

Analytical, nutritional and biological evaluation of various brands of fortified and non-fortified wheat flour

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Abstract: In Pakistan, a funded flour fortification program was launched for malnourished population, residing mainly in rural low income areas, but the urban population having comparatively better nutritional as well as economic status was focused wherein excessive intake of fortificants might cause complications. Therefore, the present study describes the physicochemical properties, elemental composition, nutritional components and hemoglobin/ferritin increasing potential of fortified and non-fortified flour. Domesticated chicken (*Gallus gallus domesticus*), either sex, age one month, weight 380 ± 18.28 g, were randomly segregated into 4 groups (n=6). The group I, II and III were fed on fortified flour, whereas group IV was fed on non-fortified flour for 30 days. The birds were weighed and blood samples of each of the birds were analyzed for determination of markers of iron status, hemoglobin (Hb) and serum ferritin (SF). Moisture, ash and iron contents were found to be lower in non-fortified flour than that of the fortified samples. Hb and SF levels in groups fed on fortified flour were significantly higher than the one received non-fortified flour ($P < 0.05$). The consumption of iron-fortified flour increases iron stores in the body without any further complication but long-term usage needs to be monitored.

Keyword: Wheat flour fortification; iron fortification, elemental composition; *in vivo* studies.

INTRODUCTION

The deficiency of micronutrients, vitamins and minerals prerequisite for proper functioning of the living system, is a serious health problem worldwide. Millions of the people are currently suffering from micronutrient deficiency due to global crisis of food dysfunction (Welch, 2005). A substantial amount of nutrients is lost during food processing. Among the micronutrients deficiencies, iron deficiency is the most common and widely spread nutritional disorder in developing as well as developed countries and affecting almost half of the world's population (Allen and Ahluwalia, 1997). Women, children and infants are the major victims of iron deficiency and its consequences (Almeida, 2003).

To combat iron deficiency both drugs and non-drug strategies can be used. The former includes the use of iron containing pharmaceutical dosage forms, whereas the later includes food fortification, food supplementation, control of infectious diseases and counseling (Lotfi *et al.*, 1996). Supplementation of iron preparations is effective and can bring immediate relief to the risk group, if taken properly (Schumann *et al.*, 1998). However, it is not possible for everybody to afford such costly products. Noncompliance is another factor reducing the importance of supplementation success (Sloan *et al.*, 1992; Mothercare, 1990). Therefore, food fortification is considered the best option as a long-term strategy to

overcome the dilemma of iron deficiency (Samuelson *et al.*, 2000).

Food fortification is the addition of one or more nutrients in food to prevent specific deficiency in a target population or to replenish the nutrients lost during food processing (Codex, 1991; Wight, 2011). Many food articles such as milk, margarine, salt and backed food stuff etc. have been used as vehicle to deliver fortificants. To achieve the best outcome of fortification, the vehicle must be safe and acceptable to most of the population. Moreover, fortification should not affect the stability and acceptability of the end product (Hurrell, 2002). Since, the most commonly used staple food by Pakistani population was wheat flour therefore, it was selected as a vehicle for fortification. Ministry of Health and Micronutrient Initiative with the help of Global Alliance for Improved Nutrition started collaborating Pakistan Flour Mill Association in 2005 to fortify wheat flour with iron and folic acid to reduce the anemia nationwide. It is reported that the addition of iron in wheat flour had reduced anemia from 36 to 16% in children of the Venezuela during two years period (Layrisse *et al.*, 1996). In Pakistan, prevalence of micronutrient deficiency is in rural areas, which is about 80% of the population (Abdullah, 1999). On the other hand, urban population is comparatively having good economic and nutritional status. The awareness of fortification program and marketing is being done mainly in urban population, which may be a risk to healthy individuals.

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There are some controversies and issues related to the use of iron, though its deficiency prevails in a number of women, man and children, but not common amongst post-menopausal women and adult men, so this group should be cautious when using fortified flour or iron supplementations due great risk of iron overload, which can cause liver cirrhosis and heart diseases (Dallman, 1986). Therefore, there is dire need to focus the target population with strict monitoring to get the desired outcome.

The impact of fortification in reducing anemia can be predicted by determining iron levels in the food vehicle or conducting studies on anemic human beings and animals. The iron status of the body can be assessed by examining the level of hemoglobin (Hb) and serum ferritin (SF) (Cook *et al.*, 1996). In the present study, we have selected animal model- domesticated chicken (*Gallus gallus domesticus*) - because human model was not feasible ethically. The domesticated chicken were preferred because these could be fed on flour and kept without any much controlled environment.

Though, literature confirmed the role of fortification in anemia and other micronutrient efficiencies in many countries, except Pakistan, whereby no quality assurance system for food safety exists. Therefore, the present study was undertaken to investigate fortified and non-fortified flour for physicochemical properties, elemental composition, nutritional components and biological studies. The finding of this study may be useful for proper implementation of wheat fortification program in Pakistan and other developed and developing countries.

MATERIALS AND METHODS

Chemicals, solvents and other supplies

The chemicals and solvents of analytical grade procured from E. Merck, included nitric acid, hydrochloric acid, zinc powder, iron powder, magnesium powder, folin-ciocalteu's reagent, lanthanum oxide, sodium carbonate, sodium hydroxide, copper sulphate, potassium sodium tartrate, sodium chloride, potassium chloride, calcium carbonate, manganese powder, bovine serum albumin, triton-X and ethanol. Other materials were ash-less filter paper No. 24 (Whatman), disposable syringes 3 mL (BD), blood collection tubes (BD), and ferritin serozyme kit (Biochem Immune System, L.L. C. Bridgetown, USA). In-house prepared de-ionized/distilled water was used in all the experiments.

Three most commonly used fortified wheat flour brands and one non-fortified flour brand (*Chakki Atta*) were purchased from the local market, and to hide the identity for ethical purposes, fortified brands were designated as brand-I, -II and -III, whereas non-fortified brand was designated as normal four.

Determination of physicochemical properties

Organoleptic properties

The color, texture and odor of the flour were evaluated by seeing under visible light, physical touching and sniffing.

Proximate analysis

The different brands of wheat flour were investigated for proximate analysis using the methods described in British Pharmacopoeia (B. P, 1980). Briefly described as follows:

Moisture contents: Two grams of flour in a tarred china dish was dried in an oven at 105°C for 30 min. Then, the dish was allowed to cool to room temperature in desiccators and weighed to calculate the moisture contents.

Total ash: A clean china dish was heated in an oven at 100°C for 10 min, allowed to cool and weighed. Then 2 g of flour was incinerated in furnace by slowly increasing the temperature to 675 ± 25°C. Once the material got free from carbon, the dish was kept in desiccators to cool to room temperature and weighed to calculate total ash contents.

Acid insoluble ash: A quantity of ash was boiled for 5 min in 25 mL of dilute HCl, the mixture was filtered using ash-less filter paper and the retained material was washed by warm distilled water. Then the filter paper along with the retained material was burnt in a tarred china dish until free from carbon. The dish was cooled to room temperature in desiccators and weighed to calculate acid insoluble ash.

Sulphated ash: Two grams of flour taken in a tarred china dish was moistened with H₂SO₄, heated till emission of white fumes ceased and then ignited in furnace at 500–600°C. The dish was then allowed to cool in desiccators, again moistened with the acid and ignited, cooled and weighed to determine ash contents.

Determination of nutritional contents

Estimation of total fat

Fifteen grams of flour contained in a thimble was macerated for 12 h in petroleum ether using soxhlet apparatus, and then extraction was carried out at 40-60°C for 24 h. The extract was filtered and the filtrate was dried in *vacuo* at 40°C in a tarred flask, which was weighed to calculate the lipid contents (Besbes *et al.*, 2004).

Estimation of total proteins

Fifteen grams of flour was macerated for 10 h in 100 mL distilled water containing 10 drops of triton-X. The extract obtained was used for the estimation of total protein (Lowery *et al.* 1951). Ten milliliters of extract in a centrifuge tube was centrifuged at 2700 rpm for 10 min. The supernatant (0.1 mL) was taken in test tube and made the volume 1 mL by adding distilled water. Then 3 mL of

reagent C –prepared by mixing 50 mL of reagent-A (2% Na_2CO_3 in 0.1N NaOH) and 1 mL of reagent-B (0.5% CuSO_4 in 1% potassium sodium tartrate) - and 0.2 mL of folin-ciocalteau's reagent were added. Then the tube was incubated for 30 min at room temperature and absorbance was measured at 600 nm against a blank that was prepared by combining all the reagents and water, in place of sample (UV-2550, Shimadzu Corporation Japan). The solutions of different concentrations of bovine serum albumin (Fraction V), treated like the sample, were used for calibration curve. All the samples and the standards were analyzed in triplicates and total protein contents were calculated from the calibration curve using linear regression.

Estimation of total carbohydrates

Carbohydrate contents were calculated using the method described by Al-Hooti *et al.* (1997) and Barminas *et al.* (1999) as 100-(Sum of percentages of moisture, ash, protein and lipids).

Determination of minerals

Preparation of standard stock solutions

Stock solutions (1000 $\mu\text{g/mL}$) of sodium, potassium, calcium and manganese were prepared by dissolving 2.5420 mg of dried sodium chloride, 1.9067 g of dried potassium chloride, 2.4963 g of calcium carbonate and 1.00 g manganese powder in an appropriate amount of 2% hydrochloric acid, separately, and making the volume 1000 mL with de-ionized water.

Stock solutions (1000 $\mu\text{g/mL}$) of zinc, iron and magnesium were prepared by dissolving 1.00 g powder of each material in 40 mL of nitric acid, separately, and making the volume 1000 mL with de-ionized water.

A stock solution of lanthanum (50 g/L) was prepared by wetting 29.32 g lanthanum oxide in 25 mL of de-ionized water, and then adding 125 mL of HCl slowly to dissolve the contents. Finally, the volume was made up 500 mL with de-ionized water. Lanthanum solution was used as releasing agent especially for the calcium and magnesium.

Preparation of working standard solutions

The working standard solutions of Zn, Mn, Na and K (1, 2, 3, 4 and 5 $\mu\text{g/mL}$), Ca and Mg (5, 10, 15, 20 and 25 $\mu\text{g/mL}$) and iron (2, 4, 6, 8 and 10 $\mu\text{g/mL}$) were prepared by diluting the respective stock solution with de-ionized water.

Preparation of sample stock solution

Five gram flour taken in silica crucible was carbonized in Muffle furnace (Carbolyte, USA) at 550°C for 1 h. The crucible was allowed to cool to room temperature and the ash was dissolved in 5 mL of 20% HCl. The contents were transferred into 50 mL volumetric flask and made-up the volume with de-ionized water. Five milliliters of 20% HCl diluted to 50 mL with de-ionized water was used as a blank.

Preparation working sample solutions

For Ca and Mg, 1mL of the sample stock solution taken in 100 mL volumetric flask was mixed with 2 mL of lanthanum solution and made up the volume with de-ionized water. For sodium and potassium, 1mL of the sample stock solution was diluted to 100 mL with 2% HCl. For iron, zinc and manganese, 1mL of the sample stock solution was diluted to 100 mL with de-ionized water.

Conditions of atomic absorption spectrophotometry

The conditions used for the determination of various types of elements are given in Table 1. The samples of flour and all the standard solutions were analyzed in triplicate using Atomic Absorption Spectrophotometer (PerkinElmer, Inc. Shelton, CT, USA). The contents of the metals were determined from calibration curves of the respective metals using linear regression.

Biological studies

Animals and grouping

Twenty four healthy, domesticated chicken (*Gallus gallus domesticus*), either sex, age 1 month, weight 380 ± 18.28 g, were purchased from local market. The birds were randomly segregated into four groups as group-I, -II, -III and -IV, each comprising 6 birds. Each group was housed in separate cage and allowed to acclimatize for 7 days in Animal House of the University College of Pharmacy, University of the Punjab, Lahore, Pakistan. During this period, commercially available broiler feed (Hi-tech Feeds Pvt. Limited, Pakistan) and water was supplied *ad libitum*.

Feeding procedure

The study was conducted as per approved protocol of the Animal Ethics Committee, University College of Pharmacy, University of the Punjab, Lahore, Pakistan. On each day, flour was mixed with small quantity of water to prepare feed. On day one, all the animals were weighed and only flour was given as feed throughout the study period. Each of the groups received 500, 750, 1000 and 1000 g flour per day in first, second, third and fourth weeks, respectively, whereas tap water was provided *ad libitum*. Group-I, -II and -III were fed on fortified flour of brand-I, -II and -III, respectively, whereas group IV was fed on non-fortified flour.

Weighing of birds and blood sampling

After 30 days, all the birds were weighed and blood samples, from blood vessel under the crest, were collected in plain blood collection tubes, which were then analyzed within 24 h of the collection for the determination of Hb (HemoCue model, Angelholm, Sweden) and SF (Serozyme kit).

STATISTICAL ANALYSIS

All the analytical tests were performed in triplicate and results were presented as mean $\pm$ SD. Levels of Hb and

SF of different groups of animals were analyzed using one way-ANOVA with Post Hoc multiple comparison, Bonferroni. A $P \leq 0.05$ was taken as significant.

RESULTS

Organoleptic properties

Organoleptic examination of different flour brands indicated that color of the normal flour was reddish brown, whereas the three fortified brands were whitish in appearance. The normal flour was coarse in nature while the fortified samples were fine powders. However, there was no difference of odor among both fortified and non-fortified samples. Therefore, we can presume the equivalence of acceptability of fortified flour.

Proximate analysis

The results of proximate analysis of different types of flour are given in Table 2. These results revealed that moisture contents of different types of flour varied from 9.20 to 10.07%. There was no significant difference of moisture contents in fortified brands, however, non-fortified flour was found to be having lower moisture contents than the fortified samples ($P < 0.05$).

The ash contents in the normal flour were lesser than that of the fortified brands. The brand-III was found to be having higher contents of the total ash, which meant that it contained the higher amount of metals. The acid insoluble ash contents were higher in brand-III as compared to other fortified brands and normal flour. However, there was no significant difference of sulphated ash among various fortified and non-fortified flour samples.

Determination of nutritional components

The major nutritional components such as fats, proteins and carbohydrates in various flour samples are presented in Table 3. These results indicate that normal flour has slightly higher amount of protein as compared to the three fortified brands. Similarly, the fats contents in all types of flour samples were almost similar. The same trend was noted in carbohydrate contents of all the brands of wheat flour.

Mineral contents

The contents of seven elements such as iron, zinc, calcium, magnesium, sodium, potassium and manganese in fortified and non-fortified samples are presented in Table 4. These results indicate that the iron contents in fortified brands are significantly higher than that of the non-fortified sample. However, the contents of all the other elements both in fortified and non-fortified brands were almost similar, except potassium in brand-I (207.74 mg/100g). The fortified brands found to contain around 34 % more iron as compared to non-fortified flour.

The contents of Hb and SF in birds of different groups fed on fortified and non-fortified flour are presented in fig. 1 and 2, respectively. It is clear from these results that the level of Hb and SF in group-I, -II and -III, fed on fortified flour, were significantly higher than that of the group-IV, fed on non-fortified flour ($P < 0.05$). However, there was no significant difference of these markers in group-I, -II and -III ($P > 0.05$). These results indicate the usefulness of fortification. However, the higher levels of iron may participate in redox system of the body producing free radicals, injurious to health.

The effect of weight gain of birds in after 30 day's treatment is shown in Fig. 3. There was no significant difference in weight gain of bird of groups fed on fortified and non-fortified flour ($P > 0.05$).

DISCUSSION

Physical properties such as texture, color and odor of flour play an important role in its acceptability. Some people like coarse, granular, flour whereas some prefer fine powdered flour. Both the texture and color of the flour depend upon the milling process. There was a difference of texture as well as color of fortified and non-fortified flour. Non-fortified flour was prepared by a less sophisticated mill called "*atta chaki*" and during milling bran was not removed. On opposite, fortified brand were prepared in roller flour mills and during grinding bran was removed producing fine and whitish flour. Fortificants might impart disagreeable odor, but in the present study there was not any noticeable difference of odor in both the types of flours, which was an evidence of good acceptability of fortified flour.

The moisture is one of the important factors determining the quality and stability of flour and micronutrients. The higher moisture hydrolyzes flour resulting degradation of dietary constituents, thus reducing usefulness of the flour. The degradation products may also not be good for health. In addition to this, it facilitates the microbial and pest growth that affects the dietary significance of the flour. The moisture contents depend on wheat storage conditions of and milling process. The lower moisture in non-fortified flour was due to the uncontrolled rise in temperature during grinding at local grinding mills as reported that moisture is reduced during grinding at local mills by frictional heat (Haridas *et al.*, 1983). However, both fortified and non-fortified flour samples were found to be having moisture within permissible limit, 12 - 13%. Hence, it can be concluded that all flour samples were of good quality.

Total ash, both physiological and non-physiological, provides evidence about the material left over after ignition. Physiological ash comes from the plant material whilst non-physiological ash is the residues of exogenous

Table 1: Conditions for the determination of metal ions by atomic absorption spectrometer

Element	Fe	Zn	Ca	Mg	Na	K	Mn
Wavelength	248.3	213.9	422.7	285.2	589.00	766.5	279.5
Slit (nm)	0.02	0.7	0.7	0.7	0.7	0.7	0.7
Lamp current	30	10	10	6	8	12	20
Replicate	3	3	3	3	3	3	3
Air flow	17	17	17	17	17	17	17
Acetylene	1.5	1.5	1.5	1.5	1.5	1.5	1.5

Table 2: Proximal analyses of various brands of wheat flour

Description of sample	Moisture %	Ash %	Acid insoluble ash %	Sulphated ash%
Brand-I	10.04	1.86	0.94	54.05
Brand-II	10.04	1.75	0.95	53.95
Brand-III	10.07	1.90	1.30	53.75
Normal flour	9.20	1.51	0.92	53.95

Brand I, II and III (fortified brands); Normal flour (non-fortified)

Table 3: Nutritional components such as proteins fats and carbohydrates in different brands of wheat flour

Sample description	Protein (%)	Fat (%)	Carbohydrates (%)
Brand-I	10.93	1.97	76.04
Brand-II	10.55	1.98	75.68
Brand-III	11.38	1.97	74.68
Normal flour	11.85	1.95	77.88

Brand I, II and III (fortified brands); Normal flour (non-fortified)

Table 4: Mineral contents (mg/100g) in various brands of wheat flour composition of wheat flour

Sample description	Fe	Zn	Ca	Na	K	Mg	Mn
Brand I	7.23	2.49	25.37	2.19	207.74	20.58	4.09
Brand II	7.39	2.76	25.43	2.86	135.28	18.73	4.05
Brand III	7.66	2.39	25.46	2.60	138.67	19.99	4.51
Normal flour	4.87	2.85	24.98	2.29	152.83	16.11	4.13

Brand I, II and III (fortified brands); Normal flour (non-fortified)

stuff such as gravel, soil attached to surface of the grain or stones of grinding parts. The higher values of the ash of the fortified brands were due to exogenous addition of certain minerals as fortificants. The findings of our study were substantiated by the previous studies conducted on various commercially available brands of wheat flour showing ash in a range of 1.30 - 1.95% (Kamal and Behere, 2003). The higher value of acid insoluble ash in brand-III was also verified by its higher total ash contents.

As far as nutritional components are concerned, fats, proteins and carbohydrates were alike both in fortified and non-fortified flour. Wheat flour contains lipase which is responsible for lipid hydrolysis, thus decreasing the fat contents (Rose and Pike, 2006). The grinding process can further reduce the fat contents because some of the fat is destroyed while grinding (Haridas *et al.*, 1983; Farooq *et al.*, 2001). The protein contents in all the four brands investigated in this study were within the specified limits

(Hussain *et al.*, 2004). Generally, wheat flour contains protein from 8-12% and this variation depends upon various genetic factors and external factors related to the crop. The presence of proteins, fats and carbohydrates in almost equal quantities indicated that all the brands, both fortified and non-fortified were equivalent in nutritional value. However, the major difference among all such brands was the minerals as indicated by ash values which might further be confirmed by elemental analysis.

Elemental analysis indicated that iron was the major fortificant present in fortified flour. The normal limit of iron in wheat flour was found to be 3.30 mg/100 g, which was lesser than that of the fortified brands, 7.23 – 7.66 mg/100 g. The consumption of 300 g of fortified flour per day can provide 21.5 mg/day iron which corresponds to its recommended daily intake (RDI), 14 -28 mg/day (FAO/WHO, 1988). However, the usefulness of this fortification may be questionable because fortified brands

are being consumed in urban areas of Pakistan whereby people have a better quality of life. In such situation, there may be chances of iron overload which may be deleterious (Mettler and Zimmermann, 2010). The body can only metabolize iron to a certain extent in a day, hence, if supplied in higher amounts, toxic effects may develop. Therefore, taking large supplement doses of iron is not recommended (Pootrakul *et al.*, 1988). Hence, fortified food articles need to be supplied to the specifically targeted population with strict monitoring.

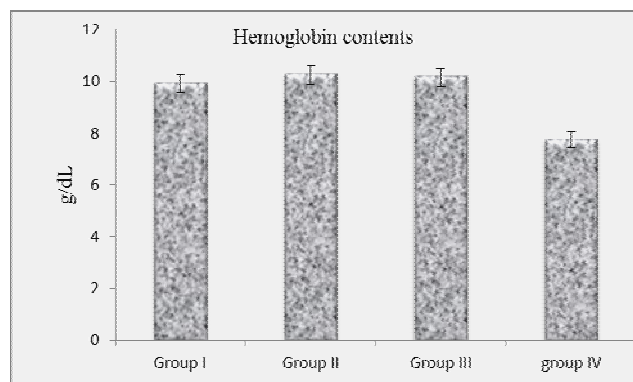


Fig. 1: Hemoglobin contents (g/dL) in birds of various groups fed on fortified and non-fortified wheat flour (n=6). Group-I, -II and -III (Fed on fortified flour of brand-I, -II and -III, respectively); Group-IV (Fed on non-fortified flour)

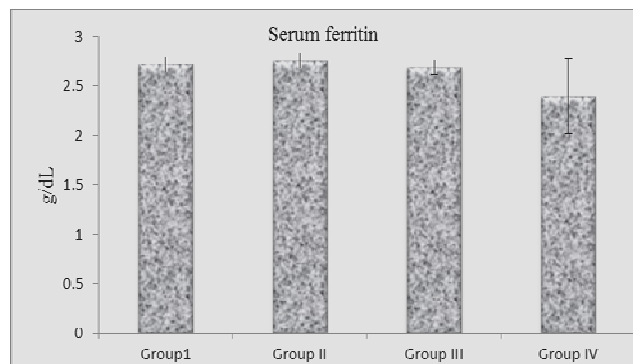


Fig. 2: Serum ferritin contents (g/dL) in birds of various groups fed on fortified and non-fortified wheat flour (n = 6). Group-I, -II and -III (Fed on fortified flour of brand-I, -II and -III, respectively); Group-IV (Fed on non-fortified flour)

The major amount of iron in the body comes from the diet which then binds to plasma transferrin to be consumed in bone marrow to form hemoglobin. Serum iron is a measure of circulating iron bound to transferrin and reflects total body iron. Ferritin is the molecule responsible for storing and detoxifying intracellular iron. The binding of iron to ferritin prevents its participation in the conversion of both hydrogen superoxide and hydrogen peroxide to reactive oxygen species and hydroxyl radicals. Ferritin is also an indicator of stored iron in the

body because it stores iron for areas that need it, especially the liver and the bone marrow. The iron ferritin level is the first in line to drop, if an individual suffers from any iron insufficiency in diet, mal-absorption or blood loss. A drop in the iron ferritin level occurs before any depletion in serum iron, as seen in iron-deficient anemia, and may decrease significantly without any obvious symptoms whatsoever. In the present study, increase in ferritin level was due to the storage of excessive iron absorbed.

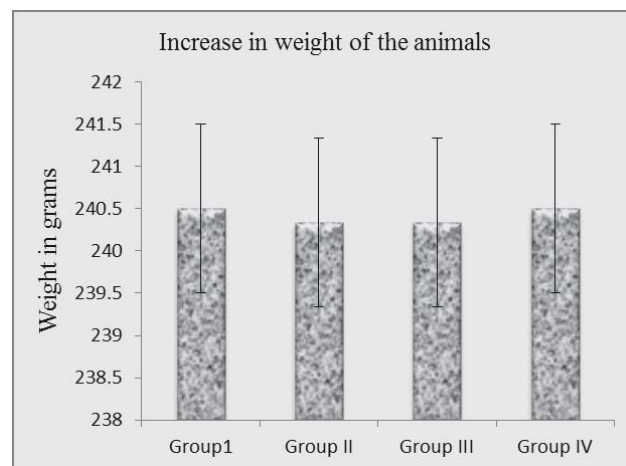


Fig. 3: Weight gain (g) of birds of various groups fed on fortified and non-fortified wheat flour (n = 6). Group-I, -II and -III (Fed on fortified flour of brand-I, -II and -III, respectively); Group-IV (Fed on non-fortified flour)

The majority of the Pakistani population suffering from malnutrition is residing in villages whereby people either cannot afford the costly fortified wheat flour due to their bad economic status or fulfill their flour demands by grinding wheat at local grinding mills. Therefore, there is a need to evaluate the usefulness of fortified flour conducting *in vivo* studies, preferably on malnourished human beings. However, due to ethical issues and limited time, the present study was conducted on animals – which could be fed on flour – to evaluate the effectiveness/iron overload of iron-fortified flour.

Hb and SF are two responsive measures of bioavailability of iron and their levels increase once the stores of iron replete and absorption of iron is sufficient. The changes in SF and Hb contents provide indirect estimation of iron over a period of time when subjects utilize experimental food fortified with iron. In our findings, Hb and SF contents in birds using fortified flour were higher than those receiving non-fortified flour. These results were consistent with the reported earlier (Hungerford and Linder, 1983; Viteri, 1995). The rise in the levels of hemoglobin using fortified flour is the evidence of its potential of treating anemia. The results of our study were supported by a study conducted on Kenyan children in randomized controlled trial whereby maize fortified with

iron was found to be highly efficacious in improving iron status of the patients (Andango *et al.*, 2007). Another study conducted in Vietnamese children had also shown similar results (Le *et al.*, 2006). Except iron, both fortified and non-fortified flour brands were found to be having almost similar contents of Zn, Ca, Na, Mg and Mn. The consumption of such brands can provide sufficient quantity of Mn (RDI=2.5 mg/day) whereas there is a need to take the remaining of the metals from other sources to meet the body requirements.

The findings of the present study are interesting because fortified flour consumption has increased the level of Hb and SF without any significant difference in weight gain of animals consuming both the types of flours. The almost equal increase in weight of birds consuming fortified flour to that of the non-fortified flour indicates that excessive iron has not caused any deleterious effect on birds. The outliers were possible but the weight gain in all the eighteen birds was the sufficient evidence to conclude that fortified flour could be taken without the fear of iron overload. Nevertheless, the study was conducted for a short time and birds were in the growing age, the effect of higher iron was not obvious. Therefore, further studies are needed to get the additional evidence.

CONCLUSION

Iron was the main fortificant found in fortified flour samples, whereas fortified and non-fortified flour samples were equivalent in terms of major nutritional components such as protein, fats and carbohydrates. Fortified flour proved to be efficacious in increasing hemoglobin and serum ferritin; however its long-term usage needs to be monitored.

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