Original Article
ISSN (Online): 2582-7472

ShodhKosh: Journal of Visual and Performing Arts
Multidisciplinary Issue on Humanities, Commerce, Science, Engineering & Technology
January-June 2023 4(MIHCSET), 16–25

SCANNING ELECTRON MICROSCOPE AND ENERGY DISPERSIVE X-RAY ELEMENTAL ANALYSIS OF GOAT MILK

Harshesh Kumar [1], Dr. Harendra Kumar [2]

[1] Research Scholar, J.S. University, Shikohabad, U.P.
[2] Associate Professor, Department of Zoology, J.S. University, Shikohabad, U.P.

DOI
10.29121/shodhkosh.v4.iMIHCSET.2023.5109

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

ABSTRACT

The leaves of mulberry are highly valued for their nutritional content and they are not only used as a source of food for silkworms but also as a top supplement for cattle. The purpose of the current study was to compare the effects of giving mulberry leaves to goat milk before and after. Goat milk contained elements such as O, Na, Mg, S, P, Cl, K, and Ca according to the energy-dispersive X-ray (EDX) spectrum. After the feeding of mulberry leaves, oxygen was found in the highest concentration (68.9%) and magnesium was found in the lowest concentration (0.96%) in goat milk. Prior to the feeding of goat milk, sulphur was found in the lowest concentration (1.28%) and oxygen was found in the highest concentration (82.64%).

Keywords: SEM, EDX, Silkworm, Spectrum, Mulberry

1. INTRODUCTION

The silkworm (Bombyx mori), for a very long time, has only consumed mulberry leaves (Morus alba). Mulberry trees can be found growing all over the world in a variety of climates, from temperate to tropical. Fresh leaves have a high protein content (18 to 25% in DM) and a high in vivo DM digestibility (75 to 85%), and their biomass output is frequently in the range of 25 to 30 tons/ha/year with a cutting interval of roughly 9 to 10 weeks (Ba et al. 2005). Mulberry leaves therefore have a great deal of potential as a protein-rich fodder addition for livestock production (Benavides, 2000).

Fresh mulberry leaf production and total dry matter production per hectare are influenced by climatic factors, soil characteristics, plant variety, plant density, fertilizer application, and harvesting methods, but mulberry produces more digestible nutrients than the majority of traditional forages (Sanchez 2000). According to the variety, level of maturity, leaf position on the branch, and fertilization level, the chemical composition of the leaves varies (Shayo 1997; Singh and Makkar 2002). Thus, when leaves mature, their levels of protein, soluble sugars, and organic acids drop while their levels of fiber, fat, and ash rise. In addition, mulberry leaves contain more moisture, protein, and carbs in temperate areas than in tropical ones (Singh and Makkar 2002). In several regions of Greece, especially in the north; mulberry is semiextensively grown primarily for the leaves to support the sericulture business. Mulberry is cultivated on a semi-extensive scale in various parts of Greece, particularly in the northern part, mainly for the leaves to support the sericulture industry.

Mulberry grows rapidly in the early stages and reaches maturity at an early age; the growth the main feed for sheep (Prasad and Reddy 1991), goats (Omar et al. 1999; Sanchez 2000; rate falls off rapidly after approximately ten years. The mulberry leaves can also be used as Schmidek et al. 2000; Bakshi and Wadhwa 2007) and rabbits Prasad et al. 2003; Martinez et

al. 2005). Moreover, they have been used to replace concentrates in dairy cattle (Sanchez 2000; Mejia 2002) or goat diets (Anbarasu et al. 2004). They can also be used as an ingredient in the diets of monogastric livestock, such as pigs (Ly et al. 2001; Leterme et al. 2006) and laying hens (Narayana and Setty 1977). Recently, Srivastava et al. (2003) investigated the potential of powdered dried mulberry leaves, which in mixture with wheat flour can be utilized for human consumption in Indian diets. Due to its high digestibility and excellent level of crude protein, mulberry foliage can be a comparable source to commercial concentrates for ruminal feeding and production. The content of total phenols is very low (1.8% as tannic acid equivalent) and tannins by the protein precipitation capacity method were not detectable (Singh and Makkar 2002).

One of the main features of mulberry leaves is their high palatability. Small ruminants avidly consume the fresh leaves and the young stems first, even if they have never been exposed to them before. Then, if the branches are offered un chopped, they may tear them off and eat the bark. Cattle consume the whole biomass if it is finely chopped. However, for ruminants, the preferred method of feeding has been branch cutting by hand, although mechanical harvesting could be employed in the future for direct feeding of fresh material on a large scale, for processing or drying. Since mulberry leaves are rich in nitrogen, sulphur and minerals (Singh and Makkar 2002) their use for ration supplementation could increase the efficiency of utilization of crop residues in ruminant feeding systems. Thus, mulberry leaves have the potential to be used as a supplementary feed for improving livestock productivity and play a valuable role in world agriculture. However, little information is available on their nutritive value and benefits, as a high-quality supplement to low-quality roughages or replacing grain- based concentrates in ruminant feeding. Therefore, studies to determine intake and optimal levels of supplementation with mulberry leaves for growing ruminants and milk yield should be carried out. Beyondb silkworm feeding, depending on regions, mulberry is also appreciated for its fruit (fresh, in juice, as preserve), for its medicinal properties (mulberry leaf tea), for landscaping, as a vegetable (leaves and young stems) and asa feed for ruminants and others animals (Zepeda, 1991).

A computer based image analysis systems having sophisticated statistical analysis software are commercially available and detailed analysis of micrographs are now more routinely on food products (Brennan and Grandison, 2012; Hui, 1992). A variety of devices have been developed in scanning electr (SEM) like (WDS), energy-dispersive (EDS) spectrometers and proton induced x-ray emission (PIXE) are based on the detection of X- (SEM) is carried out non- rays. Chemical characterization of a sample through a destructively with energy dispersive X-ray (EDX) analysis. The electron beam strikes the atoms in the sample with uniform energy and they instantaneously emit characteristic X-rays of specific energies for every element. These X-rays provide information about the elemental composition of the sample. Elements starting with the atomic number six (carbon) can be determined with this analytic technique. The detection limit with SEM-EDS is -0.1 wt%. The unique resolution of the SEM depends on the size of the electron spot which in turn on the magnetic electro-optical system which produces the scanning beam. The depends resolution is also limited by the size of the interaction volume, or the extent of material which interacts with the electron beam. The merits of SEM-EDX technique include the small sample size required (<1 mm3). In contrast, there are limitations to this method that includes its poor sensitivity to trace elements and elements lighter than Na. Also, the loss of Volatile elements (Na) during excitation is a well-recognized phenomenon (Kursula, 2000). In accordance with Reed (1996), the theoretical detection limits in SEM-EDX measurements. are about 0.08 wt%.Milk of any mammalian species has a wide range of organic and inorganic ingredients that are all interlinked in a specific manner. For example Ca and P are bound with casein micelle, Fe is related to lacto ferrin, transferring, xanthine oxidase and slightly to casein, Zn is associated with lacto ferrin Mn is bound to the milk fat membrane and Co is a key mineral in the large vitamin B12 molecule etc (Fennema, 1996; Whitney, combination to fat free milk by applying SEM technique (Kumar and Mishra, 2006). Another study was conducted using SEM to examine the relationship between skimmed milk powder and physical properties of chocolate mass (Attaie et al., 2003). In SEM analysis, sample protocol, such as drying, stabilizing through a fixative, mounting the sample on metallic holder and coating a thin layer by means of an electrical conductor are the fundamental steps (Bozzola and Russell, 1992). The aim of this study was to focus on the chemical analytical aspects of elements in the goat's whole milk with and without damaging the organic portion through exploration at microstructure level.

2. OBJECTIVES

To identify the ingredients in goat milk.

3. MATERIALS AND METHODS

3.1. COLLECTION OF SAMPLE

Two goats were selected from a farmer, Ravi Yadav Madai, Shikohabad,. One mgoat was selected from a farmer, Ram Prasad, Kantri, Shikohabad. One goat was black in colour and 2 Years old, second goat was brown in color and 3 yearold and third goat was red in color and 4 year old. The milk sample was collected before feeding of mulberry leaves, six samples were collected alternative days in a 12 day feeding period. Two samples were collected of three goats after feeding of mulberry leaves (one day gape), sample were collected in morning time in sterilebottles and stored in the freezer at -20°C.

3.2. SAMPLE COLLECTION

In the month of June during the summer, a sample of fresh goat whole milk was taken from a cattle colony. The sample was collected in a 500 ml polythene bag and refrigerated below 4°C.

3.3. SEM ANALYTICAL PROTOCOL

Two pre-treatment methods were used for SEM-EDX analysis: analysis via mineral oxides or ash and analysis through coagulated whole milk. An aliquot of 10 cc was dried on a hot plate at 105 °C and torched at 550 °C in the furnace to produce ash (AOAC, 2000). An aliquot of the milk sample was cooked on a hot plate to over 85°C for the coagulation (Twyman, 2005). A lumpy substance was produced when the moisture was eliminated.

SEM-EDX analysis

Applying scanning electron microscopy (SEM) along with an energy-dispersive X-ray (EDX) spectrometer allowed for the recording of the two observations. The samples were loaded onto the sample holder for this work using double site tape, and then they were coated using an auto coater from Joel Japan, model number JSM 6490 LV. Palladium is the coater's target metal. Until 300oA, the samples were coated. Then, samples that had been coated were put onto a SEM from Joel Japan, model JSM 6490 LV. Joel Japan's INCA x-act EDX detector was used to perform the EDX analyses.

4. RESULTS AND DISCUSSION

In the current investigation, a trial run of SEM-EDX on metal oxides (ash) did not yield any distinguishable particles. This trial suggests that the approach might not be usable in the absence of organic materials, such as milk from cows. In the second trial, SEM was used to obtain images of three different types of dried cow's milk particles and a relevant depiction of the element distribution on a surface using a different scanning resolution of dehydrated milk. The inner qualitative organization of the various sample sites is shown in Fig. 1.

Fig. 2 depicts the elemental breakdown of the surface cow milk particles. By using Standard Less Quantitative Analysis with ZAF Correction Method (Trincavelli et al., 2014), it was possible to determine the percentages of various elements, including metals and non-metals, at various places within the sample. These percentages are shown in Table 3 below. In the several scans, the amounts of all the estimated elements varied. Table 2 shows the ranges of percentages of main, trace, and hazardous elements. Ca, which is abundant in milk, was estimated twice but with a relatively high percentage compared to other components.

P was measured in all scans and highest in the 3rd scan, but its percentage was noted as the second highest after Ca. Percentages of electrolytes such as Na and K differed in all trials and their ranges were: Na 2.16 - 4. 69% and K 6.44 - 13.99%. Trace elements were found in the range of 1.37 - 1.41% (Table 2). Nevertheless, the quantities of essential trace elements (O, Mg, P, S, Ca, Cl Zr and Pt) were much lesser than that of the major elements. Fe and Cu were found in three trials, but percentage of Fe was uniform and comparatively lower than Cu. The percentages of Zn varied considerably but the maximum was measured as 0.08%. The percentages of Pt were observed in all scan in the range of 12.53-12.83.In the light of the results, it is concluded that the concentration of elements in the sample was not homogeneous in all particles In some studies, characterization with the confirmation of elemental composition through spectrum of silver nanoparticles synthesized by various sources by applying SEM-EDX technique has been reported (Guangquan et al., 2012 and Jegadeeswaran et al., 2012).

5. CONCLUSION

The influence of mulberry leaves on goat milk composition in this study should have a high crude protein content, high apparent digestibility, casein content of 1.21%, carbohydrate content of 2.41%, and lipid content of 2.89%. The feeding of mulberry leaves causes the functional groups and components of three goats' milk to grow, but more research is required to see how the goats would react to mulberry leaves being included in their meals at higher and higher levels.

Figure. 1 Sem Images of Cow Milk

Element	Weight %	Atomic %
O K	83.64	91.51
Na K	1.37	1.05
P K	4.50	2.54
K K	1.29	0.70
Cl K	2.73	1.35
K K	2.00	0.89
Ca K	4.47	1.95
Totals	100.00	

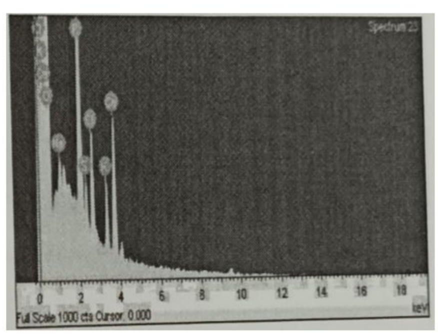


Fig. 2 And Table 1. Edx Image of Milk of Brown Goat (Beroge Feeding)

Element	Weight %	Atomic %
O K	70.14	83.52

Na K	2.00	1.66
P K	5.03	3.09
S K	1.69	1.00
Cl K	9.21	4.95
K K	8.13	3.96
Ca K	3.80	1.81
Totals	100.00	

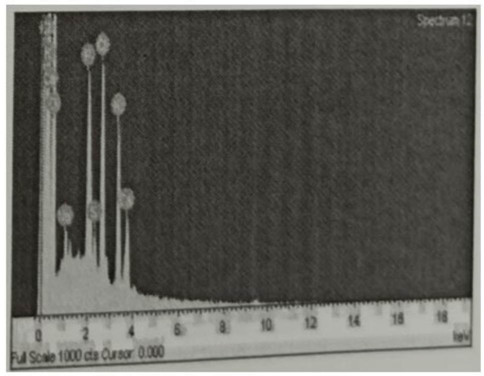


Fig. 3 And Table 2. Edx Image Of Milk Of Red Goat (Beroge Feeding)

Element	Weight %	Atomic %
O K	72.03	89.17
S K	2.64	1.63
Cl K	6.00	3.35
K K	2.68	1.36
Ca K	3.19	1.57
Zr L	13.47	2.92
Totals	100.00	

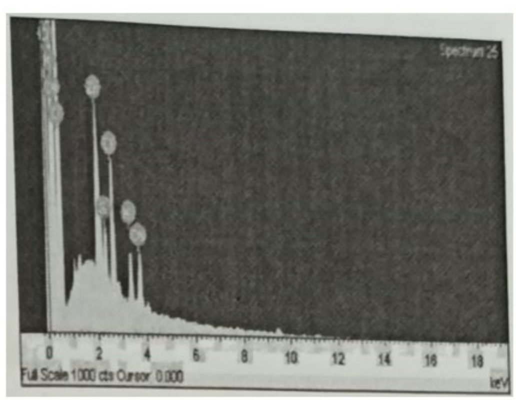


Fig. 4 And Table 3. Edx Image Of Milk Of Black Goat (Feeding Period)

Element	Weight %	Atomic %
O K	53.50	75.89

Na K	2.09	2.07
Mg K	1.37	1.28
P K	5.84	4.28
S K	2.25	1.59
Cl K	8.72	5.58
K K	6.66	3.87
Ca K	7.03	3.98
Pt M	12.53	1.46
Totals	100.00	

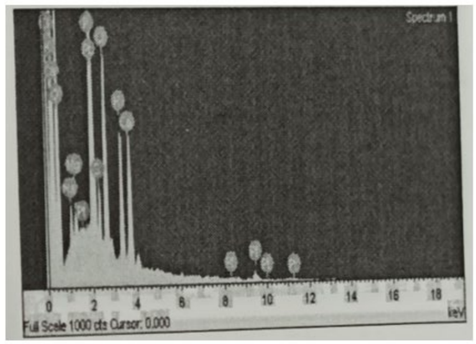


Fig. 5 And Table 4. Edx Image Of Milk Of Brown Goat (Feeding Period)

Element	Weight %	Atomic %
O K	67.38	81.65
Na K	2.01	1.69
P K	6.16	3.86
S K	2.59	1.57
Cl K	9.41	5.51
K K	5.53	2.74
Ca K	6.92	3.35
Totals	100.00	

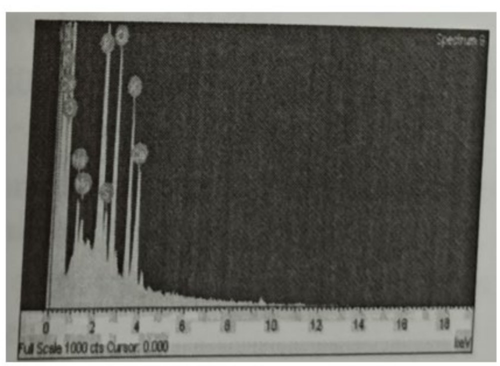


Fig. 6 And Table 5. Edx Image Of Milk Of Red Goat (Feeding Period)

Element	Weight %	Atomic %

O K	69.99	83.15
Na K	2.16	1.79
Mg K	0.97	0.76
P K	5.41	3.32
S K	2.03	1.20
Cl K	7.94	4.26
K K	6.44	3.13
Totals	100.00	

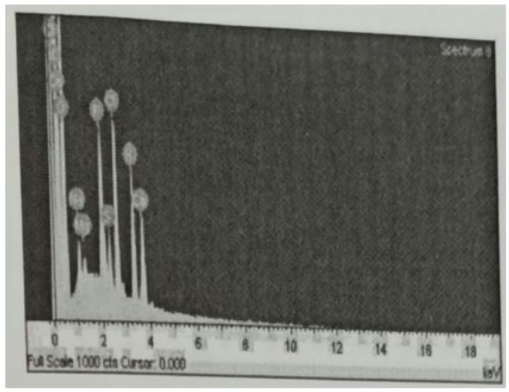


Fig. 7 And Table 6. Edx Image Of Milk Of Black Goat (After Feeding)

Element	Weight %	Atomic %
O K	68.58	86.08
Na K	1.34	1.17
P K	2.35	1.53
S K	1.31	0.82
Cl K	4.67	2.65
K K	5.62	2.89
Ca K	4.67	2.34
Zr L	11.44	2.52
Totals	100.00	

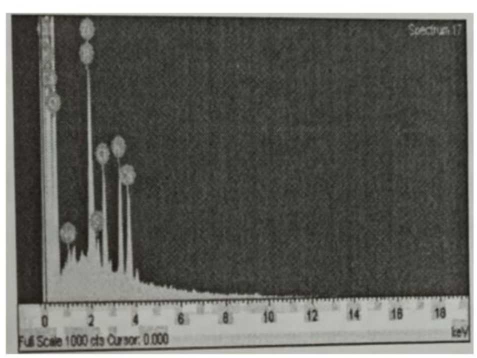


Fig. 8 And Table 7. Edx Image Of Milk Of Brown Goat (After Feeding)

Element	Weight %	Atomic %

O K	67.93	81.69
Na K	2.66	2.23
Mg K	0.98	0.78
P K	6.14	3.81
S K	1.85	1.11
Cl K	7.84	4.25
K K	7.22	3.55
Ca K	5.37	2.58
Totals	100.00	

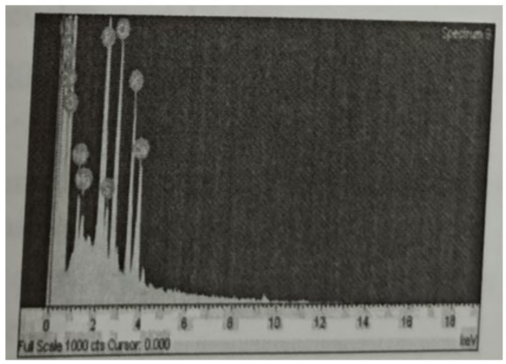
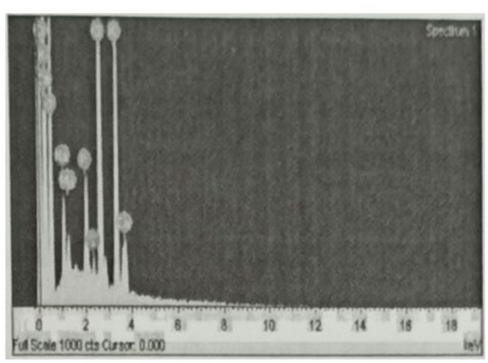


Fig. 9 And Table 8. Edx Image Of Milk Of Red Goat (After Feeding)

Element	Weight %	Atomic %
O K	57.63	74.12
Na K	4.69	4.20
Mg K	1.52	1.29
P K	3.69	2.45
S K	0.76	0.49
Cl K	14.98	8.69
K K	13.99	7.36
Ca K	2.73	1.40
Total	100.00	



CONFLICT OF INTERESTS

None.

ACKNOWLEDGMENTS

None.

REFERENCES

AOAC (Association of Official Analytical Chemists). (2000). Official Methods of Analysis, International Dairy Products. 17th Ed. Washington, DC.

Anbarasu, C., Dutta, N., Sharma,K., Rawat, M.(2004). Responce of goat to partial replacement of dietary proteinby a leaf meal mixtuere containing Leucaenaleucocephala, Morus alba and Tectonagrandis. Small Runinant Research .51 (1),47-56.

Attaie, H., Breitschuh, B., Braun, P. and Windhab, E.J. (2003). The functionality of milk powder and its relationship to chocolate mass processing, in particular the effect of milk powder manufacturing and composition on the physical properties of chocolate masses. International Journal of Food Science and Technology, 38, 325- 335.

Ba N.X., Giang V.D., Ngoan L.D. (2005).Ensiling of mulberry foliage (Morus alba) and the nutritive value of mulberry foliage silage for goats in central Vietnam. Livestock Research for Rural Development 17 (15).

Bakshi,M.P.S., Wadhwa,M. (2007). Tree leaves as complete feed for goat buck. Small Ruminant Research .69(1-3), 74-78.

Benavides,J., (2000). Utilization of mulberry in Animal Production Systems .In: Sanchez (ed), "Mulberry for Animal Production".

Bozzola, J.J.and Russell, L.D. (1992). Electron Microscopy. Jones and Bartlett Publishers Inc., Boston, Mass. Brennan.

Fennema, O.R. (1996). Food Chemistry, 3rd ed. Owen R. Fennema, Chapter no. 14.

Guangquan, L., He, D., Qian, Y., Guan, B., Gao, S., Cui, Y., Yokoyama, K. and Wang, L. (2012). Fungus-Mediated green synthesis of Silver nanoparticles using Aspergillusterreus. International Journal of Molecular Sciences, 13, 466-476.

Hui, Y.H. (1992). Encyclopedia of food science and technology. John Wiley & Sons Inc., New York, USA,2, 1112-1114.

Jegadeeswaran, P., Shivaraj, R. and Venckatesh, R. (2012). Green synthesis of silver nanoparticles from extract of PadinaTetrastromatica leaf. Digest Journal of Nanomaterials and Biostructures, 7 3, 991-998

J.G.and Grandison, A.S. (2012). Food Processing Handbook .2nd Ed. Vol.2, Wiley- VCH Verlag and Co. KGaA, Boschstr, Weinheim, Germany, 494 p.

Kumar, S.G.V. and Mishra, D.N. (2006). Yakugaku Zasshi, The Pharmacuitical Society of Japan, 126, 93-97.Kursula, P.K. (2000). Accuracy, Precision and Detection Limits of SEM

WDS, SEM-EDS and PIXE in the Multi-Elemental Analysis of Medieval Glass. X- RAY Spectrometry, 29, 111-118.

Ly, J., Yao,J., Yan, B.,Tu ,J.Q., Shi,Z.Q. (2001). Effects of mulberry leaves to replace rapessed meal onperformance of sheep feeding on ammoniated rice straw diet. Small Ruminant Research. 39, 131-136.

Ly,J., Chhay,T., phiny C., Preston ,T.R.(2001). Some aspects of the nutritive value of leaf meals ofTrichanthera gigantean and Morusalba for mongcai pigs Livestock Research for Rural Developmentn. 13 (3). Martinez, M., Motta, W., Cervera,C., Pla ,M. (2005). Feeding mulberry leaves to fattening rabbits: effect on growth, carcass characteristics and meat quality. Animal Science. 80 (3), 275-280.

Mejja,N. (2002). Potential from production of mulberry (Morus alba) in El Salvador. In: International Symposium on Silopastoral System and Second Congress on Agroforesty and Livestock Production in Latin America.

Narayana, H., Settee, S.V.S. (1997). Studies on the incorporation of mulberry leaves (Morus alba) in layers mashon health, production and egg quality. Indian journal of Animal Science.47, 212-215.

Omar,S.S.., Shayo,C.M.., Uden,P. (1999). Voluntary intake and digestibility of mulberry (Morus alba) diets by growing goats.Tropical Grassland.33, 177-181.

Palmquist, D. L., A. D. Beaulieu, and D. M. Barbano. (1993). Feed and animal factors influencing milk fatcomposition. J. Dairy Sci. 76:1753-1771.

Prasad,P.E., Reddy, M.R.(1991). Nutritive value of mulberry (Morus alba) leaves in goats sheep. Indian Journal of Animal Nutrition .8(4), 295-296.

Prasad, R., Mishra,A.K., Sankhyan, S.K., Mishra,A.S., Tripathi, M. K., Karin, S.A., Jakhmola, R.C.(2003). Growth performance and caecal fermentation in growing rabbits fed on diets containing graded levels of mulberry (Morus alba) leaves .Asian Australasian Journal of Animal Science. 16 (9),1309-1314.

Prasad, C., Preston, T.R., Ly, J.(2003). Mulberry (Morus alba) leaves as protein source for young pigs fed rice-based diets :Digestibility studies. Livestock Research for Rural Development. 15 (1).

Reed, S.J.B. (1996). Electron Microbe Analysis and Scanning Electron Microscopy in Geology.Cambridge University Press, Cambridge, UK.

Sanchez, D.Manuel. (2000). Mulberry :an exceptional forage available almost world wide!. World Animal Review.93 (1) .FAO Rome.

Sanchez, M.D. (2000). Mulberry :an exceptional forage available almost worldwide World Animal Review . 93, 1-21.

Schmidek, A., Takahashi, R., de Medeiros,A.N., de Resende, K.T. (2000).Bromatological composition and degradation rate of mulberry in goats .In: Sanchez (ed), "Mulberry for Animal Production".

Shayo, C. M.(1997). Uses, yield and nutritive value of mulberry (Morus alba) trees for rumnants in the semi-arid areas of central Tanzania: Tropical Grassland.31,599-604.

Singh,B., Makkar, H.P.S.(2002). The potential of mulberry foliage as afeed supplement in India. In: Sanchez (ed) Mulberry For animal production. FAO Animal Production and Health Paper 147.

Srivastava, S., Kapoor,R., Thathola,A., Srivastava,R.P. (2003). Mulberry (Morus alba) leaves as human food: anew dimension of sericulture. International Journal of Food Science and Nutrition .54,411-416.

Trincavelli, J., Silvina L.and Rita B.(2014). Standardless quantification methods in electron probe microanalysis. Spectrochimica Acta Part B: Atomic Spectroscopy 101, 76-85

Twyman, R.M. (2005). Microscopy Applications/Food. Elsevier Ltd. University of York, York, UK, 50, Whitney, E. and Rolfes, S.R. (2005). Understanding Nutrition. 10th ed., Thompson Wadsworth Australia, 458.

Zepeda, J. (1991). EI arbol de oro. Los mil usos de la morera.Medioambiente (Peru) 47:28-29.