



Determination of Heavy Metals in Eight Barley Cultivars Collected from Wheat Research Station Tandojam, Sindh, Pakistan

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Received 31 January 2013, Revised 19 June 2013, Accepted 29 June 2013

Abstract

Barley (*Hordeum Vulgare* L.) is one of the most important foods for animals and possesses high nutritional value. In this paper, we have focused our study to find out the chemical parameters, especially metal content of this class of food commodity and its soil, which is not frequently used for human food. Wet digestion method was used to destroy the organic matrix to determine the content of eleven metals i.e. Iron, Zinc, Manganese, Copper, Cobalt, Chromium, Nickel, Lead, Cadmium, Barium and Aluminium, from eight Pakistani barley cultivars. The highest level of elements were determined in order; Cd < Ni < Pb < Cr < Co < Cu < Ba < Al < Mn < Zn < Fe in mg/kg. All analysis was carried out by using air-acetylene except Al and Ba where as both of these metals analysed on air-acetylene and nitrous oxide flame on Atomic Absorption Spectrophotometer. Among all these entries, maximum concentration of Fe was detected in B6, Zn in B5, Mn, & Cu in B2 & B5, Co in B1, Ba in B7, Al in B8 and rest of the elements i.e. Cr, Pb, Ni and Cd were found to be with little difference of concentration among cultivars.

Keywords: Cereals; Barley; Metals; Chemical analysis.

Introduction

The barley (*Hordeum Vulgare*) is one of the oldest cultivated cereal commodities of Poaceae family that also include wheat, rice and maize. Information about the barley cultivation has been observed in soil tablets from 1700 B.C. [1]. More than 150 million tons of barley was produced in 2009, comprising 54 million hectares of agricultural land worldwide [2]. In United States, Barley is ranked third amongst cultivated cereal grains [3, 4]. In most of countries including Europe, it is cultivated in large scale and is used as cereal grain for animal as well as birds feed [5]. Barley is almost cultivated in cold areas being a cold cultivated plant. However, it also grows at hot

areas of the world by producing new varieties of the barley, which can sustain to the temperature of that specific area. The change in climatic conditions was found to change the dietary value in cultivars of barley [6]. Barley is used commercially for animal feed, to produce malt, for seed and for human food applications as it is rich in protein, carbohydrates, dietary fibers, minerals, and vitamins. We have studied the amount of essential and trace metals present in barley and its soil using atomic absorption spectroscopy, that is, to our opinion, an important advancement towards the assessment of mineral content present in this important food commodity.

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Protein storage in barley is about 50% in mature grains that is the most important nutritional and functional property of grains [7]. Different plants follow different paths of uptake and accumulation for various elements [8]. Barley accumulates a large amount of starch and is utilized as source of energy [9]. The level of protein in barley cereal was found in the ranges of 7.5 – 17 % at a dry basis where as 75% of the protein is digestible. The protein value in greater amount, about 13.6% on dry matter would not be greatly raising the level of barley to livestock feeders [10].

Calcium value was detected at low level in maize, barley and sorghum grains but barley possesses high level of phosphorus as compared to other grains [11]. However, the calcium is a basic macro-element for life, particular for human being for the formation of bones and teeth. Low level of minerals in barley cultivars needs supplement in food, which can be fulfilled by taking the supplement from mineral salt. Micro-elements deficiency is mainly an environmental crisis that can be overcome with consultation of soil specialists as well as nutritionists of the concerned quarter. The suggestions of experts are essential to resolve this problem by giving their recommendations about supplement and also type of supplement that is needed. Most of the elements and vitamins can increase the shelf life of the food; therefore, these metals and vitamins are added in barley to preserve for longer time [12]. The element contents vary area-wise on the basis of geographic variations, which badly affect the barley crop than that of seed grown in soil [13]. The demand of barley (*Hordeum vulgare* L.) is going to increase day by day due to the interest of human being as delicious food and its industrial use in addition to animal and bird's feed [14,15]. Some of the barley varieties possess soft husk which can be easily and fairly removed. New industries have been developed around to remove the husk and make it digestible for animal and poultry as their feed. Husk less contains fiber and flour and has been used in many edible products [16]. Due to rich in fiber contents, glucan and protein, husk less is getting more importance than other grains, such as oats, that is well known for its β -glucan contents [17-19]. The β -glucan compound attracts the customers for the reason

that it decreases blood cholesterol and glucose levels [20]. The contents of various essential, trace and toxic elements in barley and unpolished rice and its soil of different areas of Pakistan were studied using spectroscopic techniques [21, 22]. Various analytical methods are used to study the mineral contents and grain yield data of numerous cultivars of barley [23].

Heavy metals are essential, needed in small quantities for the health of all living things that may be animals or plants during whole life for their physiological functions, these nutrients cannot be synthesized by the organism itself but are obtained from surrounding [24]. Most of the microelements are used for enzyme cofactors, these are easily supplied through the soil to plants in a trace amounts, which is further transferred to animal and human body. Human requires less than 100 micrograms per day in diet and is known as dietary minerals. The micro-minerals or trace elements include; Iron, cobalt, chromium, copper [25], iodine, manganese, selenium, zinc and molybdenum are not usually necessary in plant fertilizers, because its much quantity may cause harm the growth of a plant. The micronutrients are also included; vitamins that are organic compounds required as nutrients in tiny amounts by an organism but these are not known as microelements but micronutrient [26].

This research was carried out to analyze the variable uptake of concentration of minerals in eight barley lines Pedigree (B1-B8) and existence of the concentration of same elements in that specific soil where these cultivars were grown.

Materials and Methods

Barley Sampling

Wheat research institute Tandojam, Sindh, Pakistan, was selected for the collection of barley samples. Eight different genotypes; Arar (B1), Rihane-03 (B2), Beca's' (B3), Beecher (B4), Orge (B5), Giza 120/5 (B6), Albert (B7), and Arizon (B8) were grown and harvested at matured stage. The samples were collected on hit or miss basis in the maturation period to make a model sample just after harvesting and samples were de-husked, packed into polyethylene bags without chemical treatment and properly labeled, finally brought into

laboratory of National Centre of Excellence in an Analytical Chemistry, University of Sindh, Jamshoro for further chemical analysis.

The growing period of barley lies in between November to January and harvesting season is by the end of March. All varieties were grown in experimental farms in 2010 at above wheat research station (WRI).

The clay loam soil was collected from experimental field where all these varieties were grown. The soil was air-dried and sieved (<0.5mm).

Chemical analysis

Different barley cultivars and soil samples where these cultivars were grown dried at variable temperatures 80°C to 110°C in oven till invariable weight was attained. Two grams of each cultivar and one gram of soil in 5 replicates were weighed in conical flasks (100ml), then were treated with HNO₃ (5ml). Same quantity of HNO₃ of same concentration was also taken in to empty conical flask that serves as a blank. All barley and soil samples were placed in conical flasks, covered with watch glasses and the samples heated lightly on an electric plate, as content cannot splash. The heating was continued for about 60 minutes till semi dried content obtained in flasks. Sample flasks and blank were removed from hot plate for few minutes until flasks were cooled. Again 5 ml of HNO₃ were added to all flasks and placed on an electric plate for further 50-60 minutes to obtain semi-dried content. As semi-dried content obtained, flasks were removed from hot plates, left to cool down for 20 minutes then were added 2 - 3

ml of 35% H₂O₂ into the contents of flasks, heating was continued for further 50-60 minutes till semi-dried contents obtained. After removing watch glasses, 50 ml of deionized water was added to each flask, and was heated until the volumes of contents evaporated up to 25 ml. The contents of the flasks were cooled up to 15°C - 20°C, filtered from first to last by Whatman # 42 paper into 25 ml volumetric flasks. The volume of the 25 ml volumetric flasks was made up to mark with deionized water by adding 5% nitric acid to preserve the sample, finally transferred the content to sample bottles, which were previously washed and soaked. Besides that an Internationally Certified Reference Material was used to maintain analytical quality assurance. The concerned standard reference substances were selected for quality control i.e. BCR CRM191 brown bread, NIST SRM1567a wheat flour, NIST RM8433 corn bran and NIST RM8436 durum wheat flour. All these sample bottles along with blank were transferred to atomic absorption spectrometry to analyze essential trace elements as well as trace and toxic elements such as: Iron, Zinc, Manganese, Copper, Cobalt, Chromium, Nickel, Lead, Cadmium, Barium and Aluminium levels. All samples were pre-treated by the wet digestion method to destroy the organic matrix. Standard Official Methods of Analysis of the Association of Official Analytical Chemists [27] were used for the analysis of above elements. Entire unsolvable metals were estimated by using Flame Atomic Absorption Spectrophotometer (FAAS), according to Approved Methods of the American Association of Cereal Chemists [28]. Statistics were accounted on a dehydrated weight origin. Results of all above measurements have been mentioned in (Table-1).

Table1. Concentration of Eleven Heavy Metals in Eight Barley Cultivars in mg/kg

Element	B1	B2	B3	B4	B5	B6	B7	B8	Soil
Fe	1546.66	1229.84	1181.84	841.01	1537.06	2146.9	1820.28	1316.24	4966.8
Zn	48.64	43.22	30.24	37.93	53.05	41.58	44.35	42.09	70.03
Mn	21.93	38.41	25.06	22.78	38.69	30.45	33.86	21.93	663.71
Cu	9.03	10.31	7.50	7.02	10.46	9.87	8.92	7.17	21.86
Co	4.41	3.45	2.73	2.73	3.45	2.26	1.66	3.29	11.15
Cr	1.04	0.84	1.03	0.88	1.22	0.83	0.99	1.00	13.09
Ni	0.91	0.80	0.70	0.56	1.23	0.92	1.04	0.97	15.69
Pb	1.21	1.10	0.78	0.82	0.98	0.55	0.78	1.19	3.49
Cd	0.45	0.49	0.33	0.42	0.34	0.23	0.43	0.32	2.94
Ba	8.32	6.76	4.43	8.78	3.96	8.32	9.25	6.30	139.41
Al	19.06	15.92	10.42	24.57	22.21	19.06	21.42	25.35	42035.7

Table 2. Instrumental Conditions for the AAS Measurement of Fe, Zn, Mn, Cu, Co, Cr, Ni, Ba, Al, Pb and Cd

Elements	Wave length (nm)	Slit width (nm)	Lamp current (mA)	Fuel flow (acetylene) (l/min)	Flow rate (Air) (l/min)	Burner height (mm)	Oxidant (Air) kg/cm ²	Fuel (Acetylene) kg/cm ²	Signal out put
Fe	248.3	0.2	9.5	2.30	9.4	7.5	1.60	0.3	100%
Zn	214.0	1.3	9.5	2.0	9.4	7.5	1.60	0.2	100%
Mn	279.8	0.4	9.5	2.0	9.4	7.5	1.60	0.2	100%
Cu	325.0	1.3	9.5	2.0	9.4	7.5	1.60	0.2	100%
Co	250.0	0.2	9.5	2.0	9.4	10.0	1.60	0.35	100%
Cr	358.2	1.3	6.0	2.0	9.4	7.0	1.60	0.30	100%
Ni	232.3	0.2	9.5	2.0	9.4	7.0	1.60	0.30	100%
Ba	553.8	1.3	9.5	5.61	5.91(N ₂ O)	7.5	1.60(N ₂ O)	0.45	100%
Al	309.5	1.3	9.5	5.61	5.91(N ₂ O)	12.5	1.60(N ₂ O)	0.45	100%
Pb	232.3	1.3	7.0	2.0	9.4	7.5	1.60	0.2	100%
Cd	229.0	1.3	7.0	2.30	9.4	7.5	1.60	0.30	100%

Instrumentation

All tabulated results of eleven heavy elements such as, Fe, Zn, Mn, Cu, Co, Cr, Ni, Ba, Al, Pb and Cd were analyzed by Hitachi Model 180-50 atomic absorption spectrophotometer. The lamps known as Hollow cathode lamps (made by Meltorika) were used as radiation energy source to find out the concentration of given elements. For the fuel, in atomization steps of elements, an air-acetylene gas was used. However, for analysis of Al and Ba, an additional nitrous oxide was used with air-acetylene-nitrous oxide as a source of high hotness. The lamp's current 9.5, 9.5, 9.5, 9.5, 9.5, 6.0, 9.5, 9.5, 9.5, 7.0, and 7.0mA respectively were maintained by hollow-cathode lamps (Mtorika) for these elements. Flow-rate of fuel was 2.21 l/min for Fe and Cd; 2.0 l/min for Zn, Mn, Cu, Co, Cr, Ni and Pb; 5.61 l/min for Ba and Al respectively. Flow-rate for air 9.40 l/min for Fe, Zn, Mn, Cu, Co, Cr, Ni, Pb, and Cd; 5.91 l/min for Al and Ba were used respectively to get a clear yellow flame. Spectrophotometer was coupled to a Hitachi recorder 056 with a range of 5mV. The signals calculated were the heights of the absorbance/division peaks. Instrumental parameters have been mentioned in (Table 2).

Reagents and Calibration

Pure HNO₃ (65% w/v), H₂O₂ (35% w/v) chemicals (Merck) and highly purified water / deionized water having zero electrical conductivity was produced with a Milli-Q system Millipore, MA, USA.

The calibration curve was got by using the external and internal standards of analyzed elements. For preparation of standard solutions, they were diluted in 1000mg/l multi element solution (ICP Multi element standard iv, Merck, Darmstadt, FRG) with similar acids, which were used for pre-treatment of sample.

All sample solutions were analysed by aspirating through capillary tube into atomic absorption spectrophotometer through absorbance / division's capacity of each element by maintaining optimum conditions for flame atomization mode.

For inter calibration, standard references; 0.5 to 10 ppm was also run along with our own made standards. Elemental concentrations were computed on an IBM compatible PC using excel computer program. The statistical calculations for standards are given in (Table 3).

Table 3. Statistical Data for Standards of Elements

Elements	Concentration range ppm (x)	Absorbance/ Division (y)	Statistical calculation $y = m x + c$		
			m	c	r^2
Fe	0 – 3	0 - 910	03016	-0.0022	0.9996
Zn	0 – 1	0 - 0.235	0.2349	-0.0027	0.9989
Mn	0 – 1	0 – 0.134	0.1324	0.0021	0.9985
Cu	0 – 2	0 – 0.138	0.0693	-0.0001	0.999
Co	0 – 1	0 – 0.053	0.0529	0.0004	0.9997
Cr	0 – 1	0 – 0.070	0.0698	0.0002	0.999
Ni	0 – 1	0 – 0.111	0.1104	0.0022	0.998
Ba	0 – 1	0 – 22 div.	22.057	-0.0001	0.9974
Al	0 – 25	0 – 0.0358	0.0014	-0.086	0.9999
Pb	0 – 1	0 – 0.063	0.0638	-0.0009	0.9992
Cd	0 – 0.125	0 – 25 div.	356.52	0.5074	0.999

Absorbance*

div. =Divisions

Results and Discussion

The concentration of the metals in the eight barley hybrids and its soil is given in Table 1. Among all eight hybrids, the value of Fe was found highest in B6 and its lowest level detected in B4 cultivar and the second highest value of iron observed in B7, while B1 and B5 got intermediate position among all cultivars and their values were very close to each other. Analytical results of iron of same plot of soil where eight genotypes were grown indicate that the soil accumulated about three times more iron as compared to their barley cultivars. It may be possible that due to high concentration of iron in soil, barley cultivars contained larger value of iron in grains than that of listed metals. Zinc was second essential heavy metal that had preserved maximum status in B5 and minimum concentration in B3. Meanwhile there was little bit difference in values B2 and B7 as well as B6 and B8 cultivars. As far as its soil was concerned, it possessed round about double concentration of the Zn.

Manganese concentration was found in the range of 21.93 – 38.69 mg/kg in which the minimum and identical value was obtained in B1 & B8 (21.93 mg/kg) and maximum value (38.69 mg/kg) calculated in B5 cultivars. However, there was significant difference level of B2 and B5 barley hybrids (38.41 and 38.69 mg/kg)

respectively, wherever soil of that specific area haunted 23 folds higher concentration (663.71 mg/kg) of same element compared to barley hybrids.

The high up take of Cu was observed in B2 and B5 (10.31 and 10.46 mg/kg) respectively, which were precise and low up take of same element was detected in B3, B4 and B8 variety (7.50, 7.02 and 7.17 mg/kg) in that order with a minute distinction and its soil keeps (21.86 mg/kg) that was 2.5 times higher level of copper as compared to their grains. Cobalt and lead are highly toxic in nature for human being and are required at trace level to play active role in metabolism of the body. Analytical results of Table 1 established that the B1 got uppermost level (4.41 mg/kg) of Co and lowest in 1.66 mg/kg in B7, at the same time as two pairs; B2 and B5 (3.45 mg/kg) and B3 and B4 (2.73 mg/kg) had identical values. In close proximity was no significant difference in values of B2 and B8 (3.45 and 3.29 mg/kg) cultivars though soil of same crop of variable genotypes accumulated 11.15 mg of Co that was higher than values of all cultivars. The maximum concentration (1.04 mg/kg) of Cr was found in B1 and minimum (0.83 mg/kg) in B6, on the other hand, no mark able deviation was observed in pairs of barley: B1 and B3 (1.04 and 1.03 mg/kg); B2 and B6 (0.84 and 0.83 mg/kg); B7 and B8 (0.99 and 1.00 mg/kg) while soil of that

specific plot contained 13.09 mg/kg. Investigated results of Ni illustrated so as to chief value was detected in B5 and bottom one in B4 although negligible distinction observed in B1, B6 and B8 varieties. Overall, trend of Ni absorption was found in decreasing order from B1 to B4 then jumps at peak level in B5 and shows zigzag trend in rest of cultivars, furthermore, randomly collected soil samples of that plot possessed 15.69 mg/kg of nickel. Moreover, soils accumulate three fold higher concentration of cobalt and lead 3.5 folds higher that of the lead. As for as Cd concentration was concerned, all barley genotypes hold below 0.5 mg/kg of Cd and the values of B1, B2, B4 and B7 (0.45, 0.49, 0.42 and 0.43 mg/kg) respectively were high and very close to one another, lowest value of Cd was found in B6 (0.23 mg/kg) and its soil have 2.94 mg which is higher (7.82 times) than that of barley level.

Comparative study of Ba in eight cultivars indicates that B7 attained uppermost assessment and B5 lowest one, on the other hand identical values calculated in B1 and B6, at the same time close concentration was detected in pairs: B2 and B8 (6.76, 6.30 mg/kg) and B3 and B5 (4.43 and 3.96 mg/kg) respectively as soil of that plot acquired 139.41 mg/kg. Aluminum got exceptional position in capacity of higher concentration among all trace and toxic elements given in table 1, but its maximum concentration (25.35 mg/kg) was observed in last variety (B8) and minimum concentration in B3 (10.42 mg/kg). However, identical data observed in B1 and B6 (19.06 mg/kg), on the other hand, insignificant level was detected in B4 and B8 (24.57 and 25.35 mg/kg) respectively, whereas experimental plot of genotypes keep hold on (42035.7 mg/kg). There was significant difference in the results of Al, Fe, Mn, Ba, Zn, Cu, Ni, Cr, Co and Pb in barley given in table 1, which were compared with previous related published work for the same elements [29-34].

The soil was collected from those locations where all these varieties were grown. Results of the soil, where these cultivars/hybrids were grown shows that it accumulates the concentration of metals in order; Al > Fe > Mn > Ba > Zn > Cu > Ni > Cr > Co > Pb and Cd respectively.

Conclusion

It is found that B6 cultivar accumulated highest concentration of iron where as lower concentration of iron, zinc and copper was found in B4. Highest level of zinc, manganese, copper, chromium and nickel was detected in B5. Maximum level of Ba and Al was found in B7 and B8 respectively. Highest level of Pb and Cd was observed in B1 and B2 respectively and lowest level of both elements accumulated in B6 variety. The value of trace, essential and toxic elements in soil was detected from double to thousands folds higher than that of barley cultivars, which grown in the same soil.

Acknowledgement

We are grateful to Syed Iqrar Hussain Shah, Muhammed Aslam Nushad (Seed Certification Officers), Seed Certification & Registration Department and Mr. Biland Rai Oad, Wheat Botanist for their help in collection of the barley samples from Wheat Research Station (WRS), Tandojam. Author is also thankful to the authorities of Shah Abdul Latif University, Khairpur, Sindh, Pakistan for financial assistance and grant of study leave.

References

1. J. R. Harland, USDA Handbook, 338 (1979) 10.
2. Takashi Matsumoto, Jsuyoshi Tanaka, Kiyosumi Hori, Kazuhiro Sato, *Plant Physiol.*, 156 (2011) 20.
3. W. Lin, S. Evans and G. Davenport. *Ag. Info. Bull.*, 477 (1984) 40.
4. Klaus, F. X. Mayer, Mihaela Martis, Pete E. Hedley and Nils Stein, *The plant Cell*, 23 (2011) 1249.
5. Ivan T. Todorov, Padha N. Philipova, Nikolai, Z., Zhelev, Asenov and Hadjolov, *Biology of the cell*, 62 (1988) 105.
6. Hoppner, K. H., B. D. Owen and F. W. Sosulski. *Can. J. Anim. Sci.*, 48 (1968) 135.
7. Milan Chnapek, Eva Palencarova, Zlenka Galova, Zelmira Balazova, Marian Tomka, *J. Microbiol., Biotech. & Food Sci.*, 1 (2012) 622.

8. A. Aksoy and D. Demirezen. *Polish J. Env. Studies*, 15:1 (2006) 27.
9. National Research Council (NRC), Recommended Dietary Allowances, 10th Ed. National Academy Press, Washington D.C. (1989).
10. C. W. Newman, C. F. McGuire and D. C. Rasmusson. *Am. Soc. Agron., Crop Sci. Soc. and Soil Sci. Soc.*, Ed. (1985) 403.
11. National Research Council (NRC), Nutrient requirements of Beef Cattle, 7th Ed. National Academy Press, Washington D.C. (1984).
12. W. E. Dinusson, D. O. Erickson, C. N. Hauge and M. L. Buchanan. *ND Farm Res.* 26:1 (1968) 3.
13. W. E. Dinusson, D. O. Erickson, D. W. Bolin, M. L. Buchanan. *ND Farm Res.* 21:7 (1960) 8.
14. M. A. O. Oscarson, R. Anderson, A. C. Salomonsson and P. Aman, *J. Cereal Sci.*, 24 (1996) 161.
15. C. Elfverson, A. M. Anderson., P. Aman. and S. Regnér. *Cer. Chem.*, 76 (1999) 434.
16. R. S. Bhatti, *Cer. Chem.*, 76 (1999) 589.
17. A. Lapveteläinen and T. Aro. *Cer. Chem.*, 71 (1994) 133
18. E. Marconi, M. Graziano. and R. Cubadda. *Cer. Chem.*, 77 (2000) 133.
19. A. De Francisco and R. M. De Sa. Beta Glucanas: Localização, propriedades utilização. In: Lajolo, F. M.; Saura-Calixto, F. D. E. Pena, E. W. DE Menezes, E. W. Fibra dietética en Iberoamérica: Tecnología y salud. Obtención, caracterización, efecto fisiológico y aplicación en alimentos. São Paulo: Varela, (2001) 91.
20. R. K. Newman, K. C. Ore., J. Abbott. and C. W. Newman. *Cereal Foods World*, 43 (1998) 23.
21. G. Q. Shar, L. A. Shar, T. G. Kazi, and I. H. Afridi, *J. res. Sci. B. Z. Univ.* 18:2 (2007) 69.
22. G. Q. Shar, L. A. Shar, T. G. Kazi, S. Sahito and S. A. Arain, *J. Chem. Soc. Pak.* 27:1 (2005) 38.
23. Soner Yuksel, Mevlut Akcura, *Turk. J. Agric.*, 36 (2012) 285.
24. Canadian UNICEF Committee, Global Child Survival and Health, (2006) 67.
25. <http://en.wikipedia.org/wiki/Copper> in health
26. S. Lieberman and N. Bruning, The Real Vitamin & Mineral Book. NY: Avery Group, 3 (1990).
27. Association of Official Analytical Chemists – AOAC. *Official methods of analysis of the Association of Official Analytical Chemists.* Washington (1995).
28. American Association of Cereal Chemists – AACC. 2000. *Approved Methods of the American Association of Cereal Chemists.* 10th Ed. St Paul (2000).
29. F. Madrid and M. B. Kirkham, 17th WCSS, (2002) 401.
30. J. Wiecek, Z. Wiecek and Bieniaszewski, *Polish. J. Env. Studies.*, 14 (2005) 535.
31. R. Juknys, M. Racaite, G. Vitkauskaitė and J. Vencloviene, *Zemdirbyste-Agric.*, 96 (2009) 111.
32. F. S. Maleki, M. R. Chaichi, D. Mazaheri, A.R. Tavakkol and G. Savaghebi, *J. Agr. Sci. Tech.*, 13 (2011) 315.
33. R. K. Singh and R. P. Sharma, *Int. J. Green & Herbal Chem.*, 1 (2012) 21.
34. M. Samaranda, D. Neculai, M. Florica, N. J. Luminita and P. Dumitru, *Animal Sci. Biotech.*, 45 (2012) 237.