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Dear Esteemed Readers, Authors, and Colleagues,

I hope this letter finds you in good health and high spirits. It is my distinct pleasure to address you as the Editor-in-Chief of Integrative Omics and Applied Biotechnology (IIOAB) Journal, a multidisciplinary scientific journal that has always placed a profound emphasis on nurturing the involvement of young scientists and championing the significance of an interdisciplinary approach.

At Integrative Omics and Applied Biotechnology (IIOAB) Journal, we firmly believe in the transformative power of science and innovation, and we recognize that it is the vigor and enthusiasm of young minds that often drive the most groundbreaking discoveries. We actively encourage students, early-career researchers, and scientists to submit their work and engage in meaningful discourse within the pages of our journal. We take pride in providing a platform for these emerging researchers to share their novel ideas and findings with the broader scientific community.

In today's rapidly evolving scientific landscape, it is increasingly evident that the challenges we face require a collaborative and interdisciplinary approach. The most complex problems demand a diverse set of perspectives and expertise. Integrative Omics and Applied Biotechnology (IIOAB) Journal has consistently promoted and celebrated this multidisciplinary ethos. We believe that by crossing traditional disciplinary boundaries, we can unlock new avenues for discovery, innovation, and progress. This philosophy has been at the heart of our journal's mission, and we remain dedicated to publishing research that exemplifies the power of interdisciplinary collaboration.

Our journal continues to serve as a hub for knowledge exchange, providing a platform for researchers from various fields to come together and share their insights, experiences, and research outcomes. The collaborative spirit within our community is truly inspiring, and I am immensely proud of the role that IIOAB journal plays in fostering such partnerships.

As we move forward, I encourage each and every one of you to continue supporting our mission. Whether you are a seasoned researcher, a young scientist embarking on your career, or a reader with a thirst for knowledge, your involvement in our journal is invaluable. By working together and embracing interdisciplinary perspectives, we can address the most pressing challenges facing humanity, from climate change and public health to technological advancements and social issues.

I would like to extend my gratitude to our authors, reviewers, editorial board members, and readers for their unwavering support. Your dedication is what makes IIOAB Journal the thriving scientific community it is today. Together, we will continue to explore the frontiers of knowledge and pioneer new approaches to solving the world's most complex problems.

Thank you for being a part of our journey, and for your commitment to advancing science through the pages of IIOAB Journal.

Yours sincerely,

Vasco Azevedo

Vasco Azevedo, Editor-in-Chief
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Editor-in-Chief

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RESEARCH ARTICLE OPEN ACCESS

MOLECULAR CLONING AND CHARACTERIZATION OF RABBIT MYOSTATIN GENE

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ABSTRACT

Myostatin is a member of the transforming growth factor-β (TGF-β) superfamily, which is expressed specifically in vertebrate skeletal muscle and functions as negative regulator of skeletal muscle growth. In the present study, the three exonic regions of myostatin gene (MSTN) were resequenced in fifteen animals of three different rabbit (Oryctolagus cuniculus) breeds (White giant, Soviet chinchilla and Desi) with a view to make comparative study of it. Resequencing and comparison of three MSTN exonic region of these animals showed only one variation (a>G) in exon I of a soviet chinchilla (S11) and one variation (g>A) in exon III of a white giant breed (W12), respectively. The translated amino acid sequences of MSTN of these animals showed first variation at start codon (valine instead of methionine) and the second variation at amino acid position 267 (aspargine instead of aspartic acid). We conclude that the myostatin gene is highly conserved within these three different breeds of rabbit as well as within the different species of animals and is little affected by these variations.

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KEY WORDS

myostatin; rabbit; exon; resequencing; codon

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[I] INTRODUCTION

Rabbits are small mammals in the family Leporidea of the order Lagomorpha found in several parts of the world and used for many purposes mainly meat, fur production etc. Several DNA markers associated with production traits in livestock have been already identified through candidate gene approach [1], which is based on the fact that variability within genes coding for protein products involved in key physiological mechanisms and metabolic pathways directly or indirectly involved in determining an economic trait (e.g. feed efficiency, muscle mass accretion, reproduction efficiency, disease resistance, etc.) and might probably explain a fraction of the genetic variability for the production trait itself [2].

The myostatin gene, also known as GDF8 (growth differentiation factor 8), is specifically expressed during embryonic development, expressed at high level in adult skeletal muscle and controls skeletal muscle growth [3]. Molecular analysis of the myostatin gene in different species has showed that it consists of three exons and two introns **[Figure–1]** and found to affect both the amount and composition of muscle fibers. For instance, the muscle mass of MSTN knockout mice is two to three times greater than that of wild-type mice [4], which is primarily due to an increased number of muscle fibers, followed by muscle cell hypertrophy and suppression of body fat accumulation [5].

Myostatin gene is one of the most conserved genes among vertebrate species [6]. However, there are many reports of mutations, disrupting the myostatin function, which cause double-muscle phenotypes in cattle and increase in body mass in mice [7]. 30% more muscle mass with less bone and fat is found in double-muscled cattle per animal on the same food intake as normal cattle [8]. Schuelke et al. [9] reported hyper muscularity and decreased fat mass in a child due to an inactivating mutation of the MSTN which further supports its role in the regulation of body composition. MSTN gene has

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been cloned and identified from a wide variety of vertebrate species including human [10], mice [4], cattle [11], chicken [12], yak [13], and zebrafish [14].

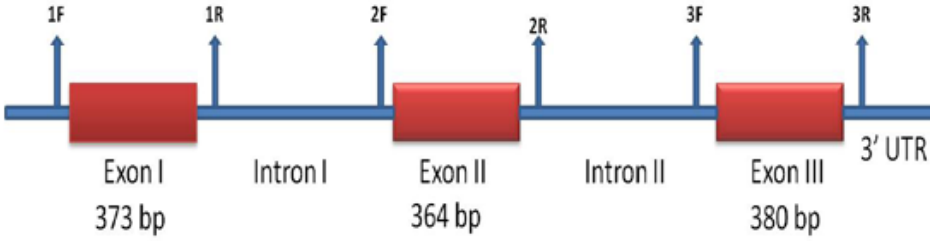

Fig: 1. Schematic representation of the rabbit myostatin gene (exons, introns and untranslated region - UTR). (A) Gene position of nucleotide of the translated region of three exons, (B) Localization of 3 pairs of primers (1F, 1R, 2F, 2R and 3F, 3R

The myostatin gene has been sequenced in other livestock and some fish to evaluate its commercial possibilities. Despite the fact that the rabbit is an animal of high economic value, myostatin has not been extensively studied and no mutation has been reported for the rabbit yet except one report by Fontanesi et al. [2]. Therefore, screening myostatin gene mutation may contribute to develop animal breeds with high meat performance. Hence, we resequenced this gene with the aim to compare myostatin gene among the three different rabbit breeds and also with other species.

[II] MATERIALS AND METHODS

2.1. Animals and DNA Isolation

Fifteen rabbits (five animals, each from three breeds i.e. White giant, Soviet chinchilla and Desi, respectively) were used for resequencing of the MSTN. DNA was isolated from blood by phenol-chloroform method [15].

2.2. Polymerase Chain Reactions (PCR)

Three PCR primer pairs were used to amplify the rabbit MSTN as previously reported by Fontanesi et al. [2] **[Table–1]**. Polymerase chain reaction (PCR) amplifications using the three primer pairs were done separately, and carried out in a final reaction volume of 25 µl containing 1X PCR Master Mix (Fermentas), 10 pmol of each primers and 50-100 ng template DNA. PCR profile was as follows: 5 min at 95°C, 35 amplification cycles of 45 sec at 95°C, 45 sec at 52°C, 45 sec at 72°C and 10 min at 72°C. The size of PCR products were of 499 bp, 570 bp and 523 bp of MSTN exon I, exon II and exon III, respectively. In every experiment, negative controls were performed containing all reagents except DNA, aiming to avoid contaminations. Assays were performed in a thermal cycler (Minicycler, MJ Research), and the amplicons were analyzed on 2% agarose containing 1 µg/ml ethidium bromide in horizontal gel electrophoresis and visualized under UV light by gel documentation system (SynGene). After gel photo-documentation, remaining PCR products were purified to remove free nucleotides, primers and enzymes by AcroprepTM 96 filterplate protocol (PALL Corporation) as manufacturer's instruction.

2.3. Cloning and sequencing

These PCR products were then ligated in pTZ57R vector using the Insta T/A cloning kit (Fermentas). The vectors containing the insert were propagated in E.coli DH5-α host following manufacturer's instructions. Transformed colonies were screened by blue white screening and the recombinant plasmids, carrying correct inserts, were isolated from the representative clone using QIAprep® Spin Miniprep kit (QIAGEN). These plasmids were subjected to BigDye® Terminator v3.1 Cycle Sequencing reaction (Applied Biosystems) and products were resolved on automated ABI PRISM® 310 Genetic Analyzer (Applied Biosystems) at Animal Biotechnology Laboratory, AAU, Anand, Gujarat, India. By using SeqScape Software (Applied Biosystems), forward and reverse sequences of representative sample of each gene fragment were assembled against most closely related reference sequence of respective gene to obtain total sequence length and similarity was looked in to the non-redundant database of GenBank with BLAST algorithms (http://www.ncbi.nlm.nih.gov/BLAST/).

2.4. Phylogenetic analysis

Sequence analysis was further validated by multiple sequences alignment of myostatin gene from different species in FASTA format using ClustalW (http://www.ebi.ac.uk/Tools/clustalw/) available at European Molecular Biology Laboratory (EMBL) website. Mutation detection was done by multiple sequence alignment of consensus sequences of rabbit in Bioedit (v 7.0.7.1) and phylogenetic tree was constructed using Neighborhood joining method of bootstrap test of phylogeny in MEGA4 [16].

[III] RESULTS AND DISCUSSION

Myostatin gene polymorphisms were detected by the cloning and sequencing of protein coding region, spanning all three exons. To cover complete MSTN coding region, these three exons (499 bp, 570 bp and 523 bp) were amplified using primers as previously reported [2] **[Figure–2]**. On the whole, resequencing of the myostatin gene in three different rabbits (Fifteen animals) generated sequence information for 1692 bp. The first 499 bp fragment included part of the 5' UTR, exon I and part of intron I, second 570 bp covered part of intron I, exon II and part of intron II and last 523 fragment bp encompassed part of intron II, exon III and part of the 3' UTR, respectively.

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Table: 1. Primers used for amplification in PCR reaction

Exon no.	Primes sequence (5'-3')	Amplicon size (bp)	Annealing temp. (^{0}C)
I	F –AATTTTGCTTGCCATTACTGA R- TCAGCAGAACTGTTGACATACAC	499	
II	F- TGCATGCATTATCCCAATAGA R- TCGGTAGTTGTTTCCCACTTT	570	52
III	F- AAAGGTATTCCAAGCAAAATGA R- GGGGAAGACCTTCCATGTTT	523	

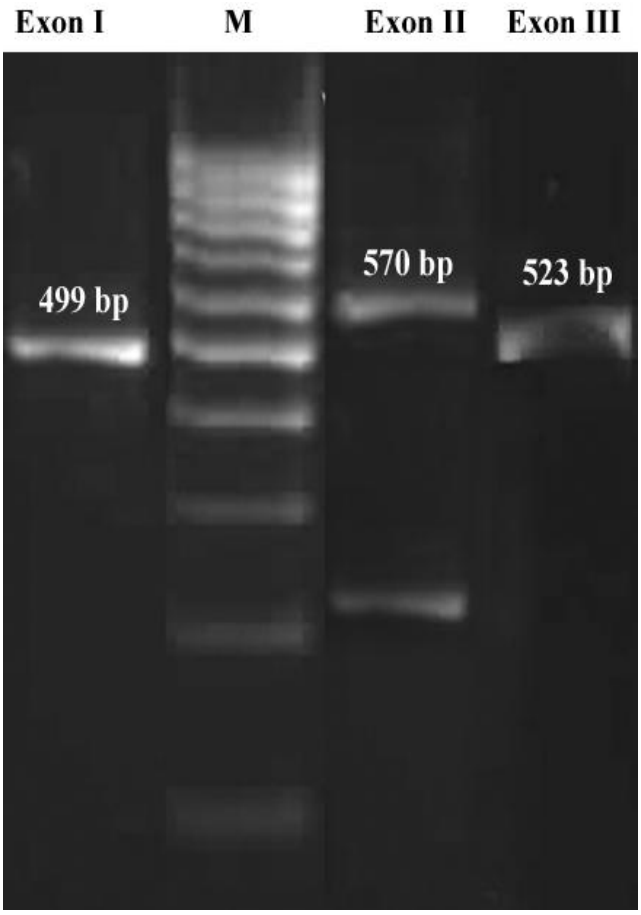

Fig: 2. 2% Agarose gel electrophoresis of exon I, II and III (499 bp, 570 bp and 523 bp) (Representative sample)

Comparing the sequences of all fifteen animals of the three rabbit breeds, only one mutation was found in exon I at nucleotide position 88 (a > G, initiation codon) in one animal of soviet chinchilla breed (S11) **[Figure–3a]**, whereas no mutation was identified in the exon II, while in case of exon

III, one mutation was found at nucleotide position 147 (g > A) in one animal of white giant breed (W12) **[Figure–3b]**. In eukaryotic systems, ATG is almost exclusive as this codon act as translational initiation, whereas in mammalian cells, ACG and CTG have also been found as initiator codons in a few mRNAs. Moreover, it has been reported that GTG acts as initiation codon in eukaryotic mitochondrial gene, bacterial ORF and yeast ORF [17]. These mutations are not always beneficial as Klein et al. [18] reported that A to G transition mutation in the ATG initiation codon of a protein coding gene may cause disease in humans.

The MSTN exon I, II and III of all the three breeds of rabbit were compared with homologous regions of Bos taurus (AAB81508), Sus scrofa (ABR08657), Capra hircus (AAR12161), Gallus gallus (ACY68210), Homo sapiens (ABI48372), Mus musculus (AAI05675) and Ovis aries (ABJ97058) using Bioedit (v 7.0.7.1), which revealed that the exonic regions are highly conserved in between species. All the three exons of reference sequence of rabbit (AM931155, AM931157 and AM931158), sample S11, sample W12 and above mentioned species were translated into protein using the Expasy Translation Bioinformatics tool. In term of amino acid, it was found that there was valine instead of methionine at start codon (a>G) and aspargine instead of aspartic acid at amino acid position 267 (g>A) **[Figure–4]**. When translated sequence were used for preparation of phylogeny, it was found that rabbit MSTN gene was nearest to M. musculus MSTN gene (AA105675) and S. scrofa (ABR88567) farthest from G. gallus MSTN gene (ACY68210) **[Figure–5]**. Apart from this, other variations were also observed in the intronic regions of myostatin gene. However, the nucleotide variations and the amino acid changes seen in the two animals of rabbits showing mutations did not affect the myostatin protein structure and conformation. Thus from this, it can be concluded that the Myostatin gene is highly conserved in between these three rabbit breeds as well as within the different species of other animals. The multiple sequence alignment of MSTN amino acid sequences of various species also showed many other variations.

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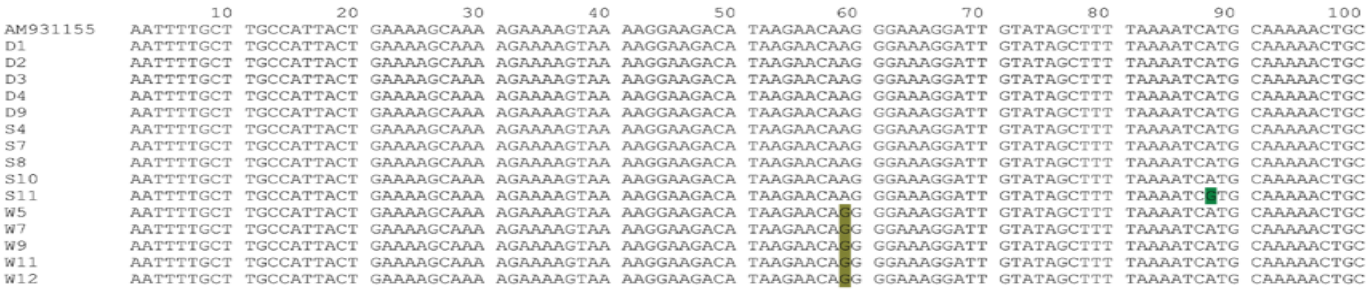

Fig: 3a. Multiple sequence alignment of all fifteen samples of Oryctolagus cuniculus MSTN Exon I showing mutation (a>G) at position 88 in one animal of Soviet Chinchilla breed (S11).

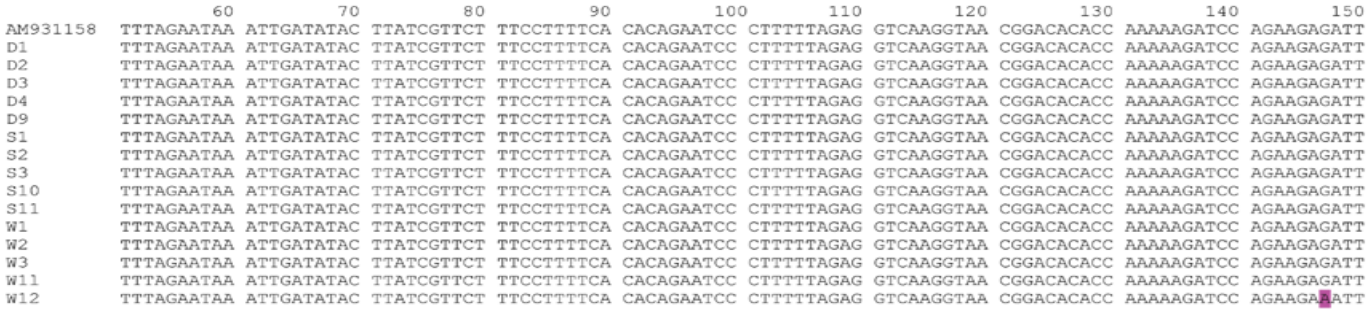

Fig: 3b. Multiple sequence alignment of all fifteen samples of *Oryctolagus cuniculus* MSTN Exon III showing mutation (g>A) at nucleotide position 147 in one animal of White Giant breed (W12).

Fontanesi et al. [2] sequenced the entire MSTN gene in the four different breeds of rabbits (Belgian hare, Burgundy fawn, Checkered giant and Giant grey) in order to identify DNA markers useful for association studies with economic traits and reported that only one single nucleotide polymorphism (C>T) in intron II of rabbit MSTN gene. Apart from this, no significant study has been done regarding the characterization of MSTN gene in different breeds of rabbits. Similarly Tay et al. [19] reported 38 nucleotide differences between the myostatin sequences in cattle and that of the goat. They found that there were 25 non-synonymous changes and 13 synonymous changes and identified three SNPs, two in exon II and one in exon III. Grisolia et al. [20] found 37 polymorphisms in the untranslated region segment, and also one SNPs in intron I and three SNPs in intron II of Nellore cattle breed. They concluded that this high degree of allelic heterogeneity in the myostatin gene could be related to its high mutation rate, and it also could be the result of a long history of artificial selection for meat production, which has probably favored such modifications and maintained them in cattle populations. Moreover, in yak myostatin gene, Jianquan et al. [13] reported one variation at encoding nucleotide position 641 in exon II which resulted into one amino acid changed between the yak and cattle, and also reported that there were 98% and 99% similarity in 5' UTR and 3' UTR respectively.

[IV] CONCLUSION

As some breeds of rabbits are significant meat producers, the double-muscling phenotype can be potentially exploited for economic gain. In summary, we have characterized the myostatin coding regions of rabbits and the identification of two nucleotide changes in the presumed myostatin protein sequence as compared to other species myostatin sequences. From an applied point of view, the identification of the myostatin gene polymorphisms in rabbits, have to be carried out in other meat species to find out DNA markers useful for association studies with economic traits.

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Fig: 4. A multiple sequence alignment of the translated myostatin protein sequences has been shown. The consensus sequences for reference myostatin compiled (AM931155, AM931157 and AM931158) and aligned with S11, W12, *Homo sapiens* (ABI48372), *Sus scrofa* (ABR08657), *Bos Taurus* (AAB81508), *Capra hircus* (AAR12161), *Mus musculus* (AAI05675), *Ovis aries* (ABJ97058) and *Gallus gallus* (ACY68210). The nucleotides in the different species which differed are written.

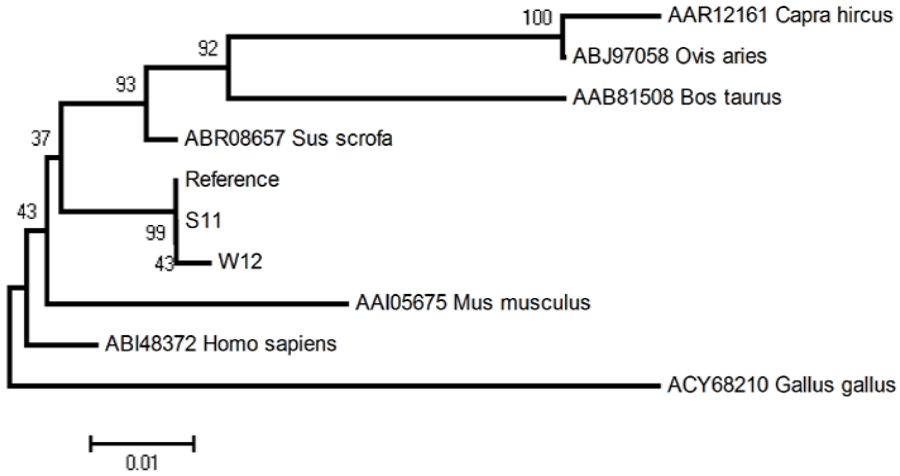

Fig: 5. Phylogenetic analysis of the amino acid sequences of MSTN gene between rabbit, human, mouse, pig, cow, goat, sheep and poultry

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AUTHOR CONTRIBUTION

Experiment was performed by A.S. Kurkute, N. Shabir, C.V. Jawale and U.V. Ramani. Analysis and manuscript writing was completed by A.K. Tripathi. Moreover, Dr. C.G Joshi, Dr. D.N. Rank and Dr. A.M. Pande are gratefully acknowledged for many helpful discussions while manuscript preparation.

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RESEARCH ARTICLE OPEN ACCESS

BULL SPERMATOZOA MOTILITY: OPTIMIZATION OF COENZYME Q10 AND ALPHA-LIPOIC ACID CONCENTRATION

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ABSTRACT

INTRODUCTION: *Coenzyme Q_{10} (CoQ_{10}) and alpha-lipoic acid (ALA) are antioxidants that play a role in ATP production and breakdown of free radicals.* ***OBJECTIVE:*** *The aim of this study was to optimize the concentration of CoQ_{10} and ALA based on motility pattern of spermatozoa.* ***METHODS:*** *The pooled bull spermatozoa were initially sub-fracted via electrophoretic separation which was performed at 20V for every two, four and six minutes. The sub-fractions were then further incubated with 0, 0.375, 0.75, 1.5, 3.0 and 6.0 µM of CoQ_{10}. The other groups of sub-fractions were incubated with 0, 0.0625, 0.0125, 0.025, 0.05, 0.1 and 1.0 mmol/L of ALA. The incubation was done at 37°C for one hour.* ***RESULTS:*** *The effect of CoQ_{10} was mostly seen on the sperm progression at every minute of separation. The only exception was sample incubated in 3 µM of CoQ_{10} which shows significant increase on VCL over 0 µM and 6 µM of CoQ_{10} at 4 minutes of separation. It was also noted that 1 mM of ALA mostly improved sperm velocity when the separation was collected at two and four minutes. Sperm population incubated with 1 mM of ALA also showed significant changes on STR and LIN at four minutes of separation.* ***CONCLUSION:*** *CoQ_{10} and ALA influence sperm progression and velocity. This finding is important in the artificial reproductive techniques especially for in vitro fertilization (IVF). This will be further validated in future study.*

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sperm; kinematic; antioxidant; CASA; bovine

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[I] INTRODUCTION

A minimal amount of reactive oxygen species (ROS) exists in the male reproductive system under normal physiological circumstances. Studies have conclusively indicated that the presence of ROS in a small amount is essential to regulate various normal sperm functions. These includes capacitation, acrosome reaction, and sperm-oocyte fusion [1]. In the presence of immature germ cell, abnormal spermatozoa, leukocytospermia, cell debris and low antioxidant levels ROS production will be high. These conditions subsequently overcome the antioxidant level and promote a condition known as oxidative stress [2].

Oxidative stress has deleterious effects on the physiology of the spermatozoa such as lipid peroxidation, DNA damage and also has been associated with destruction of sperm motility [3]. The principal means of oxidative stress to impair sperm motility is by alteration of the membrane fluidity [4]. Alteration of the membrane fluidity happened mainly due to present of polyunsaturated fatty acid (PUFAs) in high concentration at the sperm membrane. This will cause the spermatozoa becomes vulnerable to lipid peroxidation [5]. This situation is worsen by low concentration of scavenging enzyme in seminal plasma [6] and the inability of the intracellular antioxidant in providing protection to the outer layer of the membrane surrounding the head and tail of the spermatozoa [7]. Membrane fluidity plays a great role in regulating ion pump which include the ion pump that controls inwards and outwards movement of calcium ion into the spermatozoa. Alteration of the membrane fluidity will cause accumulation of calcium ion which consequently impaired sperm motility and eventually endanger the sperm survival [8].

As oxidative injury is evidently increased in male fertility, varieties of studies on antioxidant therapy have been conducted. These include study on glutathione [9], lycopene [10], vitamin C and vitamin E [11]. A number of studies also exist on the protective effects of coenzyme Q_{10} (CoQ_{10}) [12] and ALA [13] towards male oxidative injury.

CoQ_{10} is a fat-soluble molecule which is natural endogenously synthesized antioxidant in all humans and animals. CoQ_{10} also

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has the ability of being an antioxidant, enabled to quench the free radicals deteriorative effects and thus preventing lipid and protein peroxidation [14]. Moreover, CoQ_{10} is also capable of reducing alpha-tocopheroxyl radical to alpha-tocopherol. This ability of eliminating pro-oxidant radical and regenerating vitamin E will render the hyper functioning of antioxidant in the spermatozoa environment. In addition to its antioxidant properties, CoQ_{10} has been known to play an essential role in ATP production. It is supported by recent studies which had documented that CoQ_{10} is concentrated in the midpiece region [15]. Hence, deficiency in CoQ_{10} may give an indication as to why there is a reduction in sperm motility in some men. According to Lewin and Lavon, 1997, the CoQ_{10} deficiency is particularly prevalent in men suffering from asthenozoospermia [16].

Another interesting compound known as alpha-lipoic acid (ALA) also can be found naturally in mitochondria. This compound is responsible as a coenzyme for pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase. Hence its essential for energy production [17]. In addition, ALA or its reduced form, dihydrolipoic acid (DHLA) can ameliorated the harmful effect of oxidative stress both in aqueous and membrane phases. Moreover, ALA and DHLA appear to have the ability to regenerate Vitamin C and thus indirectly recycles Vitamin E [18].

Based on these previous study, treatment of spermatozoa with ALA [13] and CoQ_{10} [16] had indicated that there was improvement in sperm motility rate. However, there is only scanty information elaborating the effect of both antioxidants towards bull sperm motility. Furthermore, most of the previous study regarding the effect of antioxidant in improving male fertility problem had only evaluated on the percentage of motile sperm following treatment, improvement in sperm count, normal morphological sperm as well as the effect of the antioxidant treatment on the reproductive hormones [12]. In order to evaluate more detail on the effect of both antioxidants on sperm motility, we divided the parameter of the sperm motility into 2 categories which are sperm velocity (VCL, VSL, VAP; µm/s) and sperm progression (WOB, LIN, STR; %).

Sperm velocity was the kinematics parameter which measure how fast of sperm movement from one position to the last position it has been detected. The curvilinear velocity (VCL) has been defined as the time-average velocity of a sperm head along its actual curvilinear path while straight line velocity (VSL) was the time-average velocity of a sperm head along its straight line between its first detected position and its last. On the same note, the average path velocity (VAP) was the time-average velocity of the sperm head along its average path. This path is computed by smoothing the actual path according to algorithms in the CASA instrument [19]. On the other hand, sperm progression was the pattern of sperm movement along their path. The wobble (WOB) parameter describe side to side movement of the sperm head, linearity (LIN) describe the path curvature and straightness of trajectory (STR) shows the average distance of the sperm from its origin on the average path during all frames analyzed.

Uniquely, we are also incorporating new sperm separation techniques known as electrophoretic separation system at which the sperm is separated at 20 V and is being isolated at 2, 4 and 6 minutes. The voltage being applied was based on the preliminary study done by our group where this voltage shows a good separation. While, isolation of the sperm at 2, 4 and 6 minutes gives a better recovery of motile sperm than isolation done at 8, 10 and 12 minutes of separation. The techniques applied in this study are implemented with slight modifications to the existing model of sperm electrophoretic separation proposed by Ainsworth et al. (2005) [20]. We prefer this sperm preparation technique as the outcome indicates a positive sign in improving the weakness of other previous sperm preparation. The previous sperm preparation technique which includes Percoll® gradient centrifugation, density gradient centrifugation, and swim-up technique has been associated with the production of ROS. Hereby, this study was conducted in order to optimize both antioxidant concentrations particularly on bull spermatozoa motility.

[II] MATERIALS AND METHODS

2.1. Semen sampling

Multiple ejaculates from male adult bull were collected and pooled together. These were done by using artificial vagina at National Biotechnology Institute of Veterinary (IBVK), Jerantut, Pahang. The procedures followed have been reviewed by the institutional animal ethics research committee. Every ejaculation had yields up until 5 ml per ejaculate. Sperm motility were performed using CEROS (Hamilton Thorne Inc, Beverly, MA).

2.2. Semen preparation

About 1 ml from total semen sample was isolated for electrophoretic separations. For each electrophoretic separation, 60 µl of sample were injected into the injection site. Electrophoretic separations were performed at constant voltage of 20 V for 6 minutes. For each 2 minute interval, 20 µl of semen were taken out from the electrophoretic glass chamber. About 10 µl was analyzed for sperm motility using Hamilton Thorne Motility-CEROS version 12.1c (Hamilton Thorne Inc, Beverly, MA), and the remaining 10 µl were subjected to incubation with the selected antioxidant.

The series of concentration of CoQ10 and ALA and the incubation time were done according to the previous study with slight modification [13,16]. The incubations were done by mixing 10 µl of sperm with 10 µl of ALA or CoQ_{10} at different concentrations. Concentrations of CoQ_{10} used in this study are 6.0, 3,0, 1.5, 0.75, 0.375 and 0 µM while concentration for ALA are 1.0, 0.1, 0.05, 0.025, 0.0125, 0.0625 and 0 mmol/L. Incubation with ALA or CoQ_{10} were carried out for 1-hour using 1.5 ml microcentrifuge tube at 37 °C. For ALA, serial dilutions were performed in 0.1 % DMSO while serial dilutions of CoQ10 were carried out in Bioxcell® extender. Following 1-hour incubation, 10 µl of sample was subjected to Hamilton Thorne Motility-CEROS version 12.1c (Hamilton Thorne Inc, Beverly, MA) to assess the sperm motility. The parameter of sperm motility were classified into 2 categories which are Velocity (VCL, µm/s; VSL, µm/s; and VAP, µm/s) and Progression (WOB, %;LIN, %; and PROG, %).

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2.3. Statistical analysis

The differences between concentrations were compared and results were expressed as mean ± SEM. Analysis of variance (ANOVA) using the SPSS software version 12.0.1 with Post-hoc test was performed to verify statistical significance. The p-values of <0.05 was considered as statistically significant.

[III] RESULTS

3.1. Sperm kinematics

Following 1 hour incubation of the separated sample, the effect of CoQ10 and ALA were mostly seen on 2 and 4 minutes of separation **[Table–1 and –2]**. While only 0.375 µM of CoQ10 ($p<0.05$) gave a significant effect in sperm population isolated at 6 minutes of separation particularly on WOB.

The effect of CoQ10 dominated sperm progression at every minutes of separation **[Table–1, –2, and –3]**. However, at 4 minutes of separation, 3 µM of CoQ10 ($p<0.05$) showed a significant changes on VCL over 0 µM and 6 µM of CoQ10 **[Table–2]**.

Following 1 hour incubation of the separated sample with 1 mM of ALA caused significant changes mostly on sperm velocity isolated after 2 **[Table–1]** and 4 minutes **[Table–2]**. However, there was not much effect of ALA on sperm progression. The population incubated in 1 mM of ALA isolated at 4 minutes after separation **[Table–2]** which shows a significant changes on STR and LIN ($p<0.05$) were the only exception. The other series of ALA concentrations (0.025, 0.0125, 0.0625 and 0 mmol/L of ALA) were not demonstrated in the table. This was because spermatozoa incubated in these series of ALA concentration shows a very low motility and/or non–motile at all.

Parameter of motility	Concentration of CoQ10 (µM)						Concentration of ALA (mM)		
	0	0.375	0.75	1.5	3	6	0.05	0.1	1
Velocity (µm/s)	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
VAP	71.77±1.19	75.33±0.12	81.03±9.51	70.27±7.28	82.39±4.98	69.78±5.23	64.10±0.00	68.80±3.37	85.50±3.64[1,2]
VSL	66.90±0.34	71.13±0.43	64.73±1.77	63.93±3.21	75.16±3.67	66.54±4.21	58.00±0.00	63.50±3.47	75.50±3.29[1,2]
VCL	90.60±1.50	100.10±2.30	88.83±3.53	89.60±15.90	99.76±13.80	83.36±6.06	109.00±0.00	98.50±6.80	131.9±6.89[1]
Progression (%)									
WOB	79.23±0.35	75.33±1.88	91.70±12.25	80.80±6.81	126.72±45.50	81.02±2.68	58.80±0.00	72.00±4.97	65.60±1.97[1]
STR	93.40±1.05	94.43±0.52	82.17±9.84	92.03±5.01	91.90±1.25	95.90±1.01[c]	90.60±0.00	92.40±2.16	88.30±0.73
LIN	75.67±0.78	74.67±2.33	75.33±0.88	76.33±9.83	71.89±3.46	78.60±2.72	57.00±0.00	67.20±5.63	59.20±1.97

Table: 1. Effect of different concentration of CoQ_{10} and ALA on sperm population isolated after 2 minutes of electrophoretic separation: c= Concentration of CoQ_{10} is significantly different compared to 0.75µM of CoQ_{10} ($p<0.05$), 1= Concentration of ALA is significantly different compared to 0.05 µM of ALA ($p<0.05$), 2= Concentration of ALA is significantly different compared to 0.1 µM of ALA ($p<0.05$)

[IV] DISCUSSION

Currently male fertility status is solely dependent on the semen quality evaluation. One of the most current important parameters used to evaluate the semen quality is sperm motility. Low number of motile sperm and abnormal sperm morphology are the common contributing problems in human as well as in animal exhibiting a fertility problem. Although the evaluation of semen quality could not fully rely upon sperm motility assessment, it has been well proven that immotile and/or poorly motile sperm will yield a very low fertilization rate. In certain circumstances, there is no fertilization of the oocyte unless advanced assisted reproduction techniques (ARTs) were used.

In this study, the most significant changes on sperm motility could be seen in sperm population isolated after 2 and 4 minutes of separation. There was not much effect of both antioxidants on sperm population isolated at 6 minutes. However, only 0.375 µM of CoQ10 ($p<0.05$) had showed a significant effect in sperm population isolated at 6 minutes particularly on WOB. The different effect seen in every population isolated at each minutes might probably be due to the differences in quality of the isolated sperm population. This isolation technique was based on sperm surface charged [20] and the surface charged is inevitably dependence on the integrity the sperm membrane [21]. The integrity of the sperm membrane might further influence the motility regulation of the spermatozoa. So, here we postulated that the sperm population isolated after 2 and 4 minutes of separation is the most desirable and in good quality rather than sperm population isolated after 6 minutes.

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Parameter of motility	Concentration of CoQ10 (µM)						Concentration of ALA (mM)		
	0	0.375	0.75	1.5	3	6	0.05	0.1	1
Velocity (µm/s)	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
VAP	69.02±2.16	69.47±3.58	87.97±5.02	88.13±8.35	85.07±7.13	73.67±8.57	60.50±0.71	59.60±1.73	74.93±2.18[1,2]
VSL	65.27±2.64	66.23±2.97	75.47±3.14	83.43±8.32	73.23±5.06	72.56±8.76	57.60±0.90	56.30±0.96	66.80±1.65[1,2]
VCL	82.35±2.92	86.63±9.67	131.07±12.10	110.53±10.61	131.80±12.50[a,f]	39.10±8.78	85.00±0.55	84.70±3.71	108.40±3.61[1,2]
Progression (%)									
WOB	84.08±2.79	81.4±5.47	67.57±2.60[a]	79.77±1.19	65.68±2.43[a,b,d]	92.98±1.68[c,e]	71.10±0.38	71.50±3.10	69.60±1.06
STR	94.53±2.44	95.47±1.13	86.00±2.25[a]	94.57±0.80	87.06±2.33[a,b,d]	85.41±12.50[c,e]	95.00±0.40	94.70±1.26	89.70±1.12[1,2]
LIN	80.83±4.02	80.00±5.29	61.33±4.09	78.00±2.08	59.50±3.29	91.23±1.86	69.60±0.22	67.70±2.88	63.30±1.04[2]

Table: 2. Effect of different concentration of CoQ_{10} and ALA on sperm population isolated after 4 minutes of electrophoretic separation: a= Concentration of CoQ_{10} is significantly different compared to 0µM of CoQ_{10} ($p<0.05$), b= Concentration of CoQ_{10} is significantly different compared to 0.375µM of CoQ_{10} ($p<0.05$),c= Concentration of CoQ_{10} is significantly different compared to 0.75µM of CoQ_{10} ($p<0.05$), d= Concentration of CoQ_{10} is significantly different compared to 1.5µM of CoQ_{10} ($p<0.05$),e= Concentration of CoQ_{10} is significantly different compared to 3µM of CoQ_{10} ($p<0.05$), f= Concentration of CoQ_{10} is significantly different compared to 6µM of CoQ_{10} ($p<0.05$),1= Concentration of ALA is significantly different compared to 0.05 µM of ALA ($p<0.05$), 2= Concentration of ALA is significantly different compared to 0.1 µM of ALA ($p<0.05$)

Parameter of motility	Concentration of CoQ10 (µM)						Concentration of ALA (mM)		
	0	0.375	0.75	1.5	3	6	0.05	0.1	1
Velocity (µm/s)	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
VAP	74.43±2.26	77.37±3.62	87.97±7.42	75.23±2.83	83.96±3.80	97.83±9.27	80.80±6.12	82.60±3.41	74.90±2.99
VSL	69.03±2.11	70.93±3.04	76.23±2.80	70.40±1.27	78.10±3.48	90.10±8.44	73.20±4.19	75.20±4.15	69.50±2.68
VCL	108.93±5.08	100.03±3.83	135.90±19.04	96.33±9.11	118.23±7.77	159.43±18.50	11.50±8.39	119.20±6.29	91.00±2.93
Progression (%)									
WOB	68.90±1.05	77.30±0.81[f]	65.67±3.37	78.93±4.43	72.73±2.46	64.07±1.87	73.00±2.43	70.20±3.18	81.60±1.03
STR	92.73±0.42	91.70±1.13	87.37±4.44	93.70±1.83	93.13±0.80	92.30±0.20	91.70±1.05	90.60±1.62	93.10±0.79
LIN	66.67±0.82	73.33±0.66[f]	61.33±6.69	77.00±4.93	69.43±2.84	61.00±1.86	67.50±2.84	66.00±3.62	76.40±1.25

Table: 3. Effect of different concentration of CoQ_{10} and ALA on sperm population isolated after 6 minutes of electrophoretic separation: f= Concentration of CoQ_{10} is significantly different compared to 6µM of CoQ_{10} ($p<0.05$)

Following 1 hour incubation of the isolated spermatozoa, this study demonstrated that there were no significant changes on sperm velocity in sperm population incubated with CoQ_{10}. The only exception was the population isolated at 4 minutes of separation. In this population, incubation with 3 µM of CoQ_{10} gave a significant changes on VCL compared to 0 µM and 6 µM of CoQ_{10} ($p<0.05$) **[Table—2]**. Based on these previous evidence, we believe that any CoQ_{10} or ALA concentration which significantly affects VCL alone, is not suitable choice for conduction of ARTs especially IVF. According to Grippo et al. (1995) the increase in linearity and straightness of sperm movement are responsible for accelerated progressive movement. These patterns of motility are essential by the spermatozoa to generate force in order to penetrate zona pellucida instead of quiescent state (VCL) of the spermatozoa prior to fertilization [22]. On the same note, Rodriguez-Miranda et al. (2008) showed that there was an increased in values for VSL and LIN in extracellular ATP (ATPe)-treated capacitated sperm. The success rates of IVF using the treated sperm were also increased [23]. Therefore, the spermatozoa that swim faster and straighter are more likely to give a great impact on IVF success rate and not the curve movement of the spermatozoa.

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CoQ_{10} at 6 µM concentration caused significant changes on sperm progression in each separation time. The mechanism by which CoQ_{10} significantly affected the sperm progression is still to be determined. The possible explanation is the involvement of the CoQ_{10} in the regulation of energy production as it is concentrated within the mitochondrial midpiece [15]. Since soluble adenylyl cyclase (sAC) which is vital for sperm motility activation is also confined to the midpiece region, it is possible for this highly lipophilic antioxidant to diffuse and directly protect the sAC. Subsequently it will influence the pattern of sperm progression.

On the other hand, the effect of ALA that observed primarily on sperm velocity might be due to the properties of ALA. ALA could have provided a shield throughout the sperm membrane. ALA also offers protection for both outer leaflet (aqueous layer) and the inner leaflet of the phospholipids (lipid layer). These properties might offer a wider fortification throughout the membranes of the midpiece and principal piece. In turn, the energy production is maintained and it may also retain membrane fluidity at the principal piece. Both energy production and membrane fluidity are vital for membrane-dependant regulation particularly for the regulation of motility. The latter would be critically important for the regulation of plasma membrane Ca^{2+} channels, CatSper1 and CatSper2. This channels which are responsible for hyperactivated motility are confined to the principal piece [24]. Thus incubation of the sperm with ALA might directly accelerate the sperm velocity of the spermatozoa by influencing those channels present at the principle piece.

Based on the results we postulated that the particular effect of CoQ_{10} and ALA on sperm progression and sperm velocity respectively is most probably due to the nature of the antioxidant properties itself and the different motility regulatory system presence in the different segments of the sperm. We have demonstrated that 6 µM of CoQ_{10} shows a significant change on sperm progression in bull. In contrast 1 mM of ALA gives significant changes on the velocity of the sperm in bull. Both are crucial for improvement of sperm motility in bull population exhibiting the low motile sperm

[V] CONCLUSION

In conclusion, both CoQ_{10} and ALA may act as an ideal antioxidant for incubation of the sperm prior to ARTs. Incubation of spermatozoa with CoQ_{10} will improve the pattern of sperm movement. On the other hand, incubation of spermatozoa with ALA gives a great impact on sperm velocity. However, little is known about the exact mechanism by which CoQ10 and ALA influences the sperm progression and velocity of the sperm respectively. However, there are increasing interests to ascertain to what extent sperm motility would be affected if the concentrations of CoQ_{10} and ALA are raised above 6 µM and 1 mM respectively. This could be determined through further research in the future.

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RESEARCH ARTICLE OPEN ACCESS

PREVALENCE OF LIVER FLUKES INFECTIONS IN SLAUGHTERED ANIMALS IN KASHAN, ISFAHAN PROVINCE, CENTRAL IRAN

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ABSTRACT

Liver fluke are common parasites of herbivores in most of Middle East countries including Iran. The choroic infections of this parasite cause biliary liver cirrhosis in cattle, sheep and goats that leads to huge economic losses. This cross-sectional study was carried out to determine the prevalence of fascioliosis and dicrocoelioss in Slaughtered animals in Kashan,Isfahan Provience central Iran. A total of 267802 liver stock including 9066 cattle, 77912 sheep and 180824 goats and were slaughtered in the 2-year period were examined and overall 31954 (12%) livers were infected. Fascioliasis and dicrocoeliosis were responsible for 4.8% and 5.6% of total liver condemnations in this period, respectively.The infection rate of female sheep was more than males, but in female cattle and goats was lower than males. Data showed significant seasonal pattern for Dicrocoelium dendriticum in sheep and goats, but no for Fasciola in different animals . Liver condemnations due to fascioliasis and dicrocoeliosis were more prevalent in cattle slaughtered during spring. This survey provides baseline data for the future monitoring of these potentially important parasitic infections in this region.

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KEY WORDS

Prevalence; fascioliasis; dicrocoeliosis; kashan; isfahan Province; Iran

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[I] INTRODUCTION

Ruminant's contamination with parasites can cause reduction of milk production and many disorders such as diarrhea, loss of weight gain, abdominal pain, anemia and cachexia. In some parasitic diseases, liver is an important organ that is infected with parasites [1, 2]. Liver flukes (Fasciola sp and Dicrocoelium dendriticum) have especially economic importance by mortality, morbidity, and reduced growth rate, condemnation of liver, increased susceptibility to secondary infections and the expense of control measures and public health importance in many countries including Iran [2, 3, 4] Most of mammals are definitive hosts for these parasites such as sheep, goats and cattle are the most important animals in human environment. Due to complicated detection of these worms, definite recognizing of these parasitic diseases in live animals is performed in slaughterhouses.

The incidence of human fascioliasis has been increasing in 51 countries of the five continents [3, 5, 6]. Recent papers estimate human infection up to 2.4 million, up to 17 million people, or even higher depending on the unknown situations in many countries, mainly of Asia and Africa [5, 7]. Whereas, dicrocoeliosis occasionally affects humans [8, 9]. In Iran human fasciolosis was sporadic until 1987, when an outbreak occurred in Iran (Gilan Province) and affected more than 10,000 people [10]. The second outbreak occurred 10 years later and several thousand people were infected [10]. Reports of several hundred cases of human disease during interepidemic periods and recent years are present. In Mazandaran Province, fascioliasis has very recently shown to be a major human health problem too [9]. Recently, a minor emergence of fascioliasis, with 17 non-fatal cases, reported in the Kermanshah, western Province of Iran [11]. Human dicrocoeliosis has already been established in Iran (Isfahan Province) by Farid [9], though that is very rare. In the absence of statistically and epidemiologic data, evaluating liver fluke prevalence in livestock based on liver condemnation statistics might be useful. Information about infections of cattle, sheep and goats with liver fluke in south-western Asia were reported from some countries such as Iraq [12], Pakistan (Kashmir) [13], Saudi Arabia [14] and Turkey [15]. An old report has only been published on prevalence of Liver fasciolosis in sheep, cattle, goats and buffaloes from Ahwaz, Iran [16], although several reports exist on those in other regions of Iran [4,17]. Since in central Iran, there are high farms and there was not any data about Fasciola and Dicrocoelium, this survey was designed to study the presence and distribution of liver flukes in pastured ruminants in Kashan, Isfahan Province during 2007-2009.

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[II] MATERIALS AND METHODS

The total numbers of slaughtered animals and liver collection were recorded for cattle, sheep and goats. The weekly visits were made between 20 Apr, 2007 to 20 May, 2009. Liver of 267802 animals including 9066 cattle, 77912 sheep and 180824 goats examined according to the method described by Ogambo-Ongoma [18]. The livers of a total of 666 cattle, 7726 sheep and 23562 goats livers were inspected according to the method described to recognize fascioliasis and dicrocoeliosis.The parasites were identified by morphological characteristics of them [19, 20]. The recorded data, acquired with visualization, palpation and incision of livers, was used to extract the prevalence rate of these parasites. The prevalence rate was sorted monthly to determine the difference between distribution of infection rate and sex, season. Analysis of data was done, using Epi Info software (Version 6.0).

Seasonal pattern was investigated with chi-square ($\chi 2$) test. The P-value less than 0.05 considered statistically significant.

[III] RESULTS

Totally 267802 animals (Cattle 9066, Sheep77912 and goats 180824) and overall 31954 (12%) livers were condemned. Fasciolosis and dicrocoeliosis were responsible for 4.8 and 5.6 % of total liver. Among 666 livers of cattle, 2.4 and 2.7 %, of 7726 sheep 6.9 and 7.6 % and of 23562 goats 4.1 and 5 % were positive for Fasciola spp and Dicrocoelium dendriticum, respectively. There was highly significant difference in liver flukes infection between animals. Infection rate of Fasciola spp and Dicrocoelium in female cattle, sheep and goats was higher than males and female's sheep and goats were lower than males **[Table–1]**.

There was highly significant difference in Fasciola and Dicrocoelium infection between cattle, sheep and goats. Infection of cattle was considerably lower than sheep and goats ($p<0.001$).

Data showed significant seasonal pattern for Dicrocoelium in cattle, sheep and goats ($p<0.001$) and for Fasciola hepatica in different animals there were statistically significant differences with respect to season ($p<0.005$) **[Table– 2and 3]**.

The highest co-infection was found in goats (2.3%), followed by sheep (1.2%) and no cattle showed co- infection.

Table: 1. Prevalence of Fasciola spp and Dicrocoelium dendriticum infection in male and female animals slaughtered in Kashan, central Iran, 2007-2009.

Animals	No. of animals examined			No. of animals infected with Fasciola (%)			No. of animals infected with dicrocoelium (%)		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
Cattle	68	598	666	4(5.9)	12(2)	16(2.4)	4(5.9)	14(2.3)	18(2.7)
Sheep	810	6916	7726	50(6.1)	488(7)	538(6.9)	56(6.9)	530(7.6)	586(7.6)
Goat	2474	21088	23562	108(4.3)	870(4.1)	978(4.1)	186(7.5)	1010(4.8)	1196(5)

Table: 2. Seasonal prevalence of Fasciola spp infection in animals slaughtered in Kashan, central Iran, 2007-2009.

Animals	Fasciola spp							
	Spring		Summer		Autumn		Winter	
	Ex	Inf (%)	Ex	Inf (%)	Ex	Inf (%)	Ex	Inf (%)
Cattle	136	6(4.4)	184	4(2.2)	210	2(0.9)	136	4(2.9)
Sheep	1804	214(11.9)	2224	98(4.4)	1518	52(3.4)	2180	174(8)
Goats	5816	364(6.2)	6402	160(2.4)	5796	172(2.9)	5548	282(5)

Ex= No of examined animals INF= No infected animals
Seasonal pattern was investigated with chi-square ($\chi 2$) test.

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Table: 3. Seasonal prevalence of Dicrocoelium dendriticum infection in animals slaughtered in Kashan, central Iran, 2007-009.

Animals	Dicrocoelium dendriticum							
	Spring		Summer		Autumn		Winter	
	Ex	Inf (%)	Ex	Inf (%)	Ex	Inf (%)	Ex	Inf (%)
Cattle	136	8(5.9)	184	2(1.1)	210	4(1.9)	136	4(2.9)
Sheep	1804	224(12.4)	2224	132(5.9)	1518	62(4)	2180	168(7.7)
Goats	5816	422(7.2)	6402	236(3.7)	5796	266(4.6)	5548	272(4.9)

Ex= No of examined animals INF= No infected animals

Seasonal pattern was investigated with chi-square (χ2) test.

[IV] DISCUSSION

The prevalence rate of liver flukes in herbivores varies considerably throughout the world. Fasciola spp and Dicrocoelium dendriticum are common parasites of ruminants in different parts of Iran [2, 4, 16, 17].

At the end of the 1980s and during the 1990s several large epidemics, including thousands of human fascioliasis, were reported [10, 17] in the northern regions of Iran, where it has an endemic foci. Bandar Anzali city, Gilan Province is an endemic area. In 2000, there was a minor emergence of fasciolosis, in the Kermanshah western province of Iran [11].

In the present survey, fascioliasis and dicrocoeliosis were responsible for 4.8% and 5.6% of Total liver infected, respectively. On the other hand the mean prevalence of Fasciola spp in cattle 2.4%, sheep 6.9% and goats was 4.1% respectively. As such mean prevalence of Dicrocoelium dendriticum in cattle, sheep and goats was 2.7%, 7.6% and 5% respectively **[Table–1]**. The infection rate in female sheep was more than males, but the infection rate in female cattle and goats was lower than males **[Table–1]**, which was in agreement with the data obtained in our study. Liver condemnation due to Fasciola spp. and dicrocoelium dendriticum in slaughtered cattle during this survey was almost 2.8 and 1.7 folder than those observed in sheep and goats, respectively. The epidemiologic implication of this finding might be attributed at least partly to the sources of their main food. Main food of sheep and goats belonged to plants which are present in mountains and plains, while cattle are mainly feed with herbs close to the sources of water such as

slough, stream, creek, and swampland. It is clear that the plants which are close to water due to higher infection with Fasciola metacercaria might be attributed in more distribution fascioliasis in sheep. In a slaughterhouse survey in ruminants of Tehran, 25.5% of cattle, 31.2 % of sheep and 64.3% of goats were infected with Fasciola hepatica [21]. The overall prevalence of fascioliasis was lower than previous report in the region by Ahmadi NA et al. [16], that 35.1% of cattle, 22.8% of sheep and 11.4% of goats. Daryani et al. [4], in a study in Ardabil Province, reported that prevalence of Fasciola spp. in cattle, sheep and goats was 25.9%, 5.3%, and 4.9%; as such prevalence of Dicrocoelium dendriticum in those animals was 10.6%, 6.8%, and 12.4%, respectively. In a slaughterhouse survey of ruminants of Mazandaran Province 4.6% of cattle, 5.7% of sheep and 1.6% of goats were infected with Fasciola spp [17] Other studies were carried out in Iran, reported variable prevalence rates of Fasciola spp. and D. dendriticum in different regions of the country. A study conducted by Daryani et al. reported prevalence rate of fascioliasis in cattle and sheep in Guilan Province which were 25.9 % and 5.3 %, respectively, whereas prevalence rate of dicrocoeliosis in cattle and sheep were respectively 10.6 % and 6.8 %(6), Sahba et al. informed that 82 % and 27.1 % of cattle and sheep livers were infected in Khouzestan province by F. hepatica, respectively [22]. In a study conducted by Movassagh Ghazani and Valilou in the northwest region of Iran, 8.57 % and 20 % of sheep livers were infected by F. hepatica and D. dendriticum, respectively [23]. Saffarbani observed that 20 % and 18.6 % of sheep livers were infected with F. hepatica and D. dendriticum in a slaughterhouse in Ardabil, respectively [24], Eslami. Observed that prevalence rate of F. hepatica in ruminants of Guilan and Mazandaran provinces was 21.5 % and12 % and this rate for Tehran province was 25.5 % of cattle, 31.2 % of sheep and 64.3 % of goats were infected with F. hepatica [21]. Radfar and Sakha studied prevalence rate of fascioliasis and dicrocoeliosis in sheep which were 1.5 % and 0.22 % in Kerman slaughterhouse [25]. In a study performed in slaughterhouse of Khorram Abad in Lorestan province, 9.5% of sheep and goats were infected with liver trematodes and 1.6% of liver were condemned [26]. Almost 4.1% of sheep slaughtered in Shahr-e Kord were infected with Fasciola hepatica and infection rate in female animals was more than

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males [27]. This was in agreement with the data obtained in our study. Ansari-Lari and Moazzeni's study the prevalence rate of fasciolosis in cattle and sheep were 2.91 % and 2.10 %, respectively, whereas the prevalence rate of dicrocoeliosis were 1.00 % and 0.80 % in cattle and sheep in Shiraz, respectively[28]. In a survey of carried out on sheep slaughtered in Kerman, prevalence of Fasciola hepatica and Dicrocoelium was 1.5 and 0.22%, respectively. Co-infection rate has been reported 0.33% that 0.27% of them showed intense infection to result in total condemnation of liver [25].

Studies carried out in the neighboring countries of Iran have reported different prevalence in different animals. In Pakistan (Kashmir), infection rate of Fasciola hepatica in cattle, sheep and goats was 85.1%, 51.3%, and 14.8%, respectively [13]. In Turkey, 3.99% and 23.55% of sheep and 0.48% and 2.65% of cattle were infected with Fasciola hepatica and Dicrocoelium dendriticum, respectively [15]. Gargili et al. reported that the prevalence rate of fascioliasis and dicrocoeliosis in Turkey, were 3.99 % and 23.55 % in sheep and 0.48 %, 2.65 % in cattle, respectively [15]. In Iraq, an abattoir survey in Basrah revealed that the prevalence for hepatic fasciolosis among cattle, sheep and goats was 0.13%, 0.72%, and 3.30%, respectively [29]. The corresponding figures from Saudi Arabia fascioliasis were 1.20%, 0.04%, and 0.00% in cattle, sheep and goats, respectively [14]. In Brazil, 10.34% of cattle and 20% of buffaloes were infected with liver trematodes [30]. In a survey carried out in 7 provinces of Kenya within a period of 10 years (1990-1999), infection rate of Fasciola hepatica in cattle was 0.8% [31]. On the whole, infection with Fasciola spp and Dicrocoelium dendriticum in ruminants of Kashan was less than different researchers at more different area in Iran, in all species [4, 17]. In comparison to Iran, Pakistan (Kashmir region), a neighboring country, has shown a higher rate in all species [13], but infection with Fasciola in livestock of Isfahan Province (Kashan-Iran) was more than that in Saudi Arabia (for all species) and in Turkey (only for cattle) [14,15]. Infection rate caused by fasciolosis in small ruminants (goats) of Iraq [12] was similar to our results. In comparison to Turkey, Iran (Kashan) showed lower rate of dicrocoeliosis in all species. As it shown above, the prevalence rate of fascioliasis is higher than dicrocoeliosis in most studies but the results of this study were different and showed prevalence rate of dicrocoeliosis (1.2%) higher than fascioliasis in cattle, sheep and goats that slaughtered in Kashan slaughterhouse. This high prevalence of dicrocoeliosis can be probably due to more anti helminthes resistance of Dicrocoelium dendriticum than Fasciola spp. in the country. Data showed significant seasonal pattern for fascioliasis and dicrocoeliosis in cattle, sheep and goats **[Tables–2 and –3]**. This is close to the results reported from Ardabil by Daryani et al. [4], and Mazandaran by Moghaddam et al. [17]. As it is clear from **Tables –2 and –3**, liver condemnations due to fascioliasis and dicrocoeliosis were more prevalent in cattle slaughtered during spring (4.4% and 5.9%), sheep (11.9% and 12.4%) and goats (6.2% and 7.2%) respectively. Different weather in different seasons in Kashan area may be differences in parasitic infection.

[V] CONCLUSION

Liver Infection due to fascioliasis and dicrocoeliosis were more prevalent in cattle slaughtered during spring in sheep and goats respectively. Different weather in different seasons in Kashan area may be differences in parasitic infection

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MALE CIRCUMCISION: ITS ROLE IN HIV PREVENTION

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ABSTRACT

BACKGROUND: *The findings from observational studies, reviews and meta-analyses, supported by biological theories, that circumcised men appear less likely to acquire human immunodeficiency virus (HIV) has contributed to support for considering MC as a strategy for preventing sexually acquired infection. We sought to elucidate and appraise the global evidence from published studies that circumcision can be used as an intervention to prevent HIV infection.* ***OBJECTIVES:*** *This review summarizes the evidences for the potential of MC to prevent HIV.* ***SELECTION CRITERIA:*** *We searched for reviews and observational studies and compare acquisition rates of HIV-1 in circumcised and uncircumcised heterosexual men.* ***CONCLUSIONS:*** *We found insufficient evidence to support an interventional effect of MC on HIV acquisition in heterosexual men. The results from existing observational studies show a strong epidemiological association between MC and prevention of HIV, especially among high-risk groups. However, observational studies are inherently limited by confounding which is unlikely to be fully adjusted for. In the light of forthcoming results from RCTs, the value of IPD analysis of the included studies is doubtful. The results of these trials will need to be carefully considered before circumcision is implemented as a public health intervention for prevention of sexually transmitted HIV.*

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KEY WORDS

HIV;circumcision; sexual transmission; prevention; sexually transmitted disease

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[I] INTRODUCTION

Male Circumcision (MC) is practiced in different societies all over the world, for religious, cultural/secular, and medical reasons. MC as a religious tradition is practiced by Jews and Muslims, usually during the neonatal period [1]. Within sub-Saharan Africa, MC is most often performed for cultural reasons and is largely determined by ethnicity [2]. In all situations, cultural differences between circumcised and uncircumcised men may affect their sexual and hygienic behavior, including their exposure to various STD and HIV-1 infection. About 30% of men are estimated to be circumcised worldwide, although this rate is still less than 20% in Europe. The procedure is often done shortly before or at puberty and is considered a rite of passage to adulthood.

In United States, MC is largely a secular decision, and its frequency has changed over time. The American Academy of Pediatrics Task Force on Circumcision stated in its 1999 revision on circumcision policy that circumcision conferred potential medical benefits, which could be considered by parents but that the scientific evidence, was insufficient to warrant recommending routine neonatal circumcision [3]. Currently, 80% of men in the United States are circumcised, with higher rates for Caucasians than for African Americans and Hispanics [2]. In contrast, circumcision is less often practiced in Canada and in Europe [4] and within Asia there is considerable country-to-country variation in circumcision prevalence [5]. Circumcision has many health benefits which include: easier hygiene, decreased risk of urinary tract infections, prevention of penile problems like phimosis, decreased risk of penile cancer, decreased risk of sexually transmitted diseases including HIV-1.

In the 150 years since Moses [6] published his findings, a number of studies have evaluated the effect of circumcision on the acquisition of HIV-1 and STDs. The majority has found a protective effect of MC on acquisition of genital ulcers and HIV-1. Despite the consensus that emerges from the literature, the implementation of circumcision promotion as a population-based intervention to reduce HIV-1 and STD incidence has not been seriously entertained. We acknowledge that attitudes toward MC may be difficult to change in some settings, but we encourage behavioral scientists to conduct acceptability studies, particularly in high HIV -1 prevalence communities, to begin assessing feasibility of circumcision promotion.

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[II] EPIDEMIOLOGICAL EVIDENCES

Male Circumcision markedly decreases the acquisition of HIV-1 infection, the major epidemic of our time. This is the first biological intervention shown to prevent HIV-1 infection and will not depend upon continuing behaviour change to give protection. In 1986, five years after the description of AIDS, the first article suggesting that MC is associated with lower risk of human immunodeficiency virus (HIV-1) infection was published. During the following years, different studies almost exclusively from sub-Saharan Africa, which quickly became the centre of HIV-1 epidemic increasingly supported this hypothesis [7]. The tribes and other defined populations with low prevalence of MC had high prevalence of HIV-1 infection, suggesting a correlation between MC and HIV-1 prevention [8, 9, 10].

Several physiologic mechanism MC might explain the association between an intact foreskin and increased risk of HIV-1 and genital ulcers. In uncircumcised men, the epithelium lining the glans and preputial sac is thinner and less cornified than that of circumcised men and therefore may be more susceptible to traumatic lesions during sexual intercourse and to the transfer of microorganism between partners [11]. The environment of the preputial sac may be favorable for the survival and replication of bacteria and viruses, allowing for a longer exposure time for infections to occur. This effect may be accentuated by poor hygienic practices. Finally, the stratified squamous epithelium of the foreskin contains target cells for HIV-1 (Langerhans cells and macrophages that are coated with CD4 receptors) [12]. It has been suggested that following circumcision, the surface epithelium of the glans develops a protective keratin layer, a form of natural condom [13]. Thus, circumcision could reduce the HIV-1 incidence by directly decreasing the susceptibility of uninfected men to HIV-1. Circumcision could also reduce the incidence of HIV-1 by directly decreasing the infectivity of men with HIV-1, as suggested by the studies of tissue samples collected from macaques infected with the simian immunodeficiency virus (SIV), which showed infected mononuclear cells in the dermis and epidermis of the penile foreskin [14].

Despite that, there is still uncertainty among many scientists and public health scientific societies, mainly due to the fear that circumcised men have different (safer) sexual practices than men who are not, and that this and not MC led to lower rates of HIV-1 infection in circumcised men and in populations where circumcision is common. Furthermore, some or all sexually transmitted diseases (STD) may increase men's susceptibility to HIV-1 [15, 16]. If circumcision reduces the transmission of genital infections, either by improving local hygiene or by accelerating the healing of otherwise subpreputial lesion circumcision may also delay HIV-1 transmission [17]. Therefore, potential associations between the lack of circumcision and STD other than HIV-1 are also of interest.

Surprising to some, multiple studies have consistently shown that populations which do not traditionally circumcise their children or young adults will readily accept MC. In 13 studies carried out in sub-Saharan Africa, 65% of men were willing to be circumcised and 69% of women favoured MC for their male partners. Improved hygiene, sexual satisfaction, and partial protection from HIV-1 are cited as principal reasons. The widespread implementation of MC in Southern sub-Saharan Africa, where prevalence of MC is generally low and HIV-1 is very common, could prevent 2,000,000 infections over a 10-year period. While it is unclear if a circumcised man who is HIV-positive is less likely to transmit HIV-1 to a woman than if he is not circumcised, women in general would benefit from increasing rates of MC because fewer men would become infected with HIV-1. WHO recommends that countries where the incidence of heterosexually acquired HIV-1 infection is high and the diffusion of MC is low urgently consider implementing the access to MC services as a priority. WHO examined all the available data about the effectiveness of MC in preventing HIV-1 infection, and recognized MC as an additional important intervention to reduce the risk of heterosexually acquired HIV-1 infection in men. Adequate resources should be rapidly mobilized to support the expansion of safe MC services within the context of moving towards universal access to comprehensive HIV prevention, treatment and care [18, 19].

Over the past decade, numerous epidemiological studies have reported a significant association between lack of MC and risk for HIV -1 infection, leading to recommendations for MC to be added to the armamentarium of effective HIV-1 prevention strategies. We review the epidemiological data from studies that have investigated this association, including ecological, cross-sectional/case-control, and prospective and retrospective studies. An individual's choice to undergo MC or a community's decision to promote the practice should be made in the light of the best available scientific evidence.

According to a recent meta-analysis conducted by Vermund *et al*., MC has been shown to protect men from acquiring HIV-1 infection during sex with women. It has reduced female-to-male transmission rates by 48% to 60% in sub-Saharan Africa but that protective effect appears less reliable among men who have sex with men. It encompasses data from 15 studies conducted in seven countries, involving more than 53,000 men, most of whom were Caucasian and approximately half of whom were circumcised. The authors concluded that being circumcised reduced a man's risk of acquiring HIV-1 by 14%. Though the finding was statistically non significant, but the authors advocated that it should be regarded as a launching point for future trials. Millett's analysis found that in studies

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conducted before 1996 before the advent of highly active antiretroviral therapy circumcision was associated with a statistically significant 53% reduction in HIV-1 transmission risk, which is on par with the 48% to 60% reduction in infection rates reported by the 2007 trials in Kenya, South Africa and Uganda that studied heterosexual men. After 1996, however, when antiretroviral (ARV) drugs turned HIV-1 into a condition that people lived with rather than died from, the protective effect of circumcision became non significant [19, 20, 21].

Bailey *et al*., in 2007 conducted the two studies and found a protective effect of 53% and 60% respectively in men who were circumcised, compared to those who were not The trial enrolled 2,784 men and was carried out on the behalf of the U.S. National Institutes of Health and the Canadian Institute for Health Research, while the other study, also sponsored by the National Institutes of Health, randomized 4,996 men. Each of these trials was also stopped prematurely in December 2006, due to an extremely high efficacy rate. The findings of the studies are similar, and remarkably consistent with the protective effect (58% on average) found in a systematic review of observational studies available in medical literature. This is the first published prospective study with this finding in an occupationally based cohort, which may be more representative of the general population than cohorts recruited from STD clinics. In this prospective cohort study, uncircumcised men were at 4-fold increased risk for acquiring HIV-1 infection. In addition to HIV-1 risk, uncircumcised status was associated with a 2.5-fold increased risk of genital ulcer disease [22].

Auvert *et al*., in 2005 showed a 60% protective effect against HIV-1 infection among the men who were circumcised. The study was conducted on the behalf of the South African National Institute for Communicable Diseases (Johannesburg) and the Institute National de la Santé et de la Recherche Médicale (ANRS Paris, France), involving 3,274 men who were randomized to receive circumcision or not. The subjects were followed over a mean period of 18.1 months, and the trial was stopped prematurely because of the high efficacy observed among circumcised patients [23].

Reynolds *et al*., in 2003, in a prospective study of 2298 HIV-1uninfected men attending sexually transmitted infection clinics in India, noted that circumcision was strongly protective against HIV-1 infection (adjusted relative risk 0•15; 95% CI 0•04–0•62; p=0•0089); however, they noted no protective effect against herpes simplex virus type 2, syphilis, or gonorrhoea. The specificity of this relation suggests a biological rather than behavioural explanation for the protective effect of MC against HIV-1[24].

Szabo and Short, in their excellent review made compelling epidemiological evidence from over 40 studies which showed that MC provided significant protection against HIV-1 infection; circumcised males were two to eight times less likely to become infected with HIV [25]. Furthermore, circumcision also protected against other sexually transmitted infections, such as syphilis and gonorrhoea [26] and since people who had a sexually transmitted infection were two to five times more likely to become infected with HIV-1[27] circumcision may be even more protective. The most dramatic evidence of the protective effect of circumcision came from study of couples in Uganda who had discordant HIV-1 status; in the study the woman was HIV-1 positive and her male partner was not [28] No new infections occurred among any of the 50 circumcised men over 30 months, whereas 40 of 137 uncircumcised men became infected during this time. Both groups had been given free access to HIV testing, intensive instruction about preventing infection, and free condoms (which were continuously available), but 89% of the men never used condoms, and condom use did not seem to influence the rate of transmission of HIV-1. These findings focused the spotlight of scientific attention onto the foreskin. That is its removal reduces a man's susceptibility to HIV-1 infection [29].

Grey *et al*., determined HIV-1 acquisition in a cohort of 5507 HIV-negative Ugandan men, and in 187 HIV-negative men in discordant relationships. Transmission was determined in 223 HIV-positive men with HIV-negative partners. HIV-1 incidence per 100 person years (py) and adjusted rate ratios (RR) and 95% confidence intervals (CI) were estimated by Poisson regression. HIV-1 serum viral load was determined for the seropositive partners in HIV-1discordant couples. The prevalence of circumcision were 16.5% for all men; 99.1% in Muslims and 3.7% in non-Muslims. Circumcision was significantly associated with reduced HIV-1 acquisition in the cohort as a whole (RR 0.53, CI 0.33-0.87), but not among non-Muslim men [30].

Prepubertal circumcision significantly reduced HIV-1 acquisition (RR 0.49, CI 0.26-0.82), but post pubertal circumcision did not. In discordant couples with HIV-negative men, no seroconversions occurred in 50 circumcised men, whereas HIV-1 acquisition was 16.7 per 100 py in uncircumcised men (P = 0.004). In couples with HIV-positive men, HIV transmission was significantly reduced in circumcised men with HIV-1 viral loads less than 50 000 copies/ml (P = 0.02).Prepubertal circumcision may reduce male HIV-1 acquisition in a general population, but the protective effects are confounded by cultural and behavioral factors in Muslims. In discordant couples, circumcision reduces HIV acquisition and transmission. This analysis confined to circumcised men, suggests that Muslims may generally be at lower risk of HIV-1 acquisition than non-Muslims, particularly in the age group 20-29 years. Although Muslims have a generally lower risk profile than circumcised non-Muslims, it is unclear what specific behaviors, other than

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abstinence from alcohol, might reduce the risk among Muslim men. However, key informant interviews suggest that the Islamic practice of post-coital cleansing before prayer may be an important factor explaining the lower incidence of HIV-1 in circumcised Muslim men [31, 32].

Moses *et al*., identified 26 cross-sectional studies regarding circumcision and HIV-1 prevention. Eleven studies found a significant difference in HIV-1 prevalence between circumcised and uncircumcised men after adjusting for potential confounders, including indices of sexual behavior, with odds ratios of 1.5–5.6. Six other studies found a significant difference, but no adjustment for possible confounders was reported [33, 34].

Lavrey *et al*., in 1999, conducted another prospective cohort study involving 746 HIV-1 seronegative trucking company employees, in Mombasa, Kenya; during the course of follow-up, 43 men acquired HIV-1 antibodies, yielding an annual incidence of 3.0%. The annual incidences of genital ulcers and urethritis were 4.2% and 15.5%, respectively. In this analysis, after controlling for demographic and behavioral variables, uncircumcised status was an independent risk factor for HIV-1 infection (hazard rate ratio [HRR= 4.0; 95% confidence interval [CI], 1.9–8.3) and genital ulcer disease (HRR= 2.5; 95% CI, 1.1–5.3). Circumcision status had no effect on the acquisition of urethral infections and genital warts. Uncircumcised status was associated with increased risk of HIV-1 infection and genital ulcer disease, and these effects remained after controlling for potential confounders [35].

Another meta analysis of 27 studies, conducted by Weiss *et al*. in 1999, that included circumcision as a risk factor for HIV-1 infection among men in sub-Saharan Africa, 21 studies showed a reduced risk of HIV-1 among circumcised men, being approximately half that in uncircumcised men (crude RR = 0.52, CI 0.40-0.68). In 15 studies that adjusted for potential confounding factors, the association was even stronger (adjusted RR = 0.42, CI 0.34-0.54). The association was stronger among men at high risk of HIV-1 (crude RR = 0.27; adjusted RR = 0.29, CI 0.20-0.41) than among men in general populations (crude RR = 0.93; adjusted RR = 0.56, CI 0.44-0.70). The meta analysis showed MC is associated with a significantly reduced risk of HIV infection among men in sub-Saharan Africa, particularly those at high risk of HIV-1. These results suggest that consideration should be given to the acceptability and feasibility of providing safe services for MC as an additional HIV-1 prevention strategy in areas of Africa where men are not traditionally circumcised [36].

Cameron *et al*. Studied the effect of circumcision on the risk of HIV-1 sero conversion in a group of male STD patients in Nairobi and found a risk ratio of 8.2 for uncircumcised men after adjusting for potential confounders. In studies of STD clinic patients in New York City and in Pune, India, there were trends for uncircumcised men to be at increased risk of HIV-1 acquisition, but these associations were not statistically significant [37, 38, 39].

Various retrospective studies including partner studies were done by researchers like Guimeraes in 1991 in Brazil, (sample size 109, O.R 0.4),Moss Kenya, 1991(sample size 70, O.R 0.4), Allen Rwanda,1991(sample size 1458 O.R 1.1) showed no statistical significance of association between HIV-1 serostatus and lack of circumcision, while as studies by Fischl,USA,1988, (sample size 92 OR 9.6) Hunter Kenya 1990,(sample size 623 , OR 3.7)and Hellman Uganda 1991 (sample size 42 OR 5.4) showed statistically significant association between HIV-1 sero status and lack of circumcision. In several other retrospective studies [40-49] male populations were recruited to look for risk factors for HIV infection, four (12, 38, 40, 42) reported significant associations between the lack of circumcision and an increased susceptibility to HIV-1 infection in men.

Furthermore, in a recent study by Baeten *et al*., 2010 MC modestly reduces the risk of an HIV-positive man transmitting HIV to a female sex partner. This prospective study was carried on a total of 1096 African HIV-1-serodiscordant couples which were analyzed for the relationship between circumcision status of HIV-1-seropositive men and risk of HIV-1 acquisition among their female partners. Analysis showed that HIV incidence was approximately 40% lower in these genetically linked transmissions amongst women whose partner was circumcised (hazard ratio 0.57; 95% CI, 0.29-1.11, p = 0.10). However, this could have been down to chance as this reduction in risk was not statistically significant [50].

[III] CONCLUSION

The About 70% of men infected with HIV-1 have acquired the virus through vaginal sex, and a smaller number have acquired it from insertive anal intercourse [49]. Thus, on a global scale most men who are HIV-1 positive have acquired the virus via the penis. Of the estimated 50 million people infected with HIV worldwide, about half are men, who become infected through their penises. The inner surface of the foreskin, which is rich in HIV receptors, and the frenulum, a common site for trauma and other sexually transmitted infections, must be regarded as the most probable sites for viral entry in primary HIV-1 infection in men [50, 51]. Although condoms must remain the first choice for preventing the sexual transmission of HIV-1, they are often not used consistently or correctly, they may break during use, and there may be strong cultural and aesthetic objections to using them. Cultural and religious attitudes towards MC are even more deeply held, but in the light of the evidence presented here circumcising males seems highly desirable, especially in countries with a high prevalence of HIV-1 infection. Circumcision at puberty, as practiced by many Muslim communities, would be the most immediately

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effective intervention for reducing HIV-1 transmission since it would be done before young men are likely to become sexually active. MC may protect HIV-1 negative men from acquiring HIV -1 infection to varying degrees. The effects are more modest in the general population, in which HIV-1 exposure and incidence are relatively low. Also, the apparent protective effects of circumcision are not consistently observed in all subgroups and are largely associated with Muslim religious affiliation, which could be a marker for unmeasured differences in cultural practices or sexual behaviors. [51] However, circumcision appears to be highly protective among HIV-1 negative men in a discordant relationship with an HIV-1 positive female partner, and circumcision may reduce HIV-1 transmission from HIV-1 positive men with viral loads of less than 50, 000 copies/ml. We believe that these observational data are not sufficient to justify the promotion of voluntary circumcision for HIV-1 prevention in the general population or in high-risk groups and those clinical trials are needed before policies on circumcision for HIV-1 prevention can be established.

It is also to be noted that nobody should frame MC as some sort of panacea. But it may prove to be one more tool in the toolbox. If we can add it to behavioral risk reduction, prompt diagnosis and access to care, it may be the combination needed to really knock the socks off the HIV-1 epidemic.

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EDITORIAL OPEN ACCESS

CAN HIV BE CURED?

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Earlier, it was observed that certain individuals do not get infected with HIV, even though they are classified as high risk individuals for HIV, as some of them were previously exposed to HIV numerous times. Surprisingly, these individuals did not show any signs and symptoms of HIV infection, even though they were not under any anti-retroviral treatment. These individuals considered as having "natural resistance" against HIV infections. Natural resistance against microbial infections is not a new phenomenon, resistance against other microorganisms is well known in case of other diseases like small pox [1], etc. Certainly occurrence for natural resistance in case of HIV is a new observation with enormous clinical implications. Further studies revealed that the main reason for this natural resistance against HIV is due to the requirement of other receptors, which are expressed on T-cells and essential for HV infectivity. Commonly, CD4 is the primary receptor for HIV infections, but chemokine receptors like CXCR4 and CCR5 has been shown to be essential for HIV infection and known as co-receptors. HIV strains are also classified into two categories as per their preferences for co-receptors like X-4 and R5 strain of HIV. It was realized that natural resistance against HIV infection is attributable to mutation in co-receptor CCR5. Among resistant individuals CCR5 gene has a deletion of 32 bp sequence. Some of resistant individuals are homozygous for Δ32 deletion, while some are heterozygous for Δ32 deletion. Individual with homozygous deletion have shown to be resistant with HIV, while individual with heterozygous deletion have shown an extremely slow progression of disease after exposure to HIV. This mutation was reported with low frequencies of ~1-3% that is only among Caucasians. Certainly, it was a significant finding to revisit the mechanism for HIV infections, which may offer new insights for the development of new therapeutic strategies to treat HIV.

This important observation remained a matter of laboratory studies and this has to wait another ~10-12 years to see its implications in real-life situation. Hutter *et al*. [2] published their findings in Feb. 12, 2009 issue of New England Journal of Medicine about bone marrow transplantation in a HIV seropositive patient. They performed allogenic bone marrow transplantation in February of 2007 in a HIV seropositive. The patient was a 40 year old male HIV seropositive under HAART regimen with no signs and symptoms of HIV infection that means HIV infection was under control due to HAART. But, this patient was presented with acute meylogenous leukemia (AML) and tried with chemotherapy to treat AML. Due to toxicity of drugs, HAART was stopped during chemotherapy of AML, as a result of which rebound of HIV was noticed. Unfortunately, chemotherapy for AML was not very helpful for this patient because AML relapsed. So the best option to treat the patient was to perform bone-marrow transplantation. Hutter being a hematologist in the team of clinicians took a cognizant decision to perform bone marrow transplantation. Apart from matching donor, Hutter opted to have a donor with homozygous mutation in CCR5 co-receptors, i.e., CCR5Δ32/Δ32. There were two reasons for Hutter to opt for this particular combination, 1) it reduces chances for GVHD rejection, and 2) possibilities are there that this patient may become naturally resistant to HIV. Therefore, bone marrow transplantation can treat this patient for AML as well as HIV, it is like hitting two birds with one stone.

Successful bone marrow transplantation in this patient led to discontinuation of HAART. This patient remained negative for HIV infection. But an episode of AML relapse was the reason for another bone marrow transplantation on 391th day after the first bone-marrow transplantation. This patient was closely followed for 20 months, and during observation period this patient did not show any signs and symptoms of HIV infection. On the one hand, these results showed a success for the cure for HIV, while on the other hand it has left many questions unanswered. These questions have valid scientific and theoretical foundations some of them are like chimerism, activation of long-term HIV reservoirs in body, conversion of one form of HIV into another, etc. The answers for all these question will have to wait for its time for long term observations with this patient.

Recently in December 2010 issue of Blood [3] have published the results for 4 year follow up of the this patients, who had received CCR5Δ32/Δ32 bone marrow transplantation. In this report Allers *et al*. has declared that "Cure for HIV has been achieved". By February, 2011, this patient has lived almost ~4 years post-bone marrow transplantation and remained free of HIV without any anti-retroviral treatment. Chimerism was achieved in this patient, while no conversion of HIV has been observed, so far. A successful immune-reconstitution was also observed in this patient, which was comparable to other

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transplanted patient. A milder form of Graft-vs-Host Disease (GVHD) reaction was noticed, which is not unexpected and that was treated appropriately. HIV RNA and HIV cDNA was found to be absent in all samples, tested so far from this patient.

Without any doubts and debate, this experiment has raised a possible hope toward cure for HIV. It is also certain that just 4 years is not long enough to answer all the possible questions and concerns. However, a long term survival will have answers for all these uncomfortable and complicated questions to assure success of this procedure. These types of therapeutic interventions may have far reaching implications, which warrants further investigations with more number of patients. This strategy can be helpful for those, who do not respond to anti-retroviral treatment due to one reason or another. Some of the possible applications of this have been discussed earlier [4]. Nevertheless, one of the major limitations for this mode of treatment is the availability of limited pool of donors with this specific mutation. This mutation is restricted only to Caucasians, while ~66% of HIV infected people live in Africa. Expansion of search program to identify this mutation among different races will serve as a consolation for ever expanding numbers due to longer survivability among HIV seropositives. There is a need of active research programs to develop universal stem cell with CCR5 deletion, which can be administered to any HIV seropositive without any restriction of matching. Possibilities are limitless to make real use of this breakthrough in treatment of HIV patient.

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RESEARCH ARTICLE OPEN ACCESS

COMBINATION OF VERMICOMPOSTS AND BIOPESTICIDES AGAINST NEMATODE (PRATYLENCHUS SP.) AND THEIR EFFECT ON GROWTH AND YIELD OF TOMATO (*LYCOPERSICON ESCULENTUM*)

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ABSTRACT

Vermicomposts singly and in combination with different biopesticide were used in agricultural field to check the infestation of nematode (Pratylenchus sp.) and measured the growth and yield of tomato (Lycopersicon esculentum) crop. Significant reduction of nematode population was observed in the soil after mixing of combination of vermicompost with neem oil (95%) and custard apple leaves (83%). The combination of garlic extract with different vermicompost caused 100% control of nematode population. Vermicompost obtained from animal dung + gram bran with neem oil was also very effective against the nematode (Pratylenchus sp.). Applications of vermicompost with biopesticide increased the productivity of tomato crop up to four times with respect to control. The results clearly demonstrate that the use of vermicompost with plant product is more beneficial in organic farming. It is helpful to compensate the deficiency of nutrients in the soil as well as control of the harmful nematode.

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KEY WORDS

Vermicomposting; Eisenia foetida; biopesticide; nematodes; Lycopersicon esculentum; productivity

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[I] INTRODUCTION

Lycopersicon esculentum (Tomato), most popular cultivated fruit vegetable, belongs to family Solanaceae. Commonly it is used as soup, salad, pickles, ketchup, puree and sauses. Its pressed cake is used as fodder for cattle and as fertilizer [1]. Use of chemical fertilizers and synthetic pesticide increase the productivity of the crops, but it also leads to decline the different physico-chemical parameters of soil [2]. The regular cultivation of land without incorporation of organic matter caused deterioration of the soil quality [3]. Management of soil quality, by the use of the bio-products is a need of today. Consequently, more biological wastes are used for production of biofertilizer [4].Vermicomposting is one of them. The vermicomposting is a suitable way of waste management with help of earthworm Eisenia foetida. Organic composts have recognized as effective mean of improving soil fertility [5-7]. Vermicomposts are finally peat like materials with high porosity, aeration, drainage and water holding capacity [8].

The phytoparasitic nematodes damaged the productivity of crops [9]. The plant parasitic nematodes bearing a style which helps the nematodes to punctured the protective wall of host plant. The nematodes inject the secretion of oesophageal gland which dissolved the cell wall of the host plant, ingest the cell content. Ultimately, resulted a poor plant growth, winter injury and wilting of the tree, loss of seedlings [10]. The addition of organic material in soil has been used in managing plant parasitic nematodes, to increase the crop yield [11]. Meyer et al., [12] have reported that clove oil derived from clove plant (Synzygium aromaticum) is effective against various soil born plant parasitic nematodes.

Gupta and Sharma [13] reported that aqueous extract of garlic bulbs suppressed the hatching of Meloidagyne incognita eggs. Plant products are receiving greater attention as prophylactics against several species of plant-parasitic nematodes. Various products (oils, cakes, extracts, etc.) prepared from the leaves and seed of neem plant (Azadirachta indica) have been reported as effective protectants against nematode pests. Akhtar [14] and Akhtar and Mahamood [11] have reported that the utilization of wastes material such as oil seeds, cake, chitin, compost, livestock and poultry manures and cellulogic wastes appeared promising for reducing population of plant parasitic nematodes. They also suggested that nitrogen based amendment, plant phenolics, nematotoxic chemical, development of predators and parasites of nematodes and

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micro-organism stimulation have been considered to be promising agent for nematodes managements.

The aim of the present study is to investigate the effect of vermicompost of different animal (cow, buffalo, sheep, goat and horse) dung and agro / kitchen waste singly as well in binary combination with different biopesticides against the harmful soil nematode (*Pratylenchus* sp.) and their related growth, flowering and productivity of tomato crop.

[II] MATERIALS AND METHODS

2.1. Collection of Wastes

Different kinds of organic wastes which are used for vermicomposting as well as feeding material for earthworms Eisenia foetida, were collected from different parts of Gorakhpur district.

2.1.1. Animal wastes

Animal wastes (cow, buffalo, sheep, horse, goat dung) were collected from different farm houses of the Gorakhpur district.

2.1.2. Agro wastes

Different agro wastes (gram bran, straw, wheat bran, barley bran and rice bran) vegetable wastes were collected from rural and urban parts of Gorakhpur district. Partially decomposed mixtures of animal, agro/kitchen wastes were used for enhancement of vermicomposting efficiency. For this purpose, the mixture of organic wastes sprayed in a layer of 1-2 feet and exposed to sun light for 5 to 10 days to removing the various harmful organism and noxious gases [15].

2.2. Collection of earthworms

Earth worms Eisenia foetida an epigeic species were cultured in laboratory condition, temperature (20 to 30 0C) and aeration, moisture (40% to 60%) for proper growth and survival of earthworms by the method of Gupta [4].

2.3. Preparation of vermicomposts

Vermicomposts of different animal and agro wastes were prepared on cemented earth surface. There are 35 vermibed formed by different combinations of animal, agro / kitchen wastes in 1:1 ratio the size of each vermibed is 3m x 1m x 9cm. After formation of vermibed moist it and inoculated 2kg cultured Eisenia foetida in each bed. The beds were covered with jute pockets and moisten the bed daily up to 40 to 50 days for maintaining the moisture content. After one week interval, mixture of bed was manually turned up to 3 weeks. After 50 to 60 days granular tea like vermicompost appear on the upper surface of beds.

2.4. Collection and preparation of biopesticide

2.4.1. Neem oil

Neem oil obtained from neem seed (Azadirachta indica). Neem Oil-Azadirachtine, 00.03 %; neem oil, 90.57%; Hydroxy El, 05.00% ; Epichlorohydrine 00.50 %; Aromax, 03.9%; Multiplex agricare Pvt. Ltd.

2.4.2. Garlic extract

Aqueous extract of Garlic (10gm/100ml) obtained from garlic (Allium sativum) bulb was mixed with vermicompost in 1:100 ratio.

2.4.3. Custard apple

Leaves are collected from plant of Custard apple (Annona squamosa). Prepared aqueous extract (10gm/100ml) of leaves and mixed with vermicompost in 1:100 ratio.

2.5. Extraction of nematodes from soil

Soil sample were collected from different experimental sites. Soils from 20 cm depth were used for the analysis of nematode. A small amount of soil (100 cm3) of each samples were collected from the experimental field. Nematodes were extracted from soil using Cobb's Sieving and gravity methods [16]. The samples were passed through sieves and the finally centrifuged for one minute. Nematode was identified through their taxonomic character. Their number was counted with the help of microscope.

Vermicompost obtained from different combination of animal and agro wastes in single and binary combination with biopesticide (neem oil, leaves extract of custard apple and garlic extract) were mixed @ 2 kg/m2 experimental area. Number of nematodes at pre and after mixing of vermicomposts in soil was counted with the help of microscope.

2.6. Experimental design of crops for measurement of growth, flowering period and productivity

Measurement of growth, flowering period and productivity of crops were performed in the experimental field of Vermiculture Research Center, Department of Zoology, D.D.U. Gorakhpur University. The 40 days old seedlings of tomato (*Lycopersicon esculentum*) variety HS-102 crops were planted in the experimental field/squire meter in each. Growth of crop was measured by auxanometer after 20 days from plantation. Flowering period were observed in adults plants. Productivity (kg/m2) of tomato was measured in each experimental field.

2.7. Chemical analysis

The chemical analysis of raw organic wastes and final vermicompost were determined by standard methods. Total organic carbon (TOC) was measured by the method of [17]. Total Kjeldahl nitrogen was determined by the method of Bremner and Mulvaney [18]. Total available phosphorus (TAP) was determined by colorimetric method of Bansal and Kapoor [19]. Total Potassium and Calcium were determined by flame photometer [19]. The pH and electrical conductivity (EC) were determined by with the help of pH and conductivity meter.

2.8. Statistical analysis

The value is expressed as mean ± SE of 6 replicates. Two way analysis of variance (ANOVA) was applied to determined the significant ($P<0.05$) difference among the number of nematodes in control and treaded group. One way analysis of variance was applied to locate significant ($P<0.05$) difference between flowering and productivity of crop with respect to different formulations of vermicompost [20].

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[III] RESULTS

The combination of vermicompost with biopesticide viz. neem (Azadirachta indica) oil, aqueous extract of garlic (Allium sativum) and leaves extract of custard apple (Annona squamosa) caused a significant ($P<0.05$) reduction in pest infestation and increase in plant growth, early flowering and productivity of the tomato crop. Significant reduction in number of nematodes population was observed in the soil mixed with vermicompost containing biopsticides **[Suplimentary Table–1 and 4]**.The different combination of vermicompost with garlic extract and animal dung + gram bran with neem oil have caused the complete control of soil nematodes infestation in tomato crops **[Supplementary Tables–2 and 3].**

Growth of tomato plant in control group was 10.20, 13.70 and 20.20 cm after 20, 30 and 40 days of plantation, respectively. Combinations of different animal dung + agro/kitchen wastes vermicomposts with biopesticides in the soil caused significant increase growth of tomato plant. The highest growth of tomato (38.02 cm) was observed in soil mixed with vermicompost of buffalo dung +gram bran + garlic extract, followed by vermicomposts of buffalo dung + gram bran + neem oil and buffalo dung + gram bran +leaf extract of custard apple **[Supplementary Tables– 2, 3, and 4].**

The flowering period of tomato in control group was 102.42 days. Significant early flowering was observed in all combination of vermicompost of different animal dung + agro/kitchen wastes singly, as well as binary combination with different biopesticide. The maximum significant early flowering period of tomato was 90.57 and 92.18 day shown in combination of vermicompost of buffalo dung + gram bran/goat dung + rice bran with neem oil **[Supplementary Table–2]**.

The significant increase in productivity of tomato was observed in all the combinations of vermicomposts of different animal, agro/ kitchen wastes singly and in binary combination with neem oil garlic extract and Annona squamosa leaf extract. The combinations of buffalo dung +gram bran with aqueous extract of garlic have maximum productivity of tomato (6.30 kg/m2) in comparison to all the biopesticide **[Supplementary Tables–2 and 3].**

[IV] DISCUSSION

It is evident from result section that the use of vermicompost obtained from different combinations of animal and agro/kitchen wastes singly as well as in combination with different biopesticides like neem (Azadirachta indica) oil, aqueous extract of garlic (Allium sativum) bulb and leaf extract of custard apple (Annona squamosa) [11, 13, 21] caused significant reduction in plant parasitic nematodes infestation in the soil, which ultimately enhances the growth, early flowering and productivity of tomato crop. Vermicompost of different animal-agro wastes have significant amount of nitrogen, phosphorus, Ca++, K+ vitamins, enzyme, plant hormones etc. [22-24] and plant pesticide viz neem (Azadirachta indica) oil, aqueous extract of garlic (Allium sativum) bulb and leaf extract of custard apple (Annona squamosa) have toxic effect against nematode infestation [11, 13, 21]. Akhtar and Mahmood [11] reported that addition of nitrogen based supplement along with organic amendments alter the soil texture, consequently number of nematodes in soil significantly reduced. Earthworms feed on the egg and larvae of soil nematode pest which ultimately reduced the soil nematode population [4, 25]. Meyer et al. [12] find similar result by the use of Syzygium arometicum against root knot nematodes Meloidigyne incognita. Musabyimana and Saxena [26] reported that garlic and neem seed derivatives were very effective against plant parasitic nematode (Pratylenchus sp.). Neem; garlic and custard apple are potent actively against different nematodes [13, 14].

The different combination of vermicompost obtained from different animal agro/kitchen wastes with garlic extract and vermicompost obtain from different animal dung +gram bran with neem oil shows total control of soil nematode pest. The reduction of nematode infestation may be due to the migration, poor penetration and retardation of different activities of nematodes in plant. Chemical content by the plant extract had the ability to affect the nervous system by inhibiting the activity of acetylcholinestrase in nematodes [28].

The highest growth of tomato was observed in vermicompost obtained from buffalo dung +gram bran in all the combination with biopesticide. Vermicompost of these combinations are the rich source of enzyme, vitamins plant growth hormones such as IAA, Gibbereline, Cytokinine along with micro and macro nutrients and due to the presence of biopesticides which enhance the growth of plant [24].

There was significant reduction in flowering period of tomato in all the combination of vermicomposts of different animal and agro wastes +neem oil/garlic/custard apple extract with respect to control. The combination of vermicompost with biopesticide caused early flowering of tomato plants, possibly due to the presence of TKN, TP in the vermicompost which stimulate the early flowering of crop [29-33]. The rich amount of TKN and TP stimulate the early flowering period of Daucus carota and tomato [34-36].

The combination of buffalo dung +gram bran with aqueous extract of garlic and neem oil shows significant maximum productivity of tomato it is due to the presence of essential nutrients in vermicompost which increased the metabolic activity of plant as well as garlic extract check the tomato infestation of nematodes [13, 31]. Large amount of humic acid were produced during vermicomposting which lowers the pH of soil and ultimately affect the productivity of plant [4]. Reduction of plant parasitic nematodes directly affects the

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productivity of crops [11, 26].

[V] CONCLUSION

It can be stated from the present study, that different combinations of vermicompost obtain from buffalo dung + gram bran with different biopesticides have significant effect on control of parasitic nematodes. Simultaneously, it also increases the growth, started early flowering and enhanced the productivity of tomato up to four times with respect to control. The use of each combination in the present study is easily producible, biodegradable, less expensive and cause no environment hazards to human health. These products will be ecologically safe and culturally more acceptable among farmers and live –stock keepers.

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SUPPLEMENTARY MATERIALS (As supplied by authors)

Supplementary Table-1: Effect of vermicomposts obtained from different animal dung and agro / kitchen wastes on nematode population in soil and growth as well as growth and productivity of tomato (*Lycopersicon esculentum*).

Combination	No. of nematodes		Growth of tomato (cm.)			Flowering period	Productivity
			Days after planting			(Days)	(kg/m^2)
	Before sowing	After harvesting	20	30	40		
Control	41.2±0.3	56.4±0.2	10.20±0.38	13.70±0.81	20.20±0.56	102.42±3.20	1.23±.24
Cow							
Dung	* 40.6±0.2	32.5±0.3	# 12.66±0.36	20.13±0.69	27.27±0.64	97.61±2.25	$ 3.06±.14
Dung +Rice Bran	42.5±0.1	31.3±0.2	* 13.22±0.49	29.46±0.51	35.10±0.46	96.54±2.88	4.07±.15
Dung +Wheat Bran	41.4±0.2	30.5±0.3	13.26±0.69	21.97±0.42	30.51±0.64	96.46±3.47	4.72±.21
Dung +Straw	42.6±0.3	28.4±0.2	15.65±0.55	22.56±0.83	34.44±0.62	97.94±2.56	3.00±.16
Dung +Vegetables	43.4±0.2	31.6±0.2	12.76±0.76	24.91±0.92	32.56±0.66	96.86±2.78	3.98±.08
Dung +Barely Bran	40.2±0.3	32.9±0.1	15.32±0.82	21.94±0.44	33.28±0.92	99.25±2.64	5.08±.25
Dung +Gram Bran	43.3±0.2	28.8±0.3	13.13±0.84	22.89±0.29	30.29±0.36	100.73±2.47	4.80±.42
Buffalo							
Dung	* 40.2±0.2	31.3±0.1	# 10.52±0.44	20.03±0.24	26.17±0.16	97.26±2.24	$ 3.12±.72
Dung + Rice Bran	41.4±0.3	32.3±0.3	* 14.15±0.49	20.42±0.97	34.42±0.88	96.42±1.46	3.62±.71
Dung + Wheat Bran	42.6±0.2	31.5±0.2	13.22±0.89	20.92±0.45	33.07±0.49	96.23±2.96	4.68±.26
Dung + Straw	44.4±0.2	28.4±0.3	13.21±0.94	22.03±0.88	31.21±0.52	97.82±1.86	3.30±.38
Dung + Vegetables	42.3±0.2	28.6±0.2	14.29±0.94	23.47±0.67	33.55±0.92	96.80±2.74	3.42±.24
Dung + Barley Bran	42.4±0.2	31.4±0.2	12.39±0.66	22.79±0.56	33.50±0.33	99.49±2.54	4.80±.40
Dung + Gram Bran	40.8±0.3	27.8±0.1	15.88±0.58	24.58±0.66	**35.35±0.33**	95.47±2.22	**5.18±.46**
Sheep							
Dung	* 42.3±0.0	32.3±0.2	# 10.56±0.22	17.45±0.19	23.64±0.28	100.99±2.24	$ 3.07±.39
Dung +Rice Bran	43.5±0.3	31.5±0.2	* 09.98±0.39	17.53±0.43	23.10±0.43	101.45±2.25	4.02±.20
Dung + Wheat Bran	42.4±0.1	30.4±0.3	12.29±0.39	17.13±0.57	24.15±0.59	101.86±2.63	3.86±.30
Dung + Straw	43.3±0.1	29.7±0.1	11.28±0.96	20.18±0.88	30.26±0.43	100.55±4.85	4.09±.21
Dung + Vegetables	40.5±0.3	28.3±0.2	11.35±0.88	21.00±0.78	29.94±0.60	100.86±3.64	5.08±.26
Dung + Barley Bran	41.4±0.2	33.7±0.2	12.28±0.60	20.22±0.33	30.23±0.73	100.35±3.86	3.14±.26
Dung + Gram Bran	40.6±0.1	31.6±0.3	10.36±0.40	20.18±0.46	31.44±0.83	100.32±2.16	4.02±.43
Goat							
Dung	* 40.9±0.2	313.±0.3	# 10.56±0.30	21.00±0.64	29.30±0.60	100.53±0.87	$ 3.02±.65
Dung + Rice Bran	42.8±0.1	30.8±0.1	* 11.84±0.41	21.13±0.72	31.19±0.58	94.18±2.56	3.40±.41
Dung + Wheat Bran	42.7±0.0	34.2±0.4	10.53±0.92	20.08±0.75	30.44±0.42	100.54±0.87	4.09±.44
Dung + Straw	41.2±0.0	30.5±0.1	09.92±0.84	19.66±0.76	31.00±0.43	101.49±2.28	3.10±.62
Dung + Vegetable	41.3±0.2	31.6±0.0	11.47±0.75	20.77±0.55	28.80±0.43	99.76±2.57	3.36±.44
Dung + Barley Bran	41.2±0.1	32.8±0.1	11.28±0.52	20.19±0.57	30.72±0.55	100.55±2.54	5.00±.43
Dung + Gram Bran	43.4±0.2	32.3±0.1	10.35±0.48	20.51±0.52	29.33±0.88	100.86±3.22	4.00±.30
Horse							
Dung	* 44.2±0.1	28.4±0.2	#09.42±0.31	19.49±0.35	25.56±0.56	99.25±1.57	$ 3.04±.21
Dung + Rice Bran	42.4±0.2	29.6±0.2	* 12.32±0.75	21.33±0.76	33.89±0.60	96.57±2.43	3.85±.20
Dung + Wheat Bran	40.5±0.2	30.7±0.1	12.83±0.69	20.93±0.54	32.24±0.58	95.68±0.45	4.05±.34
Dung + Straw	42.6±0.3	29.6±0.1	13.24±0.25	21.96±0.44	31.52±0.53	97.84±0.56	2.09±.49
Dung + Vegetable	41.4±0.1	28.5±0.2	15.51±0.48	21.43±0.64	32.34±0.55	96.46±2.88	3.15±.14
Dung + Barley Bran	40.7±0.3	33.4±0.4	16.22±0.62	20.98±0.45	32.52±0.53	100.75±2.37	4.98±.21
Dung + Gram Bran	42.2±0.0	27.2±0.3	15.87±0.63	20.81±0.52	31.33±0.49	101.56±3.01	3.91±.32

Each value is the mean ± SE of six replicates.
2way ANOVA: Significant (P<0.05) * within column, # within row.
$- Significant one way ANOVA (P<0.05) within row.

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Supplementary Table-2: Effect of combinations (1:1000) of neem oil with vermicomposts obtain from different animal dung and agro / kitchen wastes on nematode population in soil as well as growth and productivity of tomato (*Lycopersicon esculentum*).

Combination	No. of nematodes		Growth of tomato (cm.)			Flowering period	Productivity
	Before sowing	After harvesting	20	30	40		
Control	41.2±0.3	56.4±0.2	10.20±0.38	13.70±0.81	20.20±0.56	102.42±3.20	1.23±.24
Cow							
Dung	* 40.4±0.2	3.6±0.1	# 13.76±0.36	22.13±0.49	29.07±0.34	95.81±0.26	$ 3.86±.24
Dung +Rice Bran	41.3±0.3	3.5±0.0	* 14.20±0.49	24.46±0.81	36.00±0.76	94.14±1.83	4.97±.25
Dung +Wheat Bran	43.3±0.2	1.6±0.0	14.96±0.59	23.97±0.52	32.01±0.54	94.56±2.27	5.12±.11
Dung +Straw	42.6±0.3	3.7±0.1	16.55±0.45	25.56±0.81	36.40±0.64	95.90±2.56	4.00±.06
Dung +Vegetables	40.5±0.1	4.4±0.2	13.76±0.66	24.71±0.97	34.59±0.46	94.82±2.77	4.98±.05
Dung +Barely Bran	43.4±0.3	4.5±0.1	16.22±0.81	24.64±0.54	35.08±0.93	97.20±1.66	5.08±.45
Dung +Gram Bran	44.8±0.2	Nil	14.13±0.84	24.89±0.69	32.89±0.39	98.75±1.45	5.50±.40
Buffalo							
Dung	* 41.7±0.0	2.6±0.1	# 12.51±0.41	22.02±0.34	28.07±0.19	95.28±1.25	$ 3.92±.70
Dung + Rice Bran	41.2±0.1	2.5±0.1	* 15.16±0.44	23.52±0.96	36.40±0.48	94.44±1.26	4.22±.71
Dung + Wheat Bran	41.6±0.2	3.8±0.3	14.26±0.89	23.82±0.49	35.09±0.59	94.21±0.96	5.08±.22
Dung + Straw	42.4±0.1	3.3±0.0	14.21±0.96	24.02±0.89	33.11±0.92	95.80±0.83	4.10±.18
Dung + Vegetables	43.6±0.3	3.5±0.1	15.29±0.90	25.47±0.61	35.45±0.93	94.70±1.54	4.12±.14
Dung + Barley Bran	40.6±0.1	4.4±0.1	15.79±0.67	24.79±0.46	35.00±0.63	97.09±1.50	5.80±.43
Dung + Gram Bran	43.3±0.2	Nil	16.86±0.53	24.78±0.86	**37.38±0.38**	90.57±1.23	**5.98±.66**
Sheep							
Dung	* 42.2±0.0	2.7±0.1	# 11.56±0.21	19.43±0.09	25.60±0.29	99.19±0.25	$ 3.97±.29
Dung +Rice Bran	41.1±0.1	3.4±0.0	* 09.98±0.31	18.83±0.40	25.00±0.42	100.15±1.15	4.92±.30
Dung + Wheat Bran	40.4±0.2	2.5±0.1	13.20±0.33	19.03±0.47	26.11±0.39	100.76±1.83	4.46±.10
Dung + Straw	41.7±0.1	Nil	13.18±0.96	22.08±0.83	32.21±0.83	101.25±2.80	5.09±.11
Dung + Vegetables	43.6±0.3	3.4±0.1	12.25±0.88	23.00±0.77	31.94±0.66	101.86±2.60	5.48±.06
Dung + Barley Bran	40.6±0.1	4.4±0.0	14.28±0.69	22.32±0.30	32.13±0.77	100.25±2.80	4.14±.06
Dung + Gram Bran	43.0±0.2	Nil	12.26±0.44	22.08±0.66	33.34±0.86	100.35±2.19	5.02±.43
Goat							
Dung	* 43.3±0.3	2.4±0.1	# 12.55±0.35	23.00±0.60	31.20±0.60	100.52±0.47	$ 3.92±.95
Dung + Rice Bran	41.2±0.2	2.5±0.0	* 13.80±0.47	23.03±0.73	33.09±0.59	92.18±1.56	4.40±.81
Dung + Wheat Bran	41.6±0.2	3.2±0.1	12.57±0.91	21.98±0.74	32.84±0.82	99.64±0.77	5.09±.74
Dung + Straw	40.4±0.0	Nil	12.98±0.83	21.66±0.79	33.00±0.41	100.19±1.18	4.10±.60
Dung + Vegetable	42.7±0.2	1.5±0.1	13.42±0.78	22.73±0.59	30.85±0.63	98.74±2.97	4.26±.54
Dung + Barley Bran	41.6±0.4	4.3±0.0	13.18±0.51	22.09±0.59	32.72±0.53	101.25±2.04	5.80±.49
Dung + Gram Bran	42.3±0.3	Nil	12.33±0.49	22.91±0.51	31.31±0.98	100.76±2.20	4.90±.34
Horse							
Dung	* 42.3±0.0	2.2±0.1	# 10.41±0.39	21.29±0.25	27.52±0.46	98.21±1.27	$ 3.94±.25
Dung + Rice Bran	45.5±0.1	Nil	* 13.31±0.76	23.43±0.78	35.79±0.68	95.17±2.03	4.45±.20
Dung + Wheat Bran	42.4±0.2	Nil	13.88±0.89	22.91±0.64	34.04±0.88	95.68±0.45	5.05±.31
Dung + Straw	43.6±0.1	Nil	15.23±0.45	23.90±0.64	33.02±0.54	96.84±0.96	2.99±.09
Dung + Vegetable	43.4±0.3	3.4±0.1	16.56±0.43	23.73±0.34	34.24±0.50	95.45±1.83	4.15±.04
Dung + Barley Bran	40.5±0.1	4.3±0.0	15.23±0.69	23.08±0.46	34.50±0.59	100.77±2.07	5.78±.01
Dung + Gram Bran	43.5±0.2	Nil	14.86±0.63	23.01±0.52	33.53±0.79	101.56±2.01	4.91±.02

Each value is the mean ± SE of six replicates.
2way ANOVA: Significant (P<0.05) * within column, # within row.
$-Significant one way ANOVA (P<0.05) within row.

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Supplementary Table-3: Effect of combinations (1:100) of aqueous extract of garlic bulb with vermicomposts obtain from different animal dung and agro / kitchen wastes on nematode population in soil as well as growth and productivity of tomato (*Lycopersicon esculentum*).

Combination	No. of nematodes		Growth of tomato (cm.)			Flowering period	Productivity
			Days after planting			(Days)	(kg/m^2)
	Before sowing	After harvesting	20	30	40		
Control	41.2±0.3	56.4±0.2	10.20±0.38	13.70±0.81	20.20±0.56	102.42±3.20	1.23±.24
Cow							
Dung	* 42.4±0.3	Nil	# 14.26±0.26	23.13±0.45	29.97±0.45	**94.36±0.45**	$ 3.98±.23
Dung +Rice Bran	40.3±0.1	Nil	* 15.22±0.43	25.02±0.47	37.20±0.16	**93.54±1.52**	5.27±.72
Dung +Wheat Bran	43.6±0.2	1.6±0.0	15.46±0.49	24.27±0.43	33.31±0.53	93.86±1.83	5.64±.53
Dung +Straw	40.8±0.1	2.2±0.1	17.54±0.46	26.16±0.43	37.10±0.43	94.90±2.43	4.52±.16
Dung +Vegetables	42.7±0.2	3.5±0.0	14.66±0.36	25.01±0.46	35.29±0.53	94.02±1.23	4.98±.05
Dung +Barely Bran	43.9±0.2	2.5±0.1	17.25±0.21	25.14±0.52	36.08±0.46	96.80±1.45	6.08±.92
Dung +Gram Bran	42.4±0.1	Nil	15.23±0.87	25.19±0.65	33.29±0.73	97.74±2.73	5.96±.43
Buffalo							
Dung	* 41.4±0.3	Nil	# 13.61±0.42	22.52±0.30	29.17±0.76	94.58±2.43	$ 4.12±.45
Dung + Rice Bran	42.5±0.3	2.4±0.1	* 16.16±0.44	24.02±0.23	37.23±0.56	93.84±2.63	4.86±.48
Dung + Wheat Bran	40.6±0.1	Nil	15.36±0.49	24.12±0.46	36.19±0.84	93.83±0.42	5.76±.56
Dung + Straw	43.5±0.2	1.4±0.0	15.61±0.36	24.52±0.69	34.01±0.49	94.83±2.54	4.84±.73
Dung + Vegetables	40.9±0.1	Nil	16.39±0.95	25.97±0.64	36.25±0.34	93.70±2.43	4.97±.74
Dung + Barley Bran	42.4±0.2	3.2±0.1	16.49±0.64	25.18±0.40	34.10±0.52	96.59±3.55	5.08±.46
Dung + Gram Bran	43.4±0.2	Nil	14.83±0.56	25.19±0.56	**38.02±0.28**	97.87±3.43	**6.30±.46**
Sheep							
Dung	* 41.6±0.3	Nil	# 12.46±0.26	19.83±0.59	26.32±0.46	97.59±2.58	$ 4.10±.54
Dung +Rice Bran	42.2±0.0	2.6±0.1	* 10.38±0.36	19.23±0.23	26.12±0.64	98.95±2.84	5.06±.82
Dung + Wheat Bran	41.3±0.1	Nil	14.26±0.34	19.83±0.46	27.23±0.09	99.76±2.83	4.96±.73
Dung + Straw	43.5±0.2	1.4±0.0	14.28±0.36	22.98±0.80	33.21±0.13	101.25±1.82	5.79±.48
Dung + Vegetables	41.7±0.1	3.5±0.l	13.24±0.48	24.00±0.74	32.54±0.47	101.86±1.64	6.12±.47
Dung + Barley Bran	42.6±0.3	Nil	15.38±0.49	23.12±0.61	33.03±0.26	101.53±1.86	4.64±.23
Dung + Gram Bran	42.9±0.1	Nil	13.66±0.54	22.88±0.64	34.14±0.46	100.73±3.42	5.52±.46
Goat							
Dung	* 42.5±0.1	Nil	# 13.15±0.25	23.50±0.64	32.00±0.36	99.62±0.47	$ 4.23±.56
Dung + Rice Bran	43.3±0.1	2.2±0.1	* 14.20±0.43	23.93±0.75	34.09±0.29	96.88±1.56	5.00±.47
Dung + Wheat Bran	42.5±0.3	Nil	13.47±0.96	22.38±0.54	32.84±0.82	99.64±0.77	5.59±.85
Dung + Straw	42.6±0.3	1.5±0.0	13.38±0.43	22.26±0.75	33.00±0.41	100.89±2.48	4.94±.90
Dung + Vegetable	40.3±0.4	Nil	14.32±0.58	23.13±0.50	30.85±0.63	97.52±3.93	4.98±.54
Dung + Barley Bran	40.8±0.2	2.2±0.l	14.48±0.52	22.89±0.49	32.72±0.53	101.45±3.04	6.16±.46
Dung + Gram Bran	41.4±0.0	Nil	13.36±0.59	23.24±0.52	31.31±0.98	101.83±0.48	5.14±.75
Horse							
Dung	* 41.3±0.3	Nil	# 11.44±0.33	21.99±0.22	27.52±0.46	97.29±2.45	$ 4.24±.46
Dung + Rice Bran	42.4±0.0	2.4±0.1	* 14.38±0.75	24.13±0.74	35.79±0.68	94.48±2.46	4.96±.56
Dung + Wheat Bran	41.3±0.1	Nil	14.23±0.80	23.96±0.60	34.04±0.88	94.98±2.82	5.85±.48
Dung + Straw	43.6±0.2	1.5±0.0	16.33±0.46	24.92±0.34	33.02±0.54	95.24±1.36	3.16±.22
Dung + Vegetable	41.4±0.1	Nil	16.66±0.42	24.53±0.64	34.24±0.50	94.46±2.80	4.85±.43
Dung + Barley Bran	42.3±0.3	Nil	16.23±0.69	24.18±0.45	34.50±0.59	101.42±1.47	6.13±.43
Dung + Gram Bran	42.4±0.1	Nil	15.16±0.23	24.01±0.53	33.53±0.79	100.72±2.51	5.15±.45

Each value is the mean ± SE of six replicates.
2way ANOVA: Significant (P<0.05) * within column, # within row.
$- Significant one way ANOVA (P<0.05) within row.

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Supplementary Table-4: Effect of combinations (1:100) of aqueous leaf extract of custard apple (Annona squamosa) with vermicomposts obtain from different animal dung and agro / kitchen wastes on nematode population in soil as well as growth and productivity of tomato (Lycopersicon *esculentum*)

Combination	No. of nematodes		Growth of tomato (cm.)			Flowering period	Productivity
			Days after planting			(Days)	(kg/m^2)
	Before sowing	After harvesting	20	30	40		
Control	41.2±0.3	56.4±0.2	10.20±0.38	3.70±0.81	20.20±0.56	102.42±3.20	1.23±.24
Cow							
Dung	* 42.2±0.3	5.1±0.2	# 13.55±0.46	20.89±0.54	27.98±0.75	97.03±2.45	$ 3.21±.23
Dung +Rice Bran	40.2±0.2	6.2±0.2	* 13.82±0.43	30.12±0.42	35.84±0.47	96.21±1.47	4.15±.15
Dung +Wheat Bran	40.3±0.3	7.1±0.3	13.93±0.43	22.46±0.47	31.12±0.48	96.42±3.42	4.82±.14
Dung +Straw	41.4±0.2	8.2±0.2	16.12±0.42	23.13±0.32	34.98±0.24	97.14±3.14	3.15±.17
Dung +Vegetables	43.2±0.3	5.3±0.3	13.13±0.43	25.24±0.34	33.12±0.28	96.24±2.23	4.02±.21
Dung +Barely Bran	42.4±0.4	5.3±0.1	15.75±0.73	22.23±0.43	33.98±0.48	99.01±2.24	5.15±.31
Dung +Gram Bran	42.3±0.3	8.5±0.2	13.45±0.23	23.43±0.28	30.85±0.17	100.04±1.24	4.97±.28
Buffalo							
Dung	* 40.2±0.3	5.4±0.4	# 10.98±0.73	20.86±0.23	26.67±0.25	97.14±3.24	$ 3.42±.14
Dung + Rice Bran	42.3±0.2	8.4±0.2	* 14.83±0.43	20.98±0.24	34.87±0.46	96.14±2.43	3.52±.42
Dung + Wheat Bran	41.4±0.1	5.2±0.5	13.86±0.34	21.23±0.43	33.75±0.15	96.27±2.96	4.76±.17
Dung + Straw	43.6±0.2	7.3±0.4	13.87±0.34	22.65±0.88	31.86±0.47	97.47±1.24	3.46±.24
Dung + Vegetables	42.3±0.3	5.2±0.4	14.97±0.45	23.98±0.42	34.13±0.45	96.15±1.28	3.54±.47
Dung + Barley Bran	41.2±0.3	6.2±0.5	12.98±0.43	23.12±0.43	33.90±0.27	99.17±2.28	4.94±.42
Dung + Gram Bran	41.5±0.3	3.2±0.4	16.14±0.45	30.23±0.45	**35.95±0.45**	95.45±2.21	**5.42±.14**
Sheep							
Dung	* 41.0±0.1	7.6±0.3	# 11.45±0.42	17.90±0.74	23.85±0.52	100.24±2.24	$ 3.12±.47
Dung +Rice Bran	42.3±0.2	5.6±0.3	* 10.24±0.42	17.96±0.45	23.64±0.24	101.45±4.21	4.23±.14
Dung + Wheat Bran	41.4±0.2	6.5±0.7	12.89±0.23	17.68±0.25	24.84±0.18	101.32±2.14	4.02±.45
Dung + Straw	42.2±0.3	7.6±0.8	11.78±0.45	20.97±0.57	30.79±0.48	100.24±2.45	4.42±.14
Dung + Vegetables	41.5±0.2	7.3±0.4	12.05±0.28	21.75±0.76	29.98±0.48	100.48±2.17	5.23±.42
Dung + Barley Bran	40.2±0.1	7.3±0.4	12.96±0.43	20.97±0.45	31.13±0.47	100.20±3.24	3.43±.18
Dung + Gram Bran	42.6±0.3	6.5±0.2	11.03±0.43	20.97±0.43	32.13±0.14	101.21±2.14	4.14±.24
Goat							
Dung	* 41.5±0.3	7.8±0.5	# 11.21±0.25	21.79±0.34	29.89±0.89	100.17±0.48	$ 3.23±.24
Dung + Rice Bran	40.8±0.2	7.5±0.4	* 12.24±0.45	21.95±0.35	31.79±0.79	**94.02±2.17**	3.43±.24
Dung + Wheat Bran	41.6±0.1	6.5±0.4	11.23±0.25	20.73±0.64	30.89±0.27	100.05±0.48	4.56±.25
Dung + Straw	42.6±0.2	6.7±0.6	10.12±0.23	20.12±0.47	31.56±0.47	101.04±2.17	3.28±.24
Dung + Vegetable	42.4±0.1	6.5±0.8	12.42±0.24	21.24±0.42	29.24±0.17	99.03±2.18	3.54±.52
Dung + Barley Bran	43.4±0.2	6.7±0.7	11.84±0.23	20.95±0.42	31.28±0.18	100.45±2.24	5.12±.15
Dung + Gram Bran	43.6±0.2	5.8±0.4	11.23±0.14	21.12±0.23	29.98±0.47	100.17±3.23	4.15±.24
Horse							
Dung	* 43.4±0.2	8.7±0.6	# 10.23±0.24	19.97±0.28	26.23±0.12	99.47±1.45	$ 3.20±.23
Dung + Rice Bran	41.8±0.3	5.7±0.4	* 13.15±0.42	21.95±0.54	34.12±0.14	96.02±2.15	3.85±.24
Dung + Wheat Bran	41.4±0.2	5.6±0.6	13.53±0.45	21.42±0.15	32.95±0.45	95.32±0.17	4.24±.14
Dung + Straw	41.6±0.2	7.5±0.7	14.12±0.32	22.42±0.45	31.90±0.14	97.24±0.15	2.14±.32
Dung + Vegetable	43.7±0.2	5.7±0.8	16.12±0.43	21.98±0.14	32.96±0.47	96.24±2.14	3.24±.14
Dung + Barley Bran	41.6±0.2	7.5±0.7	16.89±0.24	21.46±0.23	32.95±0.41	100.14±2.24	5.14±.34
Dung + Gram Bran	42.2±0.6	6.5±0.9	16.23±0.34	21.15±0.14	31.97±0.45	100.42±3.21	4.02±.24

Each value is the mean ± SE of six replicates.
2way ANOVA: Significant (P<0.05) * within column, # within row.
$- Significant one way ANOVA (P<0.05) within row.