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DIVERSITY STUDY ANALYSIS OF LEPTIN GENE IN SOME RUMINANT AND NON-RUMINANT SELECTED ANIMAL SPECIES

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Abstract. The key element of the system regulating food intake has proven to be the Leptin. It act as hunger centre in the hypothalamus and affects the regulation of appetite. It has also been shown that Leptin gene influence milk performance in sheep, cattle and reproduction performance in beef cattle. Genetic characterization to assess the existing biodiversity and differences among the important livestock breeds is an essential pre-requisite to facilitate the conservation program in an effective and meaningful way. This paper sought to study the diversity analysis of Leptin gene in some ruminant and non-ruminant animal species. A total of twenty three (23) Leptin gene sequences belonging to eight (8) species: Cattle (3), Sheep (3), Goat (3), Swine (3), Horse (2), Camel (3), Mouse (3) and Rabbit (3) were retrieved from Genbank (www.ncbi.nlm.nih.gov). Sequences alignment, translation and comparison were done using ClustalW of the MEGA 6.0. The minimum distance matrix (Dxy) value (0.02) was observed between the sequence of cattle and goat while the maximum Dxy value (2.72) was seen between cattle and sheep in ruminant species. In non-ruminant species the highest Dxy value (17.61) was seen between rabbit and camel while the minimum Dxy value (0.18) was observed between mouse and camel respectively. The smaller the distance matrix value, the closer the sequence of the species and the lesser the genetic distance among or between species whereas the larger the Dxy value, the higher the genetic distance among and between species investigated. This finding could provide basis for selection when considering evolution and differentiation among species.

Keywords: diversity study, leptin, ruminant, non-ruminant, sequences, phylogenetic analysis

Introduction

Leptin is a 16-kDa protein hormone belonging to the class-1 helical cytokine family of proteins *(Trombley et al., 2012).* Leptin was first discovered in the mouse *Mus musculus* and has a central role in the regulation of appetite, energy metabolism, body composition, immune functions and reproduction in mammals *(Trombley et al., 2012).*

Leptin is primarily produced in adipose tissue and is secreted into the blood stream after cleavage of the 21 amino acid signal peptide *(Barb et al., 2001),* secretion occurs in response to changes in body fat levels or energy status *(Barb et al., 2001).* Leptin acts as an anorexigenic signal through a negative feedback loop to the appetite centre in the hypothalamus causing long term and short-term effects on feed uptake and energy homeostasis *(Trombley et al., 2012).*

Expression of gene which encodes a Leptin receptor has been confirmed in pituitary, adipose tissue, granulosa and theca cells of the ovary, interstitial cells in testis, in heart, liver, lung, kidney, adrenal gland, small intestine and lymph nodes *(Hoggard et al., 1997).* In mammals the Leptin is considered as a hormone that regulates the body weight by maintaining the balance between food intake and energy expenditure through signalling to the brain and brings the changes in stored energy level *(Friedman et al., 1998).*

Elevated plasma Leptin levels inhibit continued feeding and regulate body weight in the long term as well as promoting postprandial satiety *(Trombley et al., 2012).* Low Leptin levels are associated with low body fat levels and starvation, signalling energy insufficiency and stimulating appetite in humans, rats *Rattus spp* and pigs *Sus spp.* The Leptin gene is highly conserved across species and is located on chromosome 7q31.3 in humans and on chromosome 4q32 in cattle *(Fatima et al., 2011)*. Leptin gene DNA sequence includes 15,000 base pairs and contains 3 exons, which are separated by 2 introns. Out of 3 exons and 2 introns, only two exons are translated into protein.

In mammals, Leptin informs the hypothalamus *(Barb et al., 2001)* about the amount of fat stored in the body through short and long forms of Leptin receptor. Leptin also plays a major role in control of body growth, adaptability, immune function, angiogenesis, renal function, haematopoiesis, reproduction, and not only acts as an endocrine signal in brain and different peripheral tissues in which Leptin receptors are expressed in fatal tissue, mammary gland, rumen, abomasum, duodenum and pituitary gland. The Leptin expression is also modulated according to different physiological and growth stages of animal *(Wallace et al., 2014).* Therefore, the Leptin could act as marker for animal growth, feed conversion efficiency and health and therefore the present study sought to explain a form of diversity study analysis of Leptin gene in-silico in some selected ruminant and non-ruminant animal species.

Materials and Methods

A total of twenty three (23) Leptin gene sequences of some selected ruminant and non-ruminant animal species as thus: Cattle (3), Sheep (3), Goat (3), Swine (3), Horse (2), Camel (3), Mouse (3) and Rabbit (3) were retrieved from the GenBank (www.ncbi.nlm.nih.gov). The GenBank accession number of these cattle, sheep, goat, swine, horse, camel, mouse and rabbit sequences were: NM_173928.2, Y11369.1, NM_001034741.1 (Cattle), NM_001009763.1, XM_004002049.3, XM_004021753.3 (Sheep), XM_018045213.1, XM_018045217.1,NM_001159756.1 (Goat), AY079082.1, EU189935.1, GBZA01000352.1 (Swine), XM_014738998.1, XM_014736686.1 (Horse), XM_010949533.1, XM_010949543.1, XM_006180441.2 (Camel), NM_026609.2, NM_025961.5, NM_145541.5 (Mouse), XM_008258163.2, XM_002709552.3, XM_002715941.3 (Rabbit).

Sequence alignments, translations and comparisons were done using ClustalW as described by *(Larkin et al., 2007).*

Neighbor-Joining trees were constructed each using P-distance model and pair wise deletion gap/missing data treatment. The construction was on the basis of genetic distances, depicting phylogenetic relationships among the Leptin nucleotide sequences of the investigated species. The reliability of the trees was also calculated by bootstrap confidence values *(Felsenstein, 1985)*, with 1000 bootstrap iterations using MEGA 6.0 software *(Tamura et al., 2013).*

Unweighted pair group method using arithmetic average (UPGMA) trees for the gene was constructed with consensus sequences using same model as that of the tree. All sequences were trimmed to equal length corresponding to same region before generating the tree.

Results

Table 1.**Leptin sequence variation between and among species**

Species	Number of sequences	Sequence length variation (bp)
Cattle	3	2042, 2060, 2930
Sheep	3	2586, 2757, 2836
Goat	3	2205, 2643, 2767
Swine	3	2060, 2123, 2642
Horse	2	2597, 2935
Camel	3	1383, 2556, 2839
Mouse	3	2357, 2474, 2609
Rabbit	3	2433, 2526, 2680

bp= base pair

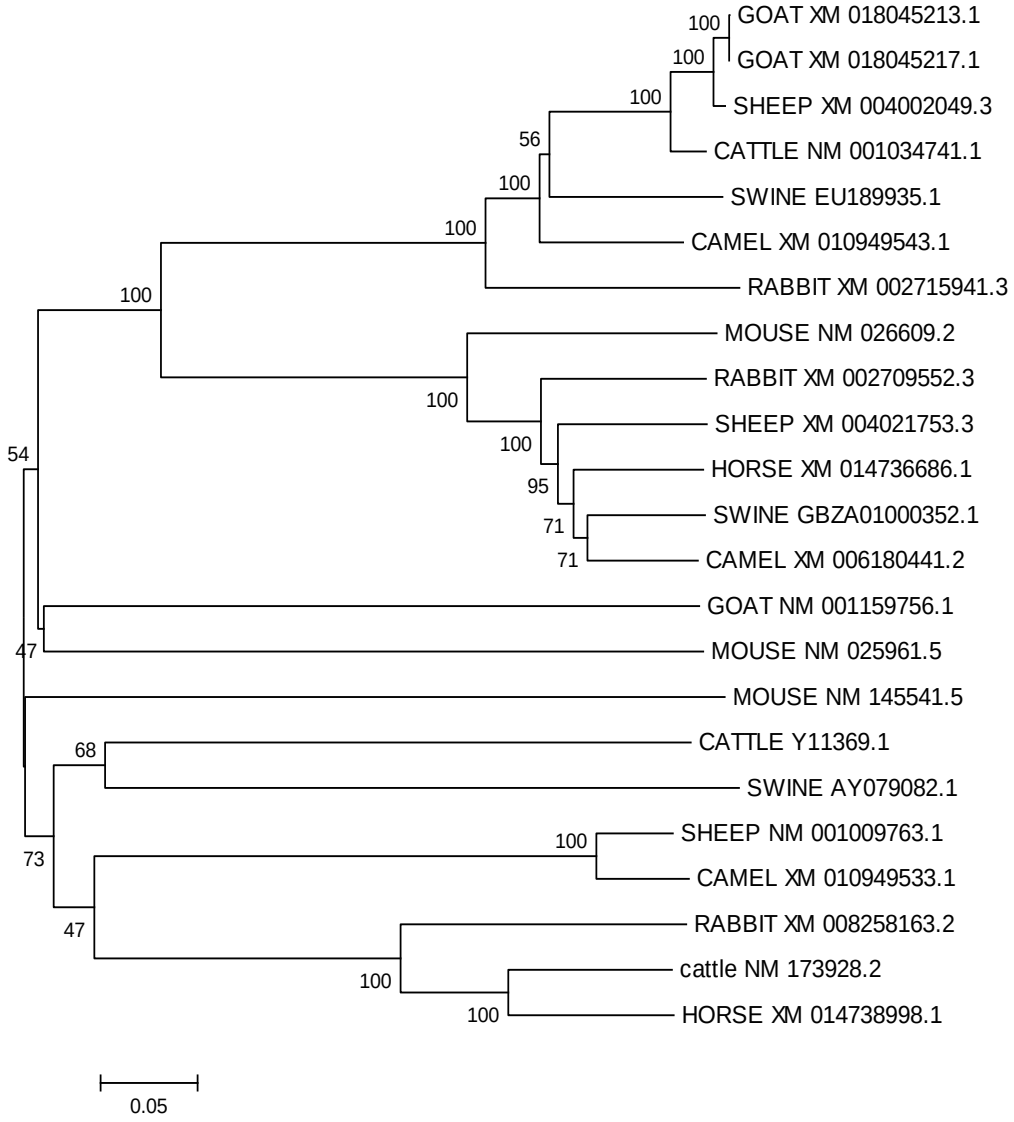

Fig 1. Phylogenetic tree of leptin gene sequences of the species selected.

The tree above showed a kind of proximity and differentiation among the ruminant and non-ruminant animal species selected.

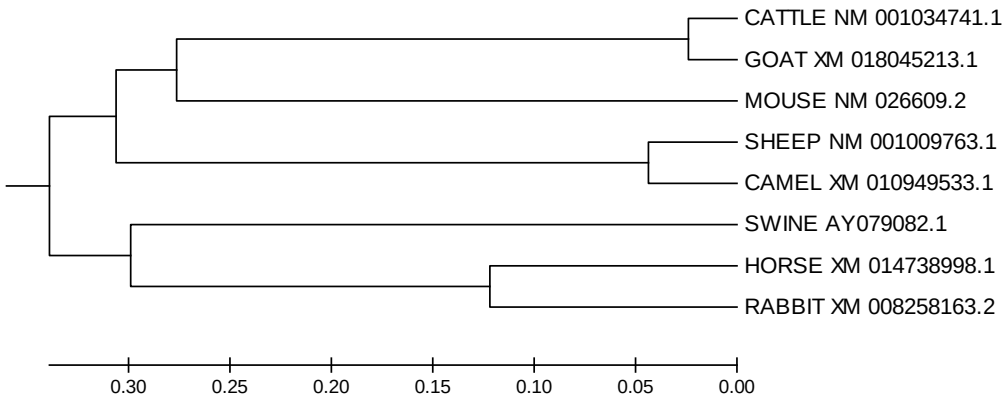

Fig.2. UPGMA tree from the consensus sequence of the phylogenetic tree

This figure showed that the sequence of Leptin gene of cattle clustered more closely with those of goats than mouse. Sequence of sheep from this figure appeared closer to those of camel than those of swine. Whereas Leptin gene sequence of horse and rabbit clustered closely than those of swine. In ruminant species, cattle and goats Leptin sequences clustered closely than those of sheep. While of those of non-ruminant, Leptin sequences of horse and rabbit clustered closely followed by those of swine and then mouse respectively and this could be explained due to species specific residues and such patterns of the sequences may be explained by gene conversion and balancing selection.

Table 2. Test of the Homogeneity of Substitution Patterns between Sequences selected

	Cattle	Sheep	Goat	Swine	Horse	Camel	Mouse	Rabbit
Cattle		0.00	0.22	0.00	0.00	0.03	1.00	0.00
Sheep	2.72		0.00	0.00	0.00	0.02	0.03	0.00
Goat	0.02	2.46		0.00	0.00	0.04	1.00	0.00
Swine	8.58	14.80	9.67		0.04	0.00	0.00	0.02
Horse	4.80	7.38	5.66	1.03		0.00	0.00	0.00
Camel	1.14	0.20	1.04	11.82	5.46		0.27	0.00
Mouse	0.00	1.37	0.00	7.56	3.35	0.18		0.00
Rabbit	14.26	21.19	16.01	1.40	3.37	17.61	13.08	

Standard error estimate is presented at the upper diagonal while average genetic distances between species is presented at the lower diagonal**.**

This distance matrix table explained better the distance between and among the leptin gene sequence of the selected animal species. The standard error above the diagonal ($P<0.05$) are considered significant.

The smaller the distance matrix value, the closer the sequence of the species and the lesser the genetic distance among or between species whereas the larger the Dxy value, the higher, the genetic distance among and between species.

Distance matrix (Dxy) of the sequence of cattle and goat (0.02) was minimum while while the maximum Dxy value (2.72) was seen between cattle and sheep in ruminant species. In non-ruminant species the highest Dxy value (17.61) was seen between rabbit and camel while the minimum Dxy value (0.18) was observed between mouse and camel respectively.

Discussion

The LEP is a cytokine-like hormone that regulates appetite, energy homeostasis, body composition, reproduction, immunity, and metabolic functions *(Ahima and Flier, 2000).* Whereas in wild animals, adaptive evolution has been shown to have occurred in pika (*Ochotona curzoniae*) Leptin in response to environmental stress (extreme cold) *(Yang et al., 2008),* in livestock, polymorphism in the Leptin gene has been found to be associated with variations in traits of economic importance *(Zhou et al., 2009).* In sheep, products of the different allele variants in the Leptin gene have been shown to differ in their biochemical and biological properties *(Reicher et al., 2011).* The presence and maintenance of Leptin genetic polymorphism in the livestock population suggests that different forms of the protein might have differential functional abilities.

The Leptin protein circulates in the serum as a free hormone or as a complex with Leptin soluble receptor (bound form). It was found that the proportion of circulating free Leptin to bound Leptin varies in different physiological conditions. In addition, it has been suggested that this variation might disrupt the binding of Leptin to its receptor *(Buchanan et al., 2002).*

Leptin gene sequence length variation of the selected species ranged from 1383–2930 base pair. The Dxy value inferred closeness and distance of the sequences of the various species.

The length variation of the Leptin gene within and among species might result from evolution and differentiation. Many length variations caused by insertions and deletions resulting in amino acid variation within species have been found by comparison with known sequences *(Faith and Owoeye, 2017).*

The presence of numerous alleles at a particular Leptin locus is evidence of the long-term evolutionary persistence of the locus. This is suggested by the fact that the alleles in one species are often more closely related to the alleles in closely related species than to the other alleles in the same species. The species wise clustering might be due to species specific residues *(Takahashi and Nei, 2000)* and such patterns of the sequences may be explained by gene conversion and balancing selection.

It has also been shown that Leptin gene influence milk performance in sheep, cattle and reproduction performance in beef cattle *(Mahmoud et al., 2014).* Studies on Leptin gene polymorphism and production traits in dairy cattle, sheep and poultry has been reported with promising results and can be considered as one of the best biological markers in animals and human beings *(Nassiry et al., 2008).*

Conclusion

The presence of numerous alleles at a particular Leptin locus is evidence of the long-term evolutionary persistence of the locus. This is suggested by the fact that the alleles in one species are often more closely related to the alleles in closely related species than to the other alleles in the same species. The species wise clustering of Leptin gene might be due to species specific residues and such patterns of the sequences may be explained by gene conversion and balancing selection.

Ispitivanje raznovrsnosti leptin gena u odabranim vrstama preživara i nepreživara

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Rezime

Leptin se pokazao kao ključni element sistema koji reguliše unošenje hrane. Deluje kao centar gladi u hipotalamusu i utiče na regulaciju apetita. Takođe je utvrđeno da leptin gen utiče na prinos mleka kod ovaca, goveda, kao i na reprodukciju u govedarstvu. Genetska karakterizacija za procenu postojećeg biodiverziteta i razlika među važnim stočarskim rasama je suštinski preduslov za olakšanje programa konzervacije na efikasan i značajan način. Ovaj rad je pokušao da prouči analizu raznolikosti leptin gena u određenoj vrsti preživara i monogastričnih životinja. Ukupno dvadeset tri (23) sekvence leptin gena koje pripadaju osam (8) vrsta: goveda (3), ovce (3), koze (3), svinje (3), konj (2), kamila (3) i zečevi (3) su preuzeti iz Genbank-e (www.ncbi.nlm.nih.gov). Usaglašavanje, prevođenje i upoređivanje sekvenci obavljeno je pomoću ClustalW - MEGA 6.0. Utvrđena je vrednost minimalne matrica rastojanja (Dxy) (0,02) između sekvence goveda i koza, dok je maksimalna vrednost Dxy (2,72) utvrđena između goveda i ovaca, kod preživara. U monogastričnim vrstama, najveća Dxy vrednost (17,61) je utvrđena između zeca i kamile, dok je minimalna Dxy vrednost (0,18) primećena između miša i kamile, respektivno. Što je manja matrica udaljenosti, to je bliža sekvenca vrste i manja je genetička razdaljina unutar ili između vrsta, dok veća vrednost Dxy, ukazuje na veću genetička razdaljina unutar i između ispitanih vrsta. Ovaj rezultat bi mogao da bude osnova za selekciju kada se razmatra evolucija i diferencijacija među vrstama.

Ključne reči: studija raznolikosti, leptin, preživari, nepreživari, sekvence, filogenetska analiza

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