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Computational analysis of milk sources from different domestic animals as supplementary food source to protect Lathyrism

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Abstract

Lathyrism, a neurological disease caused by excessive consumption of *Lathyrus sativus* due to presence of non protein amino acid beta-N-oxylamino-L-alanin acid (BOAA) or beta N-oxalyl diamino propionic acid (beta-ODAP) results in non progressive spastic paralysis in human and animals. The lathyrism thought to cause due to sulfur amino acids deficiency. To investigate the protective action of sulfur rich amino acids (Cysteine and Methionine) in four milk proteins viz. alpha S1 casein, alpha S2 casein, beta casein and kappa casein present in common milk sources of cow (*Bos Taurus*), buffalo (*Bubalus bubalis*), goat (*Capra hircus*) and sheep (*Ovis aries*). ProtParam, CLUSTALW and PSIpred were used to analyze primary structure, multiple sequence alignment and secondary structure respectively. Current study revealed that buffalo and sheep milk contain highest amount of Cysteine and Methionine (14.2%) as sulfur rich amino acids, cow milk has significant amount of Cysteine and Methionine (13.1%) while goat milk contain lowest amount of Cysteine and Methionine (13.0%). This study paves the way that buffalo and sheep milk are the best sources of sulfur amino acids than the other two and can be used as supplementary food to protect Lathyrism.

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Introduction

One of the oldest neurotoxic diseases, Lathyrism (Spencer *et al.*, 1983), known to human and domestic animals, results in the excessive consumption of *Lathyrus sativus* (also called chickling pea, Khesari dal, grass pea) and lesser degree with some other related species such as *L. cicera, L. orchous* and *L. clynenum* (Prince Leopold Institute of Tropical Medicine online database). The nutritional value of *L. sativus* was determined for growing and laying pullets and the chemical analyses indicated *L. sativus* is high in crude protein (283g/kg) and low in fat (12.0g/kg) with estimated true metabolizable energy concentration of 12.0MJ/kg (Chowdhury *et al.*, 2005). *L. sativus* seeds are deficient in the sulfur containing amino acids Cysteine (Cys) and Methionine (Met), two essential compounds protecting against oxidative stress (Shinmol and Muralidhara, 2007). It is irreversible, non progressive spastic paraparesis associated with poorly understood degenerative changes in the spinal cord (Spencer *et al.*, 1983). Lathyrism is thought to cause due to presence of non protein amino acid, beta-N-oxylamino-L-alanin acid (BOAA) (Eguchi *et al.*, 2011) or beta N-oxalyl diamino propionic acid (beta-ODAP), known as a powerful agonist of certain glutamate receptors in the nervous systems. Two forms of lathyrism-neurolathyrism and osteolathyrism which are found in animal chiefly in horses develop in the hind limbs (Spencer *et al.*, 1983); in the case of human it is commonly associated with leg weakness, leg paralysis, leg muscle rigidity, pain, leg muscle spasticity etc. This disease is prevalent in some area of Bangladesh, India, Nepal, Ethiopia and effects more men than women (Spencer *et al.*, 1993). Sulfur amino acids deficiency caused by grass pea diet plays an important role in the toxicity of l-β-ODAP by increasing the oxidative stress (Eguchi *et al.*, 2011). Recent research suggests that sulfur amino acids have a protective effect against the toxicity of β- ODAP (Sriram *et al.*, 1998). Milk is an excellent source of well balanced nutrients in which casein represents major protein fraction containing 80% of total milk protein. It was found difficult to produce aortic aneurism in the presence of dietary casein levels above 10% (Bachhuber *et al.*, 1955). A factor associated with the animal protein factor which showed effectiveness against rat lathyrism. According to the University of Maryland Medical Center website, sulfur is an important mineral component in protein-rich foods. The synthesis of protein is dependent on sulfur-containing amino acids in body. Sulfur may aid enzyme reactions, improve cellular respiration, increase blood circulation and promote muscle health. It may also repair the myelin sheath that protects nerves. This study is based on the observation that sulfur amino acid deficiency caused by grass pea diet plays an important role in toxicity of L-beta ODAP. Common sulfur rich food for human is milk, extensively available in both developing and developed countries. Present research is focused on the comparison among cow, buffalo, goat and sheep milk to find out the best one in respect of sulfur richness.

Dataset

The amino acid sequence of alpha S1, alpha S2 casein, beta-casein and kappa casein of four different mammalian species; cow (*Bos taurus*), buffalo (*Bubalis bubalis*), goat (*Capra hircus)* and sheep (*Ovis aries*) were retrieved from Swiss-prot (http://www.expasy.org/sprot) with accession numbers, along with the FASTA format.

Materials and Methods

Physico-chemical properties

The ProtParam tool at Expasy (http://www.expasy.ch/tool/ProtParam) was used to analyze physico-chemical properties i.e. amino acid compositions of four proteins in cow, buffalo, goat and sheep respectively.

Multiple sequence alignment

The clustering of proteins viz. alpha S1, alpha S2, beta and kappa casein based on their sequence similarity were carried out by CLUSTALW at European Bioinformatics Institute (http://www.ebi.ac.uk/tools/clustalw). Boxshade utility was retrieved from Embnet.ch (www.ch.embnet.org/software/BOX_form.htm) and used to shade columns according to their level of conservation.

Table 1. Selected proteins with Accession number.

Swissprot Accession Number	Protein Name	Source
P02662	Alpha S1 casein	Cow
O62823	Alpha S1 casein	Buffalo
P18626	Alpha S1 casein	Goat
P04653	Alpha S1 casein	Sheep
P02663	Alpha S2 casein	Cow
B6VPY2	Alpha S2 casein	Buffalo
P33049	Alpha S2 casein	Goat
E7BQS1	Alpha S2 casein	Sheep
P02666	Beta casein	Cow
Q9TSI0	Beta casein	Buffalo
P33048	Beta casein	Goat
P11839	Beta casein	Sheep
P02668	Kappa casein	Cow
P11840	Kappa casein	Buffalo
Q7YRX5	Kappa casein	Goat
P02669	Kappa casein	Sheep

Results and discussion

Milk contains four casein proteins viz. alpha S1, alpha S2, beta and kappa which are about 80% of total milk protein. The amino acid numbers of these proteins slightly vary from species to species (Fig. 1-4).

Multiple sequence alignment

The four casein proteins were compared in four species with MSA using the software CLUSTALW. Boxshade utility was used to manipulate the MSA where "Black" shade indicates identical amino acids or nucleotides and "Gray" shade indicates similar amino acids. From figures (1-4) of MSA of four casein proteins, it was clear that maximum number of amino acids showed identity in all four species i.e. cow, buffalo, goat and sheep.

ProtParam result

ProtParam results revealed that the amount of Cys and Met in alpha S1 protein were exact the same (3.3%) in all four species. In the case of alpha S2, cow and buffalo have the same amount of sulfur rich amino acids (3.7%) while goat and sheep have 3.5% sulfur rich amino acids each. ProtParam result of beta casein showed that cow has 3.5% sulfur rich amino acids (Cys and Met) while buffalo has 4.0%. Beta casein of goat and sheep contain 3.7% sulfur rich amino acids. Moreover, ProtParam results of Kappa casein showed cow and buffalo have 3.2% Cys and Met while goat and sheep have 2.5% and 3.7% respectively.

Previous studies showed that sulfur rich amino acids have protective action against lathyrism (Eguchi *et al.*, 2011). For that reason, we conducted our study to find available sulfur rich amino acid Sources. According to Harvard University, milk has comparatively more sulfur rich amino acid. In such a case, available sulfur rich sources can be predicted by the primary and secondary structure analysis of different milk proteins. In order to find highest amount of Cys and Met (sulfur rich amino acid), primary structure of four milk proteins were done by ProtParam. Among milk of four species cow milk contains 13.1% sulfur rich amino acid, buffalo 14.2%, goat 13.0% and sheep 14.2% (Table: 2). The secondary structure of these milk proteins revealed that there are few structural differences (Table: 3-6). Both buffalo and sheep milk have the highest amount (14.2%) of Cys and Met which are higher than the cow (13.1%) and goat (13.0%). So, buffalo and sheep milk have greater probability to protect lathyrism than cow and goat milk.

Secondary structure prediction

The secondary structure of Alpha S1 casein, Alpha S2, beta casein and kappa casein of four different species were obtained from ExPASy (bioinf.cs.ucl.ac.uk/psipred/).

Fig. 1. Multiple sequence alignment of Alpha S1 casein. Here, black shade indicates identical amino acids or nucleotides and gray shade indicates similar amino acids.

Fig. 2. Multiple sequence alignment of Alpha S2 casein. Here, black shade indicates identical amino acids or nucleotides and gray shade indicates similar amino acids.

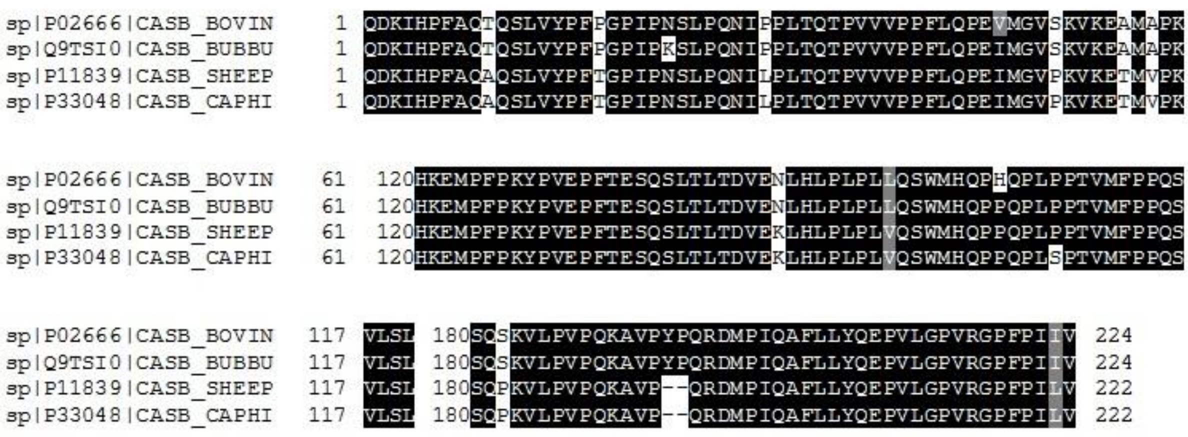

Fig. 3. Multiple sequence alignment of beta casein. Here, black shade indicates identical amino acids or nucleotides and gray shade indicates similar amino acids.

Fig. 4. Multiple sequence alignment of Kappa casein Here, black shade indicates identical amino acids or nucleotides and gray shade indicates similar amino acids.

Table 2. The table shows the amount of different amino acids in four different milk proteins (e.g. alpha S1, alpha S2, beta and kappa casein) of four mammalian species. The amount of sulfur rich amino acids (Cys and Met) showed in bold characters.

	Alpha S1 casein				**Alpha S2 casein**				**Beta casein**				**Kappa casein**			
Amino acids	Cow	Buffalo	Goat	Sheep	Cow	Buffalo	Goat	Sheep	Cow	Buffalo	Goat	Sheep	Cow	Buffalo	Goat	Sheep
Ala (A)	5.6%	5.6%	7.0%	7.0%	5.0%	4.5%	5.4%	5.4%	4.0%	4.0%	3.6%	3.6%	8.4%	7.9%	9.9%	10.9%
Arg (R)	2.8%	2.3%	2.8%	2.8%	2.7%	2.3%	3.1%	3.1%	1.8%	1.3%	1.4%	1.4%	2.6%	2.6%	3.1%	3.1%
Asn (N)	3.7%	3.7%	5.1%	4.7%	6.3%	5.9%	5.8%	5.8%	2.2%	1.8%	1.8%	1.8%	4.2%	5.3%	4.9%	4.7%
Asp (D)	3.3%	3.3%	3.3%	3.3%	1.8%	2.3%	2.2%	2.2%	1.8%	1.8%	1.8%	1.8%	2.1%	1.1%	4.3%	3.6%
Cys (C)	**0.5%**	**0.5%**	**0.5%**	**0.5%**	**1.4%**	**1.4%**	**1.3%**	**1.3%**	**0.4%**	**0.4%**	**0.5%**	**0.5%**	**1.1%**	**1.1%**	**1.9%**	**1.6%**
Gln (Q)	6.5%	7.9%	7.5%	7.0%	7.2%	7.2%	7.2%	7.2%	8.9%	8.9%	9.5%	9.5%	7.9%	7.9%	6.8%	7.8%
Glu (E)	11.7%	10.7%	8.9%	9.3%	10.8%	10.4%	11.2%	11.2%	8.5%	8.5%	8.6%	8.6%	6.3%	6.8%	5.6%	5.7%
Gly (G)	4.2%	4.7%	4.2%	2.2%	0.9%	0.9%	0.9%	0.9%	2.2%	2.2%	2.3%	2.3%	1.6%	1.1%	0.6%	1.0%
His (H)	2.3%	1.9%	1.9%	1.9%	1.4%	2.3%	2.2%	2.2%	2.2%	2.2%	2.3%	2.3%	1.6%	1.6%	2.5%	2.1%
Ile (I)	5.6%	6.1%	4.7%	5.6%	5.4%	5.9%	5.8%	5.4%	4.9%	5.4%	5.0%	4.5%	6.8%	6.8%	6.8%	5.7%

Leu (L)	10.3%	10.7%	10.7%	10.7%	7.2%	6.8%	5.8%	6.3%	12.1%	12.1%	11.7%	12.2%	6.8%	6.8%	4.9%	6.2%
Lys (K)	7.0%	6.1%	6.5%	7.0%	11.3%	10.8%	11.2%	11.2%	5.4%	5.8%	5.9%	5.9%	5.3%	5.3%	4.9%	4.7%
Met (M)	**2.8%**	**2.8%**	**2.8%**	**2.8%**	**2.3%**	**2.3%**	**2.2%**	**2.2%**	**3.1%**	**3.6%**	**3.2%**	**3.2%**	**2.1%**	**2.1%**	**0.6%**	**2.1%**
Phe (F)	3.7%	3.7%	3.3%	3.3%	4.1%	3.6%	4.5%	4.0%	4.0%	4.0%	4.1%	4.1%	3.7%	3.7%	2.5%	3.6%
Pro (P)	7.9%	8.4%	8.4%	7.9%	4.5%	4.5%	5.8%	5.4%	15.6%	16.1%	14.9%	15.3%	11.1%	10.5%	11.7%	10.4%
Ser (S)	7.5%	7.0%	8.4%	8.9%	7.7%	7.7%	6.3%	6.7%	7.1%	7.1%	6.8%	6.3%	7.4%	6.3%	7.4%	6.8%
Thr (T)	2.8%	2.8%	2.8%	1.9%	7.2%	8.1%	6.7%	6.7%	4.0%	3.6%	5.0%	5.0%	8.9%	10.0%	9.3%	7.3%
Trp (W)	0.9%	0.9%	0.9%	0.9%	0.9%	1.4%	1.3%	1.3%	0.4%	0.4%	0.5%	0.5%	0.5%	0.5%	0.6%	0.5%
Tyr (Y)	4.7%	4.7%	5.1%	5.1%	5.4%	5.9%	5.4%	5.4%	1.8%	1.8%	1.4%	1.4%	4.7%	4.7%	5.6%	4.7%
Val (V)	6.1%	6.1%	5.1%	5.1%	6.8%	6.3%	5.4%	5.8%	9.4%	8.9%	10.4%	10.4%	6.8%	7.9%	6.2%	7.3%

Secondary structure analysis

The PSIPRED results of four casein proteins in four species showed changes in secondary structure in different base positions. As cow is the most common milk source, comparison have been made cow to buffalo and cow to sheep. As goat milk contain the lowest amount of sulfur rich amino acids among four species, it has been discarded from secondary structure analysis.

Essential amino acids that are present at insufficient level in grass pea are the limiting factors for the low amino acid score, the sulfur containing amino acids, cysteine and methionine, needed for our protection against oxidative stress (Lambein and Ahmed, 2005). Oxidative stress is involved in neuronal cell death that occurs in the upper motor neurons in neurolathyrism (Rao and Balachandran, 2002). Recently identified protective factors are the addition of onion, ginger or garlic to the preparations, or the addition of one third cereals rich in sulfur amino acids to the grass pea (Getahun and Lambein, 2005). Epidemiological studies have also shown that mixing the food with gravy containing antioxidants or with cereals rich in sulfur amino acids reduces its prevalence significantly (Haimanot and Kidane, 1990; Haque and Hossain., 1996; Getahun and Lambein, 2005).

The rural population depends on the exclusive consumption of large amounts of *L. sativus*. An alternative road for making grasspea based diets more efficient and to prevent lathyrism is the improvement of amino acid balance by the addition of sulfur rich condiments into the diet. Milk is an ideal source of sulfur rich amino acid. Milk serves several physiological functions, including protective (immunoglobulins and other antibacterial agents), digestive aids (enzymes and enzyme inhibitors, binding or carrier proteins) and growth factors/hormones (Fox and McSweeney, 2003). Supplementing methionine to an exclusive raw grass pea seed diet fed to young broiler chicks confirmed that methionine significantly improved the growth and prevented the neurological symptoms compared to the control chicks without methionine addition in the feed (Fikre *et al.*, 2010). In the coastal areas of Bangladesh where fish is also eaten, no case of neurolathyrism is reported, although the consumption grass pea is at similar level to the inland (Ghent University, Belgium, Institute of Plant Biotechnology for Developing Countries).

Table 3. The table shows change in secondary structure of alpha S1 casein in different base positions from cow to buffalo and cow to sheep. Here, hyphen (-) represents the change in secondary structure and blank space means no change. H represents helical structure while C and E represent random coil and extended respectively.

Base change	Change in secondary structure (Cow-Buffalo)	Change in secondary structure (Cow-Sheep)
27	H-C	H-C
28	H-C	
29	H-C	
57		C-H
78		H-C
91	C-H	C-H
95		C-H
152	C-H	C-H
163	H-C	H-C

Table 4. The table shows change in secondary structure of alpha S2 casein in different base positions from cow to buffalo and cow to sheep. Here, hyphen (-) represents the change in secondary structure and blank space means no change. H represents helical structure while C and E represent random coil and extended respectively.

Base change	Change in secondary structure in alpha S2 casein (Cow-Buffalo)	Change in secondary structure in alpha S2 casein (Cow-Sheep)
31		H-C
41		E-C
44		C-E
61	C-H	C-H
62		C-H
65		C-H
66		C-H
67		C-H
68		C-H
69		C-H
70		C-H
76		H-C
77		H-C
78		H-C
79	H-C	H-C
80	H-C	H-C
81		H-C
82		H-C
83		H-C
115	C-H	C-H
116		C-H
145		H-C
149		C-H
159	H-C	
164		C-H
165		C-H
188		C-H
189		C-H
191		H-C
201	C-H	C-H
202	C-H	C-H
203	C-H	C-H
204		C-H
205		C-H

Table 5. The table shows change in secondary structure of beta casein in different base positions from cow to buffalo and cow to sheep. Here, hyphen (-) represents the change in secondary structure and blank space means no change. H represents helical structure while C and E represent random coil and extended respectively.

Base change	Change in secondary structure in beta casein (Cow-Buffalo)	Change in secondary structure in beta casein (Cow-Sheep)
27		H-C
56	C-H	

Table 6. The table shows change in secondary structure of kappa casein in different base positions from cow to buffalo and cow to sheep. Here, hyphen (-) represents the change in secondary structure and blank space means no change. H represents helical structure while C and E represent random coil and extended respectively.

Base change	Change in secondary structure in kappa casein (Cow-Buffalo)	Change in secondary structure in kappa casein (Cow-Sheep)
147		C-E
162	C-H	C-E
163		C-E
167		C-E
173	C-E	
182		E-C

Therefore it is important to get concerned authorities' attention to lathyrism and public awareness are needed to deal with storages of food richer in sulfur containing amino acids. Increasing the content of sulfur rich amino acid to improve the nutritional quality of grass pea based diets may ultimately be the cheapest solution for the people surviving on *L. sativus* where other crops are less productive or expensive.

Conclusion

Since the buffalo and sheep milk have the highest amount of sulfur rich amino acids both are suggested to use as supplementary diet for those who take *L. sativus* (Khesary dal) extensively. As cow and goat also have similar amount of sulfur rich amino acids and are more available as milk source, can be also be used for reducing the effect of lathyrism.

References

Bachhuber TE, Lalich JJ, Angevine DM, Schilling ED, StrongFM. 1955. Lathyrus factor activity of aminopropionitrile and related compounds. Proceedings of the Society for Experimental Biology and Medicine **89**, 224

Chowdhury SD , Sultana Z, Ahammed M, Chowdhury BL, Das SC, Roy. 2005. The Nutritional Value of Khesari (Lathyrus sativus) for Growing and Laying Pullets.The Jorurnal of Poultry Science **42(4)**, 308-320.

Eguchi KK, Yoshino N, Minoura A, Watanabe K, Kusama T, Lambein F, Ikegam F. 2011. Sulfur amino acids deficiency caused by grass pea diet plays an important role in the toxicity of l-β-ODAP by increasing the oxidative stress:Studies on a motor neuron cell line. Food and Chemical Toxicology **49(3)**. 636–643.

Fikre A, Yami A, Kuo YH, Ahmed S, Gheysen G, Lambein F. 2010. Effect of methionine supplement on physical responses and neurological symptoms in broiler chicks fed grass pea (Lathyrus sativus)-based starter ratio.Food and Chemical Toxicology **48**, 11–17.

Fox PF, McSweeney PLH. 1998. Milk proteins. In: Dairy chemis. and biochemistry. Blackie Academic & Professional, London, 146.

Getahun H, Lambein F. 2005. Neurolathyrism risk depends on type of grass pea preparation and on mixing with cereals and antioxidants.Tropical Medicine and International Health **10**, 169-178.

Haimanot RT, Kidane Y. 1990. Lathyrism in rural northwestern Ethiopia: a highly prevalent neurotoxic disorder. International Journal of Epidemiology. **19(3)**, 664-72.

Haque A, Hossain M. 1996. Epidemiological study of lathyrism in northwestern districts of Bangladesh.Neuroepidemiology **15(2)**, 83-91.

Lambein F and Ahmed S. 2005. The same goal, a different approach: a new Belgian-Ethiopian project. Lathyrus Lathyrism Newsletter.

Rao AV, Balachandran B. 2002. Role of oxidative stress and antioxidants in neurodegenerative diseases. Nutritional Neuroscience **5**, 291-309.

Shinomol, G. K. and Muralidhara. 2007. Differential induction of oxidative impairments in brain regions of male mice following subchronic consumption of Khesari dhal (Lathyrus sativus) and detoxified Khesari dhal.Neurotoxicology **18(4)**, 798-806.

Spencer PS, Ludolph AC, Kisby GE. 1993. Neurologic diseases associated with use of plant components with toxic potential. Environmental Research **62(1)**, 106–113.

Spencer PS, Schaumburg HH. 1983. Lathyrism: a neurotoxic disease. Neurobehavioral Toxicology and Teratology **5(6).** 625-9.

Sriram K, Shankar SK, Boyd MR, Ravindranath V. 1998. Thiol Oxidation and Loss of Mitochondrial Complex I Precede Excitatory Amino Acid-Mediated Neurodegeneration.The Journal of Neuroscience **18 (24)**.. 10287–10296.