that daily per capita availability of milk in Bangladesh is only 34 ml. against the requirement of 250 ml. and the annual production is estimated at 1.6 million metric tons where as the requirement stands at about 9.9 million metric tons (DLS, 1998). This situation indicates the depth of the requirement for raising milk production in the country for a healthy nation. In developing economy like Bangladesh, the consumption pattern of household is expected to undergo a change with the rising aggregate income. The basis unit of demand theory is primary consumer who attempts to maximize utility by spending his income. Therefore, consumer demand for a particular commodity or commodity group necessitates the understanding of the consumption behavior of the aggregate consumers or households in the consumption area. Consumption behavior of dairy consumers depends upon income, prices and availability of the milk and dairy products (Sweetmeats, Dahi, Ghee, Powder milk, Ice-cream etc.). The products consumption depend in turn on interaction of among many other factors; on their socio-economic, physical environment, its composition, cultural background (Mukherjee, 1938; Crotty, 1980), preferences (Baker, 1959), economic needs and orientation of the products to the consumers (Reberte *et al.*, 1996). So, obviously, a

large number of factors directly affect the consumption expenditure such as income, prices of individual commodities, size and composition of household etc. However this study will help to measure the present level of consumption pattern of rural and urban (Municipality town and Metropolitan city) people as well as will help the government to formulate policy for the welfare of the people of Bangladesh.

Materials and methods

The study was based on the income group or family budget and data were collected from the three selected different areas of Bangladesh namely Rural, Municipality town and Metropolitan city. This three areas were generally considered the main representative units of whole Bangladesh consumption pattern situation. Six villages namely Maniknagar, Shaylapara (Ishurdi, Pabna), Garidha (Sherpur, Bogra), Rasombari, (Ullahpara, Sirajgonj), Garaikhuti (Muktaghacca, Mymensingh) and Shahapur madhapara (Matihar, Rajshahi) were selected as rural area, the different households of four Municipality town namely Ishurdi (Pabna), Bagerhat, Comilla, and Lakshampur, and as well as four Metropolitan city namely Khulna (Altapur, Ray para), Barishal (Shikdarpara), Sylhet (Tilaghar) and Rajshahi (Kumerpara) were selected for this purpose. The data were randomly collected through personal interviewing during September' 01 to May 2002 by the Principal investigator using a pre-tested questionnaire. In this study, a total of 179 primary sampling units, 44 from Rural, 61 from Municipality town and 74 from Metropolitan city, were selected. The selected sample households were post classified into 5

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Table 1: Distribution of samples among different income groups in rural, Municipality town and Metropolitan city areas

| Category | Income groups (Tk.) | Number of observations | | | Total |
|----------|---------------------|------------------------|-------------------|-------------------|-------|
| | | Rural | Municipality town | Metropolitan city | |
| I | Below 3000 | 13 | 13 | 9 | 35 |
| II | 3001-6000 | 7 | 13 | 17 | 37 |
| III | 6001-9000 | 11 | 6 | 16 | 33 |
| IV | 9001-12000 | 6 | 14 | 16 | 36 |
| V | 12000 & above | 7 | 15 | 16 | 38 |
| Total | | 44 | 61 | 74 | 179 |

Table 2: Distribution of average income and number of family member among different income groups of household in rural, Municipality town and Metropolitan city areas

| Category | Income group | Average income (Tk.) | | | Average | Average family member (no.) | | | Average |
|----------|---------------|----------------------|-----------|--------------|---------|-----------------------------|-----------|--------------|---------|
| | | Rural | Municipal | Metropolitan | | Rural | Municipal | Metropolitan | |
| I | Below 3000 | 1769 | 2183 | 2664 | 2153 | 5.23 | 4.84 | 3.88 | 4.74 |
| II | 3001-6000 | 4420 | 4603 | 4672 | 4600 | 7.7 | 5.15 | 4.82 | 5.48 |
| III | 6001-9000 | 7306 | 7464 | 7667 | 7510 | 7.72 | 5.16 | 3.87 | 5.39 |
| IV | 9001-12000 | 10845 | 10679 | 10621 | 10681 | 8.83 | 5.28 | 4.75 | 5.64 |
| V | 12000 & above | 15125 | 35125 | 20016 | 25076 | 8 | 6.46 | 4.62 | 5.97 |
| Average | | 6935 | 13268 | 9679 | | 7.18 | 4.44 | 4.44 | |

Table 3. Average monthly consumption of milk and milk products at different areas of Bangladesh (litre or kg or g/month)

| Parameters | Areas | | | | | | Level of significance |
|---------------------|--------------------|-------|----------------------|-------|----------------------|-------|-----------------------|
| | Rural | | Municipality town | | Metropolitan cities | | |
| | Mean | SE | Mean | SE | Mean | SE | |
| Liquid milk (litre) | 21.58 | 2.8 | 23.52 | 2.4 | 17.71 | 2.13 | NS |
| Condensed milk (g) | 0.00 ^b | 0.00 | 88. ^{ab} | 44.14 | 154.64 ^a | 38.97 | * |
| Powder milk (g) | 74.89 ^a | 64.08 | 204.24 ^b | 55.10 | 363.81 ^c | 48.65 | ** |
| Sweetmeats (kg) | 1.14 | 0.21 | 1.34 | 0.18 | 1.25 | 0.16 | NS |
| Dahi (kg) | 0.86 | 0.18 | 0.91 | 0.16 | 0.85 | 0.14 | NS |
| Ghee (g) | 69.19 ^b | 31.25 | 137.43 ^{ab} | 26.87 | 163 ^a | 23.73 | * |
| Ice-cream (g) | 0.00 ^b | 0.00 | 243.95 ^a | 92.04 | 204.40 ^{ab} | 81.25 | * |

Means with different superscript (s) in the same row differ significantly, (p < 0.05) or (P < 0.01)

NS = Non significant, * = Significant at 5% level (p < 0.05), ** = Significant at 1% level (p < 0.01)

income categories based on monthly household income such as (I) < Tk. 3000 (II) Tk. 3001-6000 (III) Tk. 6001-9000 (IV) Tk. 9001-12000 and (V) > Tk. 12000. The samples drawn from different categories of people in Rural, Municipality town and Metropolitan city is shown in Table 1. and distribution of average income and number of family member among different income groups are also shown in Table 2. Various types of milk and milk products were selected for the study which were thought to be commonly consumed by different types of consumers in Bangladesh. The selected milk and milk products are liquid milk, powder milk, condensed milk, sweetmeats, dahi, ghee and ice cream.

Statistical analysis: After data collection the data were analyzed by single variate General Linear Model of SPSS

(Snedecor and Cochran, 1989) 7.5 for windows (SPSS) statistical package. The main effects were the effects of different areas and different income groups on liquid milk, condensed milk, sweetmeats, dahi, ghee etc. The model used was as follows:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ij}$$

Here, μ is the general mean, α_i is the i th, area β_j is the j th income group, $\alpha\beta_{ij}$ is the observed variable at i th area in j th income group, e_{ij} is the random error and Y_{ij} is the observed variable in question.

Results and Discussion

Liquid Milk: Average monthly consumption of liquid milk for all income groups of Rural, Municipality and Metropolitan areas were estimated 21.58, 23.52 and 17.71 litre (Table 3) and its cost was Tk. 277.26, 460.78 and

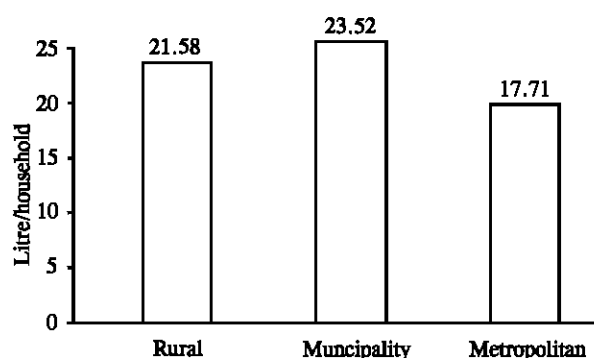


Fig. 1: Monthly consumption of milk per household at different areas of Bangladesh

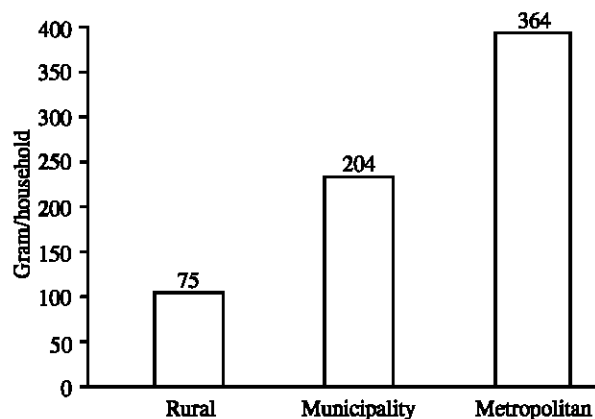


Fig. 2: Monthly powder milk consumption per household at different areas of Bangladesh

409.85 (Table 6) respectively. The percentage of total expenditure spent on liquid milk were 3.99 for Rural, 3.47 for Municipality town and 4.23 for Metropolitan households. The lowest milk consumption was Metropolitan city because they consume more powder milk in comparison to other two areas (Fig.1 and 2). The analysis of variance showed different areas had no significant effect on liquid milk consumption for all income groups. On the other hand, monthly liquid milk consumption for all three areas of various income (Tk/months) levels (below 3000, 3001-6000, 6001-9000, 9001-12000 and above 12000) were estimated 10.09, 11.49, 17.33, 24.37 and 41.41 litre (Table 4) respectively. The analysis of variance showed that different income levels had a highly significant effect on liquid milk consumption ($p < 0.01$) for all areas (Table 4). Quantity consumed as well as expenditure on milk was the highest for V income groups and the lowest for (I) income groups for all areas. Table 5 shows the positive correlation of consumption with income i.e. of income rises in different areas the quantity of milk consumption increases. Table 6 reflects that as income increases the price paid for milk by different income groups of different areas increases.

Powder and condensed milk.

The average monthly consumption of condensed milk and powder milk for all income groups were estimated at 0.00; 74.89, 88; 204.24 and 44.14; 55.10 g for Rural, municipality town and metropolitan city area, respectively (Table 3) and its cost was Tk. 0.00; 13.98, 8.37; 71.85 and 9.95; 109.28 (Table 6) respectively. All income groups of rural area did not consume condensed milk, but in Metropolitan city the household consume higher than Municipality town. Both two area condensed milk used by households as a tea whitener. The analysis of variance showed that both area and income groups had significant effect ($p < 0.05$) on condensed milk consumption. Incase of income groups LSD test indicates that the mean (I) income groups differ significantly ($p < 0.05$) with (III) income group (Table 4). Incase of powder milk consumption, the analysis of variance showed both areas and income groups had a highly significant effect ($p < 0.01$). Quantity consumed as well as expenditure on powder milk was the highest for highest income group except Rural area. (Table 5)

Sweetmeats: The monthly consumption of sweetmeats for all income groups of Rural, Municipality town and Metropolitan city areas were estimated 1.14, 1.34 and 1.25 kg and its expenditure was Tk. 61.35, 107.37 and 105.98 respectively. The analysis of variance showed that different income levels had a highly significant effect on sweetmeat consumption ($p < 0.01$) for all areas (Table 4) i.e. whenever income rise the level of consumption of sweetmeats obviously increased. Quantity consumed as well as expenditure on sweetmeats was the highest for the highest income group and the lowest for the lowest income group (Table 4, 5).

Dahi: The average quantity consumed and money spent on dahi were 0.86, 0.91 and 0.85 kg and Tk. 31.69, 53.04 and 58.78 respectively for Rural, Municipality town and Metropolitan city areas. The ANOVA showed that different income levels had a significant effect on dahi consumption ($p < 0.01$) for all areas. The (II) income class people of rural area consumed more dahi than other income class people. In Municipality town area income groups of (V) and (IV) consumed more dahi than others and in Metropolitan city area, income groups of (V) and (II) consumed more dahi than the rest in terms of quantity and expenditure. Table 5 and 6 shows that dahi consumption and expenditure had no regular sequence in all three areas.

Ghee: The monthly consumption and money spent on ghee were 69.19, 137.43 and 163g and Tk. 18.22, 38.08 and 61.65 respectively for Rural, Municipality and Metropolitan city areas. Analysis of variance showed that both areas and different income levels had a significant effect on ghee consumption (Table 4). Incase of areas,

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Table 4: Average monthly consumption of milk and milk products at various income levels (litre or kg/month)

| Parameters | Income levels (Taka) | | | | | | | | | | Level of Significance |
|---------------------|----------------------|-------|---------------------|------|----------------------|------|----------------------|------|---------------------|------|-----------------------|
| | Below 3000 (I) | | 30001-6000 (II) | | 6001-9000 (III) | | 9001-12000 (IV) | | Above 12000 (V) | | |
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | |
| Liquid milk (litre) | 10.09 ^a | 3.0 | 11.49 ^a | 3.1 | 17.33 ^a | 3.3 | 24.37 ^b | 3.3 | 41.41 ^c | 3.1 | ** |
| Condensed milk (g) | 1.71 ^a | 55.9 | 81.70 ^{ac} | 57.3 | 146.87 ^{bc} | 61.4 | 76.00 ^{ac} | 59.5 | 98.52 ^{bc} | 56.6 | * |
| Powder milk (g) | 58.95 ^a | 69.8 | 159.24 ^c | 71.5 | 198.59 ^{de} | 76.7 | 240.67 ^{ae} | 74.3 | 414.12 ^b | 70.7 | ** |
| Sweetmeats (kg) | 0.48 ^a | 0.2 | 1.09 ^{ac} | 0.2 | 1.11 ^{bc} | 0.2 | 1.26 ^{bc} | 0.2 | 2.27 ^{ed} | 0.23 | ** |
| Dahi (kg) | 0.33 ^a | 0.20 | 0.89 ^a | 0.2 | 0.70 ^a | 0.2 | 0.70 ^a | 0.2 | 1.74 ^b | 0.2 | ** |
| Ghee (g) | 57.63 ^a | 34.0 | 59.45 ^a | 34.9 | 149.55 ^a | 37.4 | 116.19 ^a | 36.2 | 233 ^b | 34.5 | ** |
| Ice-cream (g) | 40.60 ^a | 116.6 | 66.49 ^a | 119 | 135.59 ^{ac} | 128 | 111.4 ^a | 124 | 393 ^{bc} | 118 | * |

Means with different superscript (s) in the same row differ significantly ($p < 0.05$) or ($p < 0.01$)

* = Significant at 5% level ($p < 0.05$), ** = Significant at 1% level ($p < 0.01$)

Table 5 : Average monthly consumption of milk and milk products at various income levels in different areas (litre or kg or g /month)

| Category | Income group (Tk.) | Liquid milk (l) | Powder milk (g) | Condensed milk (g) | Sweetmeat (kg) | Dahi (kg) | Ghee (g) | Ice-cream (g) |
|-------------------|--------------------|-----------------|-----------------|--------------------|----------------|-------------|----------|---------------|
| Rural area I | | | | | | | | |
| | < 3000 | 7.22 ± 4.9 | 3 ± 112 | - | 0.51 ± 0.37 | 0.19 ± 0.3 | 6.4 ± 55 | - |
| II | 3001-6000 | 6.84 ± 6.7 | - | - | 1.25 ± 0.5 | 1.33 ± 0.4 | 77 ± 75 | - |
| III | 6001-9000 | 19.94 ± 5.4 | 28.4 ± 123 | - | 1.07 ± 0.4 | 0.86 ± 0.36 | 98 ± 60 | - |
| IV | 9001-12000 | 25.00 ± 7.3 | 333 ± 166 | - | 1.110 ± 0.5 | 0.69 ± 0.48 | 63 ± 81 | - |
| V | > 12000 | 48.93 ± 6.7 | 9.5 ± 154 | - | 1.78 ± 0.5 | 1.2 ± 0.45 | 101 ± 75 | - |
| Municipality town | | | | | | | | |
| I | < 3000 | 15.61 ± 4.9 | 38.5 ± 113 | 5 ± 90 | 0.49 ± 0.37 | 0.56 ± 0.3 | 120 ± 55 | 38 ± 188 |
| II | 3001-6000 | 11.93 ± 4.9 | 311 ± 113 | 20 ± 70 | 0.84 ± 0.37 | 0.47 ± 0.33 | 25 ± 55 | 77 ± 188 |
| III | 6001-9000 | 22.44 ± 7.3 | 11 ± 166 | 133 ± 133 | 0.78 ± 0.5 | 0.42 ± 0.48 | 165 ± 81 | 50 ± 227 |
| IV | 9001-12000 | 23.09 ± 4.7 | 101 ± 109 | 157 ± 87 | 1.49 ± 0.36 | 0.74 ± 0.31 | 80 ± 53 | 215 ± 181 |
| V | > 12000 | 44.51 ± 4.6 | 559 ± 105 | 146 ± 84 | 3.09 ± 0.34 | 2.37 ± 0.3 | 296 ± 51 | 839 ± 175 |
| Metropolitan city | | | | | | | | |
| I | < 3000 | 7.45 ± 5.9 | 135 ± 135 | - | 0.44 ± 0.44 | 0.22 ± 0.39 | 46 ± 66 | 83 ± 226 |
| II | 3001-6000 | 15.71 ± 4.3 | 166 ± 99 | 245 ± 79 | 1.20 ± 0.32 | 0.87 ± 0.28 | 76 ± 48 | 122 ± 165 |
| III | 6001-9000 | 9.60 ± 4.4 | 556 ± 102 | 307 ± 81 | 1.48 ± 0.33 | 0.83 ± 0.29 | 185 ± 49 | 357 ± 170 |
| IV | 9001-12000 | 25.00 ± 4.4 | 287 ± 102 | 71 ± 81 | 1.19 ± 0.33 | 0.67 ± 0.29 | 206 ± 49 | 119 ± 170 |
| V | > 12000 | 30.79 ± 4.4 | 674 ± 102 | 150 ± 81 | 1.93 ± 0.33 | 1.63 ± 0.3 | 302 ± 49 | 341 ± 170 |

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Table 6. Average monthly expenditure on milk and milk products at various income levels in different areas (Taka/month)

| Category | Income group (Tk.) | Liquid milk | Powder milk | Condensed milk | Sweet meat | Curd/Dahi | Ghee | ice cream | Total expenditure(Tk.) |
|--------------------------|--------------------|-------------|-------------|----------------|------------|-----------|--------|-----------|------------------------|
| Rural area | | | | | | | | | |
| I | < 3000 | 103 | 0.64 | - | 29.61 | 7.51 | 1.86 | - | 142.6 |
| II | 3001-6000 | 100 | - | - | 76 | 51 | 26.43 | - | 253.4 |
| III | 6001-9000 | 289 | 6 | - | 61.59 | 40.83 | 26.81 | - | 424.2 |
| IV | 9001-12000 | 373 | 87 | - | 59.48 | 28.05 | 16.94 | - | 564.4 |
| V | > 12000 | 677 | 2.62 | - | 106.43 | 46.40 | 27.97 | - | 860.4 |
| All income groups | | 277.26 | 13.98 | - | 61.35 | 31.69 | 18.22 | - | 402.5 |
| Municipality town | | | | | | | | | |
| I | < 3000 | 317 | 9.61 | 2.31 | 36.95 | 30.76 | 30.13 | 3.46 | 430.2 |
| II | 3001-6000 | 245 | 85.43 | 9.5 | 50.32 | 23.88 | 8.39 | 7.69 | 430.19 |
| III | 6001-9000 | 394 | 3.27 | 9.66 | 47.78 | 20.07 | 53.75 | 8.77 | 537.27 |
| IV | 9001-12000 | 439 | 29 | 11.00 | 107 | 38 | 30 | 31 | 685 |
| V | > 12000 | 820 | 181 | 9 | 242 | 125 | 72 | 90 | 1539 |
| All income groups | | 460.78 | 71.85 | 8.37 | 107.37 | 53.04 | 38.08 | 32.52 | 772.01 |
| Metropolitan city | | | | | | | | | |
| I | < 3000 | 121 | 39 | - | 30.92 | 14.07 | 12.03 | 10.18 | 207.82 |
| II | 3001-6000 | 335 | 43.73 | 11.40 | 93.38 | 50.83 | 26.29 | 52.26 | 612.85 |
| III | 6001-9000 | 225 | 160 | 17.62 | 113.09 | 58.43 | 68.80 | 125.40 | 768.32 |
| IV | 9001-12000 | 545 | 89.06 | 10.67 | 96.79 | 40 | 81.20 | 24.25 | 886.99 |
| V | > 12000 | 701 | 188 | 5.62 | 163.70 | 111.51 | 100.42 | 54.68 | 1325.32 |
| All income groups | | 409.85 | 109.28 | 9.95 | 105.98 | 58.78 | 61.65 | 57.40 | 812.89 |

LSD test indicates that the mean ghee consumption of Metropolitan cities differ significantly ($p < 0.05$) with the consumption of Rural and Municipality town (Table 3). But incase of income levels, LSD test indicates, the mean consumption of (V) income groups differ significantly ($p < 0.01$) with (I), (II), (III) and (IV) income groups (Table 4). It indicates that as income increased consumption of ghee increased positively. As the price paid by the different consumer groups was not same, the variation of expenditure on ghee was not similar to the variation in quantity. It may be said that, as the rich people are more conscious about nutrition, they purchase more ghee.

Ice-cream: Average consumer in Municipality and Metropolitan city areas consumed 243.95 and 204.40 g of ice-cream per month, in Rural area no one found to take milk prepared ice-cream. Both areas and income levels had significant effect ($p < 0.05$) on ice-cream consumption (Table 3 and 4). Ice-cream consumed and expenditure on it was the highest for income group (V) and the lowest for

income group (I) in municipality town.

All these results are more or less similar with the findings of Goswami (1994). He concluded expenditure on milk increased with income group and maximum expenditure was on liquid milk in all groups. Another experiments carried out by Gupta *et al.* (1995) on consumption pattern of milk and milk products. He concluded that consumption of milk and milk products increased with income; in all groups.

Assessment of the factors influencing consumption pattern of milk and milk products: The consumers of Rural, Municipality town and Metropolitan city areas were asked to report their opinions about the factors related to consumption problems that they had to face in purchasing or consuming milk and milk products. In Rural areas those factors which affects consumption pattern included less income, non-availability of milk product, not available of sweet shop, high cost, not habituated with the products, indigestion, and allergic reaction. In

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Municipality town, factors included crisis of pure milk, not available of quality sweets and other milk products and allergic reaction. In Metropolitan city, the constraining factors included crisis of pure milk, high cost, and adulteration of milk and milk products.

Conclusion: The study has shown the consumption and expenditure level of milk and different milk products with respect to different income classes. The study shows that the Municipality town households consume more milk, sweetmeats and dahi than Rural and Metropolitan city. On the other hand, Metropolitan households consume more powder milk, condensed milk, ghee and ice cream. Milk and some milk products consumption and expenditure on it increased substantially, with the increase of income in all the areas.

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