in-silico analysis of essential amino acids of Alpha-lactalbumin in different species and its association with regulatory proteins for the normal cellular metabolism for growing newborn.

[1*]Vivek Singh, [1]Ranjana Singh[1], [2]Abbas Ali Mahdi.
[1]Department of Biochemistry, King George's Medical University, Lucknow, UP, India.
[2]Department of Biochemistry, Era Medical University, Lucknow, UP, India.

Abstract

Alpha-lactalbumin consists of all the essential and nonessential amino acids, which play a dramatic role in developing newborn baby and be strong enough to fight any kind of the diseases by enhancing the immunity from neonatal baby to 6 years old child. It is a one of the crucial protein in the milk that participates in lactose synthesis and facilitates in milk production. However, the conscientious role of alpha-lactalbumin in interaction with different regulatory proteins and comparative nutritional value in different species are not much known.

Here we showed that the percentage of essential amino acids composition in different species along with their interaction with regulatory proteins B4GALT, GLB1, GALE, LCT, GALT, FUT, NOTCH. We found the percentage of essential amino acids composition of alpha-lactalbumin by in-silico (prot-param) study in Human-50%, Bovine49.2%, pig-50.3%, Sheep-49.2%, Dog-48.6%, Horse-46.5, Rat-42.7%, Rabbit-50.3%, Yellow baboon-43.8% and edge confidence of regulatory protein interaction with alpha-lactalbumin are B4GALT1-0.996, GLB10.916, LCT-0.903, GALE-0.903, GALT-0.900 by String (a tool to predict the protein-protein interactions). Our result demonstrates the percentage composition of essential amino acids is higher in Pig and Rabbit in compare to other species in nutritional perspective while the edge confidence strongly inferred that alpha-lactalbumin regulate the process of cellular biosynthesis process, protein glycosylation and UDP catalytic activity etc. Furthermore the result of string also indicates that regulation of fut chaperone by alpha-lactalbumin, which is known for the post-translational modification (fucosylation) of Notch receptor protein.

Introduction-

Alpha-Lactalbumin is a milk protein which constitutes 2-5% of the total protein present in milk, it forms the regulatory subunit of the lactose synthase (LS) heterodimer and β 1,4 galactosyltransferase (β4 GALT1) forms the catalytic unit both of this protein facilitate to produce lactose by transferring galactose moieties to glucose. Regulatory subunit of lactose synthase, change the substrate specificity of galactosyltransferase in the mammary gland

making glucose a good acceptor substrate for the enzyme (UniProtKB B-P00709). Alpha-Lactalbumin protein is made up of approximately 142 amino acid sequences and its molecular mass is about 16,225 Da (Dalton). Amino acid sequences of alpha-Lactalbumin contain some essential amino acids that cannot be synthesized in our body and thus it must be supplied in our diet and also binds with divalent cations (Ca2+, Zn2+) and may facilitate the absorption of essential minerals. However, the fragments form during proteolysis of alpha-lactalbumin that has shown antibacterial and immunostimulatory properties, which is possibly helping in protection against wide range of bacterial infections (Nicoleta et al. 2010). Total numbers of essential amino acid are phenylalanine, Histidine, leucine, isoleucine, threonine, valine, tryptophan, lysine and methionine. Deficiency of these amino acids in our body may show many health-related problems like neurological disorder, failure of kidney, skin and hair related health problems (Dietary ref from WHO). Phenylalanine is an essential amino acid, which is converted into L-tyrosine that is used for the biosynthesis of Dopamine and norepinephrine. Both are vital neurotransmitter molecule. Its deficiency may cause the severe mental disorder. Methionine is also helping in the formation of new blood vessels, it is also involved in many detoxifying processes. Daily intake of this amino acid may be benefitted from that suffers from Parkinson's disease, allergies and depression. Methionine is sulphur containing amino acid and it improves the skin tone, hair and strengthens nails (wood et al. 2009). L-methionine is sometimes given to the dog as supplements to minimizing the chance of stone in dogs. Other essential amino acids like valine, leucine, isoleucine, threonine, tryptophan, lysine and histidine are also compulsory for proper fat metabolism, muscle growth, immunity, gastric secretion and sexual function respectively. Histidine is considered to be an indispensable amino acid because of the detrimental effects on haemoglobin concentration that have been observed when individuals are feed with histidine free diet (Kriengsinyos et al. 2006) Thus alpha-Lactalbumin (a milk protein) may play an effective role in blood sugar metabolism & growth regulation (PubChem).

Some polypeptides are formed by proteolytic digestion of alpha-lactalbumin by trypsin and chymotrypsin shows the bactericidal properties (Lopez et al. 2006). At physiological PH of bovine and human milk, lactalbumin associates with lysozyme and this complex exhibit a higher antibacterial activity against both gram-positive and gram-negative bacteria. It was also suppressive effect against the increased release of proinflammatory cytokines such as IL-1, IL-6 and tumour necrosis factor- alpha (Yamaguchi et al. 2007).

Material and method-

The material was used for this study fully dependent on computational biology in which we used a crunch of bioinformatics software like Expasy, uniprot/swisport, protparam, clustal Omega, etc.

- Expasy is the SIB bioinformatics resource portal which provides the acess to scientific databases and software tools in different areas of biological science including proteomics, genomics, phylogeny, system biology, population genetics, transcriptomics etc.
- The Uniprot knowledgebase is the central hub for the collection of functional information on protein with accurate, consistent and rich annotation. It consists of Uniprot/swiss-Prot (manually-annotated records and curator-evaluated computational analysis) and UniProtKB/TrEMBL (computationally analysed records awaiting manual annotation).
- ProtParam is a tool which allows the computation of various physical and chemical parameters for a given protein stored in Swiss-prot or TrEMBL or for a user entered protein sequence. The computed parametres include molecular weight, theoretical pI, amino acid composition, atomic composition, extinnction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydrophobicity (GRAVY).
- Clustal Omega is a new multiple sequence alignment program that uses seeded guide trees and HMM profile-profile techniques to generate alignments between three or more sequences.
- String- Interactions network database showing the experimental interactions and functional interactions such as predictions, co-occurance and data mining etc.

We had taken alpha-lactalbumin amino acids sequences of Human, Bovine, Goat, Pig, Sheep, Horse, Rat, Rabbit, yellow baboon from database of uniprot;

Entry	Entry Name	Protein name	Orgnasim	Gene Name
P00709	LALBA_HUMAN	Alpha-lactalbumin	Homo sapiens (Human)	LALBA LYZL7
P00711	LALBA_BOVIN	Alpha-lactalbumin	Bos taurus (Bovine)	LALBA ALACTA
P00712	LALBA_CAPHI	Alpha-lactalbumin	Capra hircus (Goat)	LALBA
P18137	LALBA_PIG	Alpha-lactalbumin	Sus scrofa (Pig)	LALBA
P09462	LALBA_SHEEP	Alpha-lactalbumin	Ovis aries (Sheep)	LALBA
P08896	LALBA2_HORSE	Alpha-lactalbumin B/C	Equus caballus (Horse)	
P00714	LALBA_RAT	Alpha-lactalbumin	Rattus norvegicus (Rat)	lalba

P00716	LALBA_RABBIT	Alpha-lactalbumin	Orytolagus cuniculus (Rabbit)	LALBA
P12065	LALBA_PAPCY	Alpha-lactalbumin	Papio cynocephalus (Yellow baboon)	LALBA
Q9N2G9	LALBA_CANLF	Alpha-lactalbumin	Canis lupus Familiaris (Dog)	LALBA

Sequence of amino acids-

Human-

>sp|P00709|LALBA_HUMAN Alpha-lactalbumin OS=Homo sapiens GN=LALBA PE=1 SV=1
MRFFVPLFLVGILFPAILAKQFTKCELSQLLKDIDGYGGIALPELICTMFHTSGYDTQ
AIVENNESTEYGLFQISNKLWCKSSQVPQSRNICDISCDKFLDDDITDDIMCAKKILDI
KGIDYWLAHKALCTEKLEQWLCEKL

Bovine-

>sp|P00711|LALBA_BOVIN Alpha-lactalbumin OS=Bos taurus GN=LALBA PE=1 SV=2
MMSFVSLLLVGILFHATQAEQLTKCEVFRELKDLKGYGGVSLPEWVCTTFHTSGYD
TQAIVQNNDSTEYGLFQINNKIWCKDDQNPHSSNICNISCDKFLDDDLTDDIMCVKK
ILDKVGINYWLAHKALCSEKLDQWLCEKL

Goat-

>sp|P00712|LALBA_CAPHI Alpha-lactalbumin OS=Capra hircus GN=LALBA PE=1 SV=1
MMSFVSLLLVGILFHATQAEQLTKCEVFQKLKDLKDYGGVSLPEWVCTAFHTSGY
DTQAIVQNNDSTEYGLFQINNKIWCKDDQNPHSRNICNISCDKFLDDDLTDDIVCAK
KILDKVGINYWLAHKALCSEKLDQWLCEKL

Pig-

>sp|P18137|LALBA_PIG Alpha-lactalbumin OS=Sus scrofa GN=LALBA PE=1 SV=2
MMSFVSLLVVGILFPAIQAKQFTKCELSQVLKDMDGYGDITLPEWICTIFHISGYDTK
TIVHDNGSTEYGLFQINNKLWCRDNQIQSKNICGISCDKFLDDDLTDDMMCAKKILD
NEGIDYWLAHKALCSEKLDQWLCEKM

Sheep-

>sp|P09462|LALBA_SHEEP Alpha-lactalbumin OS=Ovis aries GN=LALBA PE=1 SV=2
MMSFVSLLLVGILFHATQAEQLTKCEVFQELKDLKDYGGVSLPEWVCTAFHTSGYD
TQAIVQNNDSTEYGLFQINNKIWCKDDQNPHSRNICNISCDKFLDDDLTDDIMCVKK
ILDKVGINYWLAHKALCSEKLDQWLCEKL

Horse-

>sp|P08896|LALB2_HORSE Alpha-lactalbumin B/C OS=Equus caballus PE=1 SV=1
KQFTKCQLSQVLKSMDGYKGVTLPEWICTIFHNSGYDTQTIVKNNGKTEYGLFEINN
KMWCRDNQILPSRNICGISCNKFLDDDLTDDVMCAKKDLDSEGIDYWLAHKPLCSE
KLEQWLCEEL

Rat-

>sp|P00714|LALBA_RAT Alpha-lactalbumin OS=Rattus norvegicus GN=Lalba PE=1 SV=1
MMRFVPLFLACISLPAFQATEFTKCEVSHAIEDMDGYQGISLLEWTCVLFHTSGYDS
QAIVKNNGSTEYGLFQISNRNWCKSSEFPESENICDISCDKFLDDELADDIVCAKKIV
AIKGIDYWKAHKPMCSEKLEQWRCEKPGAPALVVPALNSETPVP

Rabbit-

>sp|P00716|LALBA_RABIT Alpha-lactalbumin OS=Oryctolagus cuniculus GN=LALBA PE=1 SV=2
MMPLVPLLLVSIVFPGIQATQLTRCELTEKLKELDGYRDISMSEWICTLFHTSGLDTK
ITVNNNGSTEYGIFQISDKLWCVSKQNPQSKNICDTPCENFLDDNLTDDVKCAMKIL
DKEGIDHWLAHKPLCSENLEQWVCKK

Yellow baboon-

>sp|P12065|LALBA_PAPCY Alpha-lactalbumin OS=Papio cynocephalus GN=LALBA PE=1 SV=1
KQFTKCELSQNLYDIDGYGRIALPELICTMFHTSGYDTQAIVENNESTEYGLFQISNA
LWCKSSQSPQSRNICDITCDKFLDDDITDDIMCAKKILDIKGIDYWIAHKALCTEKLE
QWLCEKE

Dog-

>sp|Q9N2G9|LALBA_CANLF Alpha-lactalbumin OS=Canis lupus familiaris GN=LALBA PE=2 SV=1
MMSFVSLLLVSILFPAIQAKQFTKCELPQVLKDMDGFGGIALPEWICTIFHTSGYDTQ
TIVNNNGGTDYGLFQISNKFWCKDDQNLQSRNICDISCDKFLDDDLTDDMICAKKIL
DKEGIDYWLAHKPLCSEKLEQWRCEKL

Result-

After taking all the sequences of alpha-lactalbumin from UniProtKB database we had estimated the percentage composition of the essential amino acid in alpha-lactalbumin by using prot-param tools and side-by-side we had evaluated the percentage of identity and relationship of amino acids sequence of alpha-lactalbumin of different species by using Clustal-omega and interaction with other important regulatory proteins.

Data from prot-param-

Human:

Number of amino acids: 142 **Molecular weight:** 16224.90 **Theoretical pI:** 4.83

Amino acid composition:

Ala (A) 7 4.9% Arg (R) 2 1.4% Asn (N) 4 2.8% Asp (D) 12 8.5% Cys (C) 8 5.6% Gln (Q) 7 4.9% Glu (E) 8 5.6% Gly (G) 7 4.9% His (H) 2 1.4% Ile (I) 14 9.9% Leu (L) 18 12.7% Lys (K) 12 8.5% Met (M) 3 2.1% Phe (F) 8 5.6% Pro (P) 4 2.8% Ser (S) 8 5.6% Thr (T) 7 4.9% Trp (W) 3 2.1% Tyr (Y) 4 2.8% Val (V) 4 2.8% Pyl (O) 0 0.0% Sec (U) 0 0.0% (B) 0 0.0% (Z) 0 0.0% (X) 0 0.0%

Total number of negatively charged residues (Asp + Glu): 20 **Total number of positively charged residues (Arg + Lys):** 14 **Atomic composition:**

Carbon C 734 Hydrogen H 1146 Nitrogen N 178 Oxygen O 213 Sulfur S 11
Formula: $C_{734}H_{1146}N_{178}O_{213}S_{11}$ **Total number of atoms:** 2282

Bovine;
Number of amino acids: 142 **Molecular weight:** 16246.61 **Theoretical pI:** 4.92
Amino acid composition:
Ala (A) 5 3.5% Arg (R) 1 0.7% Asn (N) 8 5.6% Asp (D) 13 9.2% Cys (C) 8 5.6% Gln (Q) 7 4.9% Glu (E) 7 4.9% Gly (G) 7 4.9% His (H) 4 2.8% Ile (I) 9 6.3% Leu (L) 17 12.0% Lys (K) 12 8.5% Met (M) 3 2.1% Phe (F) 6 4.2% Pro (P) 2 1.4% Ser (S) 9 6.3% Thr (T) 8 5.6% Trp (W) 4 2.8% Tyr (Y) 4 2.8% Val (V) 8 5.6% Pyl (O) 0 0.0% Sec (U) 0 0.0% (B) 0 0.0% (Z) 0 0.0% (X) 0 0.0%
Total number of negatively charged residues (Asp + Glu): 20 **Total number of positively charged residues (Arg + Lys):** 13 **Atomic composition:**
Carbon C 723 Hydrogen H 1120 Nitrogen N 184 Oxygen O 219 Sulfur S 11 **Formula:** $C_{723}H_{1120}N_{184}O_{219}S_{11}$ **Total number of atoms:** 2257

Goat;
Number of amino acids: 142 **Molecular weight:** 16254.61 **Theoretical pI:** 5.06
Amino acid composition:
Ala (A) 7 4.9% Arg (R) 1 0.7% Asn (N) 8 5.6% Asp (D) 14 9.9% Cys (C) 8 5.6% Gln (Q) 8 5.6% Glu (E) 6 4.2% Gly (G) 6 4.2% His (H) 4 2.8% Ile (I) 9 6.3% Leu (L) 17 12.0% Lys (K) 13 9.2% Met (M) 2 1.4% Phe (F) 6 4.2% Pro (P) 2 1.4% Ser (S) 8 5.6% Thr (T) 7 4.9% Trp (W) 4 2.8% Tyr (Y) 4 2.8% Val (V) 8 5.6% Pyl (O) 0 0.0% Sec (U) 0 0.0% (B) 0 0.0% (Z) 0 0.0% (X) 0 0.0%
Total number of negatively charged residues (Asp + Glu): 20 **Total number of positively charged residues (Arg + Lys):** 14 **Atomic composition:**
Carbon C 725 Hydrogen H 1124 Nitrogen N 186 Oxygen O 218 Sulfur S 10 **Formula:** $C_{725}H_{1124}N_{186}O_{218}S_{10}$ **Total number of atoms:** 2263

Pig;
Number of amino acids: 141 **Molecular weight:** 16173.69 **Theoretical pI:** 4.68
Amino acid composition:
Ala (A) 5 3.5% Arg (R) 1 0.7% Asn (N) 6 4.3% Asp (D) 15 10.6% Cys (C) 8 5.7% Gln (Q) 7 5.0% Glu (E) 6 4.3% Gly (G) 8 5.7% His (H) 3 2.1% Ile (I) 13 9.2% Leu (L) 15 10.6% Lys (K) 12 8.5% Met (M) 6 4.3% Phe (F) 6 4.3% Pro (P) 2 1.4% Ser (S) 8 5.7% Thr (T) 7 5.0% Trp (W) 4 2.8% Tyr (Y) 4 2.8% Val (V) 5 3.5% Pyl (O) 0 0.0% Sec (U) 0 0.0% (B) 0 0.0% (Z) 0 0.0% (X) 0 0.0%
Total number of negatively charged residues (Asp + Glu): 21 **Total number of positively charged residues (Arg + Lys):** 13 **Atomic composition:**
Carbon C 719 Hydrogen H 1117 Nitrogen N 179 Oxygen O 216 Sulfur S 14 **Formula:** $C_{719}H_{1117}N_{179}O_{216}S_{14}$ **Total number of atoms:** 2245

Sheep;
Number of amino acids: 142 **Molecular weight:** 16315.67 **Theoretical pI:** 4.81
Amino acid composition:
Ala (A) 6 4.2% Arg (R) 1 0.7% Asn (N) 8 5.6% Asp (D) 14 9.9% Cys (C) 8 5.6% Gln (Q) 8 5.6% Glu (E) 7 4.9% Gly (G) 6 4.2% His (H) 4 2.8% Ile (I) 9 6.3% Leu (L) 17 12.0% Lys (K) 12 8.5% Met (M) 3 2.1% Phe (F) 6 4.2% Pro (P) 2 1.4% Ser (S) 8 5.6% Thr (T) 7 4.9% Trp (W) 4 2.8% Tyr (Y) 4 2.8% Val (V) 8 5.6% Pyl (O) 0 0.0% Sec (U) 0 0.0% (B) 0 0.0% (Z) 0 0.0% (X) 0 0.0%
Total number of negatively charged residues (Asp + Glu): 21 **Total number of positively charged residues (Arg + Lys):** 13 **Atomic composition:** Carbon C 726 Hydrogen H 1123 Nitrogen N 185 Oxygen O 220 Sulfur S 11 **Formula:** $C_{726}H_{1123}N_{185}O_{220}S_{11}$ **Total number of atoms:** 2265

Horse;
Number of amino acids: 123 **Molecular weight:** 14251.26 **Theoretical pI:** 4.95
Amino acid composition:
Ala (A) 2 1.6% Arg (R) 2 1.6% Asn (N) 8 6.5% Asp (D) 11 8.9% Cys (C) 8 6.5% Gln (Q) 6 4.9% Glu (E) 8 6.5% Gly (G) 7 5.7% His (H) 2 1.6% Ile (I) 8 6.5% Leu (L) 13 10.6% Lys (K) 12 9.8% Met (M) 3 2.4% Phe (F) 4 3.3% Pro (P) 3 2.4% Ser (S) 7 5.7% Thr (T) 7 5.7% Trp (W) 4 3.3% Tyr (Y) 4 3.3% Val (V) 4 3.3% Pyl (O) 0 0.0% Sec (U) 0 0.0% (B) 0 0.0% (Z) 0 0.0% (X) 0 0.0%
Total number of negatively charged residues (Asp + Glu): 19 **Total number of positively charged residues (Arg + Lys):** 14 **Atomic composition:** Carbon C 627 Hydrogen H 973 Nitrogen N 163 Oxygen O 194 Sulfur S 11 **Formula:** $C_{627}H_{973}N_{163}O_{194}S_{11}$ **Total number of atoms:** 1968

Rat;
Number of amino acids: 159 **Molecular weight:** 17850.44 **Theoretical pI:** 4.74
Amino acid composition:
Ala (A) 12 7.5% Arg (R) 3 1.9% Asn (N) 6 3.8% Asp (D) 10 6.3% Cys (C) 9 5.7% Gln (Q) 5 3.1% Glu (E) 13 8.2% Gly (G) 7 4.4% His (H) 3 1.9% Ile (I) 11 6.9% Leu (L) 12 7.5% Lys (K) 11 6.9% Met (M) 4 2.5% Phe (F) 8 5.0% Pro (P) 9 5.7% Ser (S) 13 8.2% Thr (T) 6 3.8% Trp (W) 4 2.5% Tyr (Y) 4 2.5% Val (V) 9 5.7% Pyl (O) 0 0.0% Sec (U) 0 0.0% (B) 0 0.0% (Z) 0 0.0% (X) 0 0.0%
Total number of negatively charged residues (Asp + Glu): 23 **Total number of positively charged residues (Arg + Lys):** 14 **Atomic composition:** Carbon C 796 Hydrogen H 1222 Nitrogen N 200 Oxygen O 240 Sulfur S 13 **Formula:** $C_{796}H_{1222}N_{200}O_{240}S_{13}$ **Total number of atoms:** 2471

Rabbit;
Number of amino acids: 141 **Molecular weight:** 16081.56 **Theoretical pI:** 4.97

Amino acid composition:
Ala (A) 3 2.1% Arg (R) 2 1.4% Asn (N) 8 5.7% Asp (D) 11 7.8% Cys (C) 8 5.7% Gln (Q) 6 4.3% Glu (E) 9 6.4% Gly (G) 6 4.3% His (H) 3 2.1% Ile (I) 10 7.1% Leu (L) 17 12.1% Lys (K) 12 8.5% Met (M) 4 2.8% Phe (F) 4 2.8% Pro (P) 6 4.3% Ser (S) 9 6.4% Thr (T) 10 7.1% Trp (W) 4 2.8% Tyr (Y) 2 1.4% Val (V) 7 5.0% Pyl (O) 0 0.0% Sec (U) 0 0.0% (B) 0 0.0% (Z) 0 0.0% (X) 0 0.0%

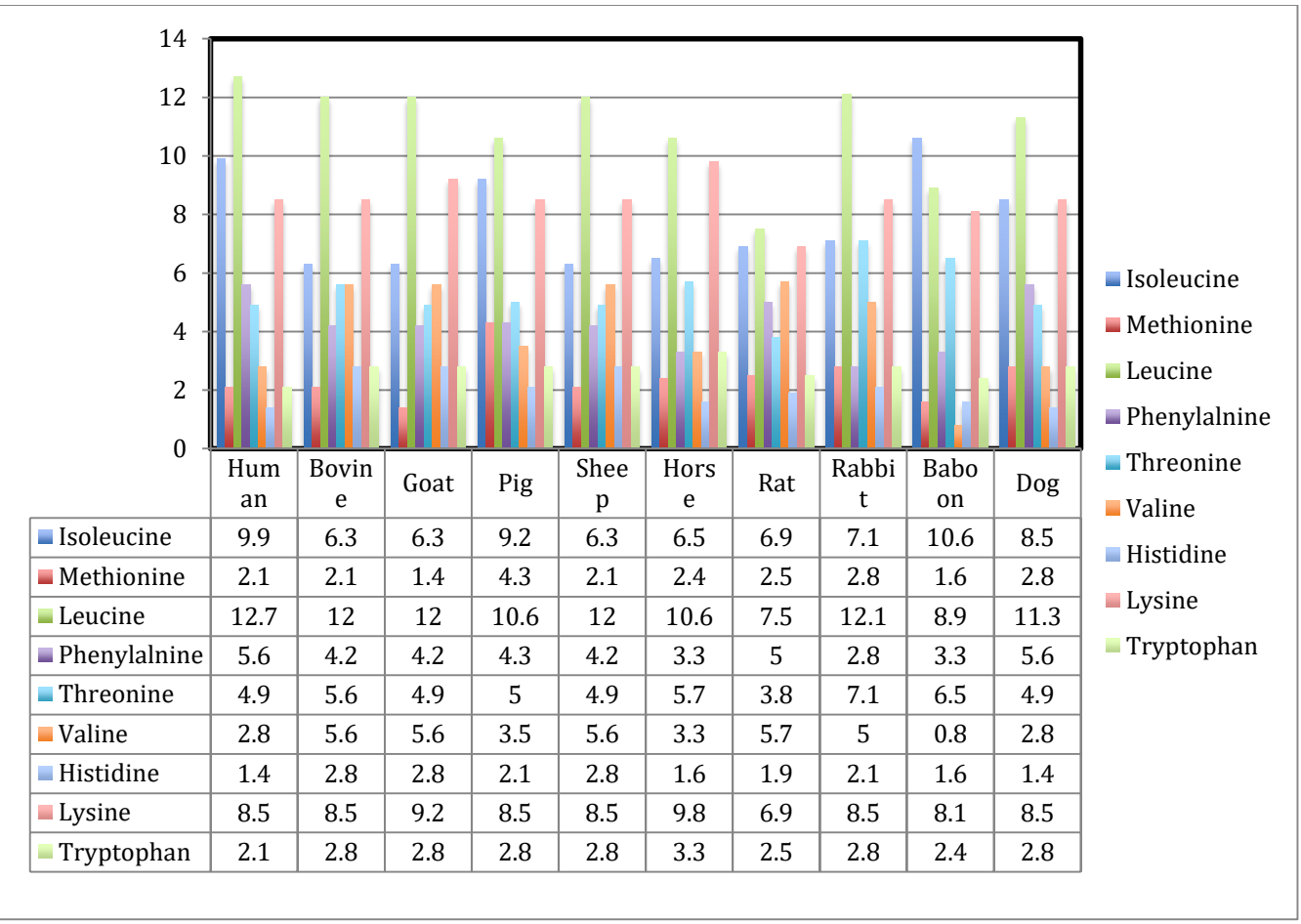
Total number of negatively charged residues (Asp + Glu): 20 **Total number of positively charged residues (Arg + Lys):** 14 **Atomic composition:** Carbon C 710 Hydrogen H 1125 Nitrogen N 183 Oxygen O 217 Sulfur S 12 **Formula:** $C_{710}H_{1125}N_{183}O_{217}S_{12}$ **Total number of atoms:** 2247

Baboon;
Number of amino acids: 123 **Molecular weight:** 14174.06 **Theoretical pI:** 4.54
Amino acid composition:
Ala (A) 6 4.9% Arg (R) 2 1.6% Asn (N) 5 4.1% Asp (D) 12 9.8% Cys (C) 8 6.5% Gln (Q) 7 5.7% Glu (E) 9 7.3% Gly (G) 5 4.1% His (H) 2 1.6% Ile (I) 13 10.6% Leu (L) 11 8.9% Lys (K) 10 8.1% Met (M) 2 1.6% Phe (F) 4 3.3% Pro (P) 2 1.6% Ser (S) 8 6.5% Thr (T) 8 6.5% Trp (W) 3 2.4% Tyr (Y) 5 4.1% Val (V) 1 0.8% Pyl (O) 0 0.0% Sec (U) 0 0.0% (B) 0 0.0% (Z) 0 0.0% (X) 0 0.0%

Total number of negatively charged residues (Asp + Glu): 21 **Total number of positively charged residues (Arg + Lys):** 12 **Atomic composition:** Carbon C 623 Hydrogen H 966 Nitrogen N 158 Oxygen O 199 Sulfur S 10 **Formula:** $C_{623}H_{966}N_{158}O_{199}S_{10}$ **Total number of atoms:** 1956

Dog;
Number of amino acids: 142 **Molecular weight:** 16284.76 **Theoretical pI:** 4.68
Amino acid composition:
Ala (A) 5 3.5% Arg (R) 2 1.4% Asn (N) 6 4.2% Asp (D) 15 10.6% Cys (C) 8 5.6% Gln (Q) 8 5.6% Glu (E) 6 4.2% Gly (G) 8 5.6% His (H) 2 1.4% Ile (I) 12 8.5% Leu (L) 16 11.3% Lys (K) 12 8.5% Met (M) 4 2.8% Phe (F) 8 5.6% Pro (P) 4 2.8% Ser (S) 8 5.6% Thr (T) 7 4.9% Trp (W) 4 2.8% Tyr (Y) 3 2.1% Val (V) 4 2.8% Pyl (O) 0 0.0% Sec (U) 0 0.0% (B) 0 0.0% (Z) 0 0.0% (X) 0 0.0%

Total number of negatively charged residues (Asp + Glu): 21 **Total number of positively charged residues (Arg + Lys):** 14 **Atomic composition:** Carbon C 728 Hydrogen H 1126 Nitrogen N 182 Oxygen O 217 Sulfur S 12 **Formula:** $C_{728}H_{1126}N_{182}O_{217}S_{12}$ **Total number of atoms:** 2265



	Human	Bovine	Goat	Pig	Sheep	Horse	Rat	Rabbit	Baboon	Dog
Isoleucine	9.9	6.3	6.3	9.2	6.3	6.5	6.9	7.1	10.6	8.5
Methionine	2.1	2.1	1.4	4.3	2.1	2.4	2.5	2.8	1.6	2.8
Leucine	12.7	12	12	10.6	12	10.6	7.5	12.1	8.9	11.3
Phenylalnine	5.6	4.2	4.2	4.3	4.2	3.3	5	2.8	3.3	5.6
Threonine	4.9	5.6	4.9	5	4.9	5.7	3.8	7.1	6.5	4.9
Valine	2.8	5.6	5.6	3.5	5.6	3.3	5.7	5	0.8	2.8
Histidine	1.4	2.8	2.8	2.1	2.8	1.6	1.9	2.1	1.6	1.4
Lysine	8.5	8.5	9.2	8.5	8.5	9.8	6.9	8.5	8.1	8.5
Tryptophan	2.1	2.8	2.8	2.8	2.8	3.3	2.5	2.8	2.4	2.8

Bar diagram showed here the composition of essential amino acids (in percent composition) in different species

S.No.	Species	% of essential amino acid composition in alpha-lactalbumin protein.
1.	Human	50%
2.	Bovine	49.2%
3.	Pig	50.3%
4.	Sheep	49.2%
5.	Horse	46.5%
6.	Rat	42.7%
7.	Rabbit	50.3%
8.	Yellow Baboon	43.8%
9.	Dog	48.6%

Chart 1. Showed the total percentage of essential amino acids composition in alpha-lactalbumin of different species.

Data from Clustal Omega-

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CLUSTAL O(1.2.4) multiple sequence alignment

sp|P00716|LALBA_RABIT      MMPLVPLLLVSIVFPGIQATQLTRCELTEKLKELDGYRDISMSEWICTLFHTSGLDTKIT
sp|P00714|LALBA_RAT        MMRFVPLFLACISLPAFQATEFTKCEVSHAIEDMDGYQGISLLEWTCVLFHTSGYDSQAI
sp|P00711|LALBA_BOVIN      MMSFVSLLLVGILFHATQAEQLTKCEVFRELKDLKGYGGVSLPEWVCTTFHTSGYDTQAI
sp|P00712|LALBA_CAPHI      MMSFVSLLLVGILFHATQAEQLTKCEVFQKLKDLKDYGGVSLPEWVCTAFHTSGYDTQAI
sp|P09462|LALBA_SHEEP      MMSFVSLLLVGILFHATQAEQLTKCEVFQELKDLKDYGGVSLPEWVCTAFHTSGYDTQAI
sp|P00709|LALBA_HUMAN      MRFFVPLFLVGILFPAILAKQFTKCELSQLLKDIDGYGGIALPELICTMFHTSGYDTQAI
sp|P12065|LALBA_PAPCY      ------------------KQFTKCELSQNLYDIDGYGRIALPELICTMFHTSGYDTQAI
sp|P18137|LALBA_PIG        MMSFVSLLVVGILFPAIQAKQFTKCELSQVLKDMDGYGDITLPEWICTIFHISGYDTKTI
sp|Q9N2G9|LALBA_CANLF      MMSFVSLLLVSILFPAIQAKQFTKCELPQVLKDMDGFGGIALPEWICTIFHTSGYDTQTI
sp|P08896|LALB2_HORSE      ------------------KQFTKCQLSQVLKSMDGYKGVTLPEWICTIFHNSGYDTQTI
                                             ::*:*:: . : .:..:  ::: *  *. ** ** *::

sp|P00716|LALBA_RABIT      VNNNGSTEYGIFQISDKLWCVSKQNPQSKNICDTPCENFLDDNLTDDVKCAMKILDKEGI
sp|P00714|LALBA_RAT        VKNNGSTEYGLFQISNRNWCKSSEFPESENICDISCDKFLDDELADDIVCAKKIVAIKGI
sp|P00711|LALBA_BOVIN      VQNNDSTEYGLFQINNKIWCKDDQNPHSSNICNISCDKFLDDDLTDDIMCVKKILDKVGI
sp|P00712|LALBA_CAPHI      VQNNDSTEYGLFQINNKIWCKDDQNPHSRNICNISCDKFLDDDLTDDIVCAKKILDKVGI
sp|P09462|LALBA_SHEEP      VQNNDSTEYGLFQINNKIWCKDDQNPHSRNICNISCDKFLDDDLTDDIMCVKKILDKVGI
sp|P00709|LALBA_HUMAN      VENNESTEYGLFQISNKLWCKSSQVPQSRNICDISCDKFLDDDITDDIMCAKKILDIKGI
sp|P12065|LALBA_PAPCY      VENNESTEYGLFQISNALWCKSSQSPQSRNICDITCDKFLDDDITDDIMCAKKILDIKGI
sp|P18137|LALBA_PIG        VHDNGSTEYGLFQINNKLWCRDNQ-IQSKNICGISCDKFLDDDLTDDMMCAKKILDNEGI
sp|Q9N2G9|LALBA_CANLF      VNNNGGTDYGLFQISNKFWCKDDQNLQSRNICDISCDKFLDDDLTDDMICAKKILDKEGI
sp|P08896|LALB2_HORSE      VKNNGKTEYGLFEINNKMWCRDNQILPSRNICGISCNKFLDDDLTDDVMCAKKDLDSEGI
                           *.:*  *:**:*:*.:  ** ..:   * ***.  *::****:::**: *. * :   **

sp|P00716|LALBA_RABIT      DHWLAHKPLCSENLEQWVCKK----------------
sp|P00714|LALBA_RAT        DYWKAHKPMCSEKLEQWRCEKPGAPALVVPALNSETPVP
sp|P00711|LALBA_BOVIN      NYWLAHKALCSEKLDQWLCEKL-----------------
sp|P00712|LALBA_CAPHI      NYWLAHKALCSEKLDQWLCEKL-----------------
sp|P09462|LALBA_SHEEP      NYWLAHKALCSEKLDQWLCEKL-----------------
sp|P00709|LALBA_HUMAN      DYWLAHKALCTEKLEQWLCEKL-----------------
sp|P12065|LALBA_PAPCY      DYWIAHKALCTEKLEQWLCEKE-----------------
sp|P18137|LALBA_PIG        DYWLAHKALCSEKLDQWLCEKM-----------------
sp|Q9N2G9|LALBA_CANLF      DYWLAHKPLCSEKLEQWRCEKL-----------------
sp|P08896|LALB2_HORSE      DYWLAHKPLCSEKLEQWLCEEL-----------------
                           ::* *** :*:*:*:** *::
```

Percent Identity Matrix - created by Clustal2.1

1: P00709:	100.00	73.94	74.65	77.30	74.65	73.17	69.01	63.12	93.50	78.87
2: P00711:	73.94	100.00	95.07	75.18	97.18	69.11	62.68	60.28	70.73	75.35
3: P00712:	74.65	95.07	100.00	75.18	97.89	69.92	63.38	60.99	71.54	76.76
4: P18137:	77.30	75.18	75.18	100.00	75.18	81.15	61.70	65.00	75.41	82.27
5: P09462:	74.65	97.18	97.89	75.18	100.00	69.92	61.97	59.57	71.54	76.06
6: P08896:	73.17	69.11	69.92	81.15	69.92	100.00	60.98	59.02	68.29	78.86
7: P00714:	69.01	62.68	63.38	61.70	61.97	60.98	100.00	56.74	69.11	66.20
8: P00716:	63.12	60.28	60.99	65.00	59.57	59.02	56.74	100.00	61.48	67.38
9: P12065:	93.50	70.73	71.54	75.41	71.54	68.29	69.11	61.48	100.00	75.61
10:Q9N2G9:	78.87	75.35	76.76	82.27	76.06	78.86	66.20	67.38	75.61	100.00

Alignments of protein sequences;
IDENTICAL POSITIONS-43
IDENTITY-27.044%
SIMILAR POSITION-45

PROGRAM- CLUSTAL0

STRING DATA-

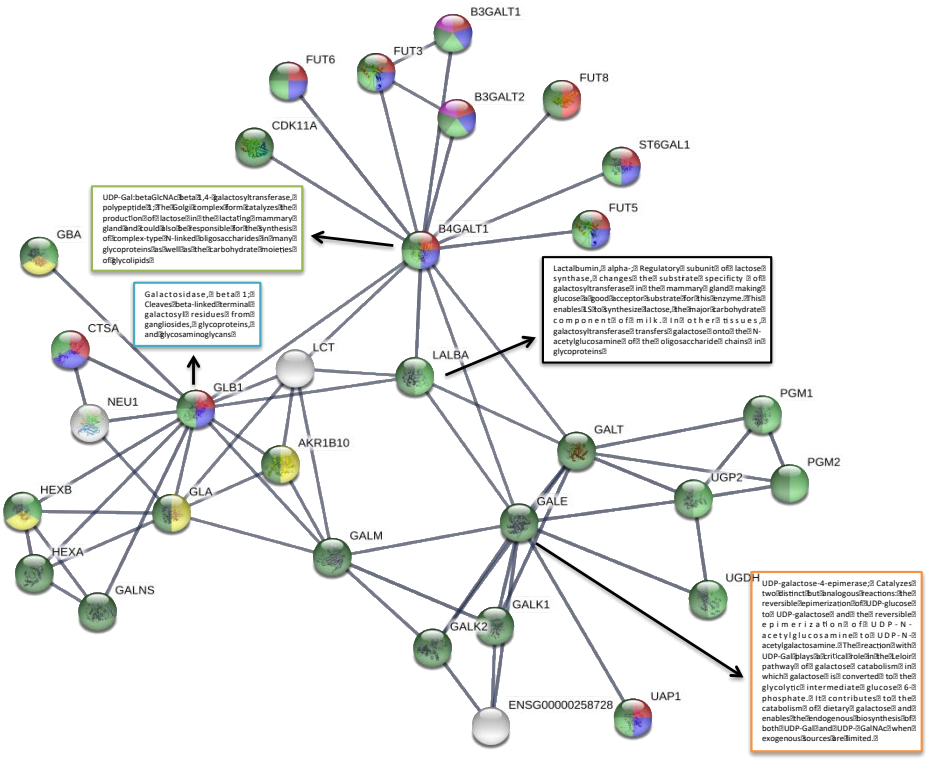


FIGURE OF STRING SHOWS-

Nodes- network nodes represent protein splice isoforms or post translational modifications are collapsed i.e. each node represent all the proteins produced by a single, protein-coding gene locus.

Node colours-

Coloured nodes- query proteins and first shell of interaction.

white nodes: second shell of interactions

Node content-

Empty nodes- proteins of unknown 3D structure.

Filled nodes- some 3D structure is known as predicted.

Glycosylation- Red colour

Protein glycosylation- Blue colour

Cellular biosynthetic process- Green colour

Cellular lipid catabolic process- Yellow colour

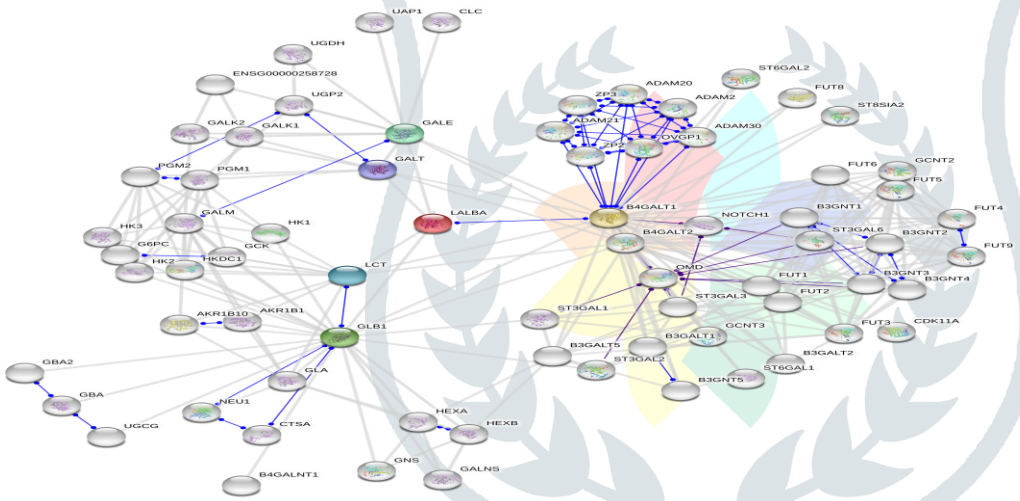
UDP-galactose, beta-N-acetylglucosamine-1,3-galctosyl-transferase- Violet colour

Catalytic activity- dark green colour.

Edge confidence- represents protein-protein interactions represent associations are meant to be specific a meanigful i.e. proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding each other.

Edge confidence- the result of edge score showed that highest range which is highly accepted value in relation with interaction.

S.No.	Protein	Edge score
1.	B4GALT1	0.996
2.	GLB1	0.916
3.	GALE	0.903
4.	LCT	0.903
5.	GACT	0.900



String- the result shows that the interaction of alpha-lactalbumin with ADAM, BGALT1, NOTCH1 & *fut* etc.

Discussion-

The sequences of alpha-lactalbumin of Human (P00709), Bovine (P00711), Goat (P00712), Pig (P18137), Sheep (P09462), Horse (P08896), Rat (P00714), Rabbit (P00716), Yellow baboon (P12065) and Dog (Q9N2G9) taken from the database of UniProtKB and after this accessing the sequence in prot-param from where we got the percentage composition of essential amino acids in alpha-lactalbumin. The above result showed that the maximum composition of essential amino acids present in the pig=rabbit>human> bovine=sheep >Dog> horse> yellow baboon> Rat, this indicated that the milk of pig and rabbit have more nutritional value than other species, but estimating at the individual essential amino acids composition every individual have its own variance which showed that the percentage composition of isoleucine-10.6%- baboon, methionine-4.3%-pig, leucine-12.7%-human,

phenylalanine-5.6%-Human and Dog, Threonine-6.5%-baboon, valine-5.7%-rat, histidine-2.8%-Bovine, goat and Sheep, lysine-9.8%-Horse, tryptophan-3.3%-Horse all these result indicated that the nutritional value of alpha-lactalbumin which is very important for the growth of newborn baby and resist different kind of perilous disease like leukemia, meningitis etc. alpha-lactalbumin is one of the protein which have well balanced composition of all essential amino acids which is shown in bar diagram. According to the WHO (USDA national nutrient database) report the daily recommended intakes for children aged three years and older is 10% to 20% higher than the adult levels and those for infants can be as much as 150% higher in the first year of life. Cysteine (or sulfur containing amino acids), tyrosine (or aromatic amino acids) and arginine are always required for infants and growing children in taking this perspective the result here showed that cysteine-6.5%-baboon and horse, Methionine-4.3%-pig, phenylalnine-5.6%-human and dog, tyrosine-4.1%-baboon, arginine-1.9%-rat etc. The result of clustal omega showed the multiple alignment and percent of identity in different species which showed that how identical the composition of amino acids in alpha-lactalbumin of different species however, it also showed the 43 identical positions are present in alpha-lactalbumin of different species with percentage of identity- 27.044% and similar position- 45.

The string (protein-protein interactions) showed that how alpha-lactalbumin interacted with other regulatory proteins which are involved in production of milk at molecular and cellular level that is also essential for preventing the disease in neonatal baby. Indeed we showed result in edges that represent protein-protein association which is meant to be specific and meaningful i.e. protein jointly contribute to a shared function; this does not necessarily mean that they are physically binds to each other so here my input is alpha-lactalbumin (query protein) which is regulatory subunit of lactose synthase, changes the substrate specificity of galatosyltransferase in the mammary gland making glucose a good acceptor substrate for this enzyme. This enables LS to synthesis lactose, the major carbohydrate component of milk. In other tissues, galatosyltransferase transfer galactose onto the N-acteylglucosamine of the oligosaccharide chains in the glycoproteins and their predicted functional partners are B4GALT1 (.996), GLB1 (.916), GALE (.903), LCT (.903), GALT (.900), all the score showed have highest edge confidence value which is very important for metabolism, cell signaling etc. Although the string result inferred that importance of alpha-lactalbumin as in consequence of containing essential and non essential amino acids which is important for normal cellular metabolism in neonatal baby.

The result of string also indicate that regulation of *fut* chaperone, Notch1, ADAM etc., by alpha-lactalbumin which is known for fucosylation of notch receptor protein however the notch pathway is involved in regulation of the cell differentiation, proliferation, stem cell and stem niche maintenance, cell fate specification and cell death.

Conclusion- The sequence of alpha-lactalbumin taken from swiss-uniprot and was further accessed by Prot-pram, clustal omega and string. This resulted into study the percentage amino acids composition, multiple alignment, percent of identity and the last one interaction of alpha-lactalbumin with other proteins. As we know that the importance of alpha-lactalbumin in neonatal baby which provide immunity, anti-bacterial activity and helping in development of the neonatal brain as in the form milk, so the amino acid percentage composition is important as the WHO parameter for the neonatal so the pig and rabbit alpha-

lactalbumin provide a very good composition of essential amino acids than the other species and side by side alpha-lactalbumin also regulate the Notch signaling pathway which is one of the important signaling pathway for the cell differentiation, proliferation, stem cell and stem niche maintenance, cell fate specification and cell death in neonatal baby.

Future perspective-

The World Health Organization recommends that breast feeding should start within an hour of birth and that children should be exclusively breast fed, on demand, for the first six months. After six months complementary foods should be added, and breast feeding should continue up to age 2 or beyond (WHO report).
In 2015 India scored 78/150 in the WBTi's assessment of 15 factors in "policy and programmes" and "infant and young child feeding"—only a small improvement from 68/150 in 2005. India ranks poorly alongside other South Asian countries such as Bangladesh, Sri Lanka, and even Afghanistan, which made significant progress in its assessment from 30/150 in 2005 to 99/150 in 2012. In looking this problem this data will provide a better option for providing the cocktail of alpha-lactalbumin in the consequence of essential amino acids from different species for the growth of new born baby.

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