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(Formerly Tamil Nadu Journal of Veterinary and Animal Sciences)

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INDIAN JOURNAL OF VETERINARY AND ANIMAL SCIENCES RESEARCH
(Formerly Tamil Nadu Journal of Veterinary and Animal Sciences)

Vol. 46 **May - June 2017** **No. 3**

Review article

1. BIOREMEDIATION OF POULTRY FEATHER WASTE- A REVIEW 964
D.Thyagarajan, A.Manivannan, S.Sakthivadivu and S.Bharathi

Full length articles

2. PLASMA TAURINE ESTIMATION IN DILATED CARDIOMYOPATHY OF LABRADOR RETRIEVERS 975
Jeyaraja.K, Sarath Chandra,G, Hamsa Yamini, S and Thirunavukkarasu, P.S

3. EFFECT OF FARMYARD MANURE ON BIOMASS YIELD, CARBON ASSIMILATION POTENTIAL AND CORRELATION COEFFICIENT IN FODDER MAIZE (Zea mays L.) IN DIFFERENT AGROCLIMATIC ZONES 982
B.Rajesh Kumar, Thanga. Thamil Vanan, T.Sivakumar, K.N.Selvakumar, V.M.Sankaran and S.Saraswathi

4. GROWTH PERFORMANCE OF LAMBS UNDER INTENSIVE, CONTINUOUS AND ROTATIONAL GRAZING SYSTEMS OF MANAGEMENT 992
M.S. Ram Prasad, S. Meenakshi Sundaram, P. Tensingh Gnanraj, C. Bandeswaran, T.J. Harikrishnan and T. Sivakumar

5. ASSESSMENT OF MICROBIAL LOAD IN RAW MEAT SAMPLES FROM OPEN MARKETS OF PRODDATUR, Y.S.R. KADAPA DISTRICT, ANDHRA PRADESH 1003
Z Naveen, RA Supriya, BR Naik and MA Raj

Short Communication

6. BIODEGRADABLE DUNKS TO CONTROL CULICINE LARVAE 1015
R K Anish, Bhaskaran Ravi Latha, R Palani Dorai

7. EFFECT OF SEASON ON REPRODUCTIVE PERFORMANCE OF OSTRICH (STRUTHIO CAMELUS) 1020
C.Pandian, S.T Selvan, R.Venkataramanan and H.Gopi

8. A RARE CASE REPORT OF PEROSOMUS ELUMBIS WITH CONCURRENT VISCERAL ABNORMALITIES IN A JERSEY CROSS BRED CALF 1025
R. Siva Shankar, M. Gokulakrishnan, M.S. Sabarish Babu, S. Dinesh Kumar, Viji Vijayan and Ravi Sundar George

Review article

BIOREMEDIATION OF POULTRY FEATHER WASTE- A REVIEW

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ABSTRACT

Poultry farming is growing at 8% Compound Annual Growth Rate (CAGR) in layer and 12% CAGR in broiler farming. Consequent to the growth of the poultry farming activity, eco-friendly approach to utilize the waste into value added products are of current importance. Waste on one hand is responsible for adding pollutants to the environment; on the other hand it causes major problems in handling. In domesticated birds, feather waste accounts to 7% of the body weight of the birds. At present it is being disposed off without any solid waste treatment. Feather is having advantage of containing valuable sulphur containing amino acids like cysteine and methionine. Over the years, feather meals are produced using thermal and chemical processes. However these methods are having disadvantages of energy expensive and poor end products. Now lot of research work are attempted through bioremediation of the feather meal. Even though much of the investigations are on the laboratory side, applications of this technology are yet to be taken up, either due to lack of detailed knowledge on these technologies or lack of information about various bacteria and fungi available for this purpose. In this paper various bacteria like *Bacillus subtilis*, *Bacillus pumilis*, *Bacillus altitudinis*, *Bacillus licheniformis*, *Pseudomonas microphilus*, *Leuconostoc*, *Thermus aquaticus* and *Fervidobacterium pennavorans* for their potential as keratinolytic activity are discussed. Similarly role of certain fungi like *Aspergillus niger*, *Penicillium citrinum*, *Trichoderma viride*, *Alternaria tenuissima*, *Chrysosporium tropicum* and *Fusarium culmorum* in bioremediation of the feather to feather meal are also elaborated. Altogether bioremediation of feather meal concurrently adhere to integrated eco-friendly waste disposal with due importance to maintaining biodiversity of the living organism in the ecosystem.

INTRODUCTION

Chicken feather is one of the major waste product getting accumulated without processing in the poultry processing industries. The quantity of feathers generated as waste cause major environmental hazards due to the decomposition and uncared disposal. Right now, chicken feathers are disposed as landfills or burnt without any defied planning. Rather, feather could be converted as feather meal through different processing techniques like, chemical hydrolysis, thermal processing and bioremediation. Feather meal produced by this technique contains valuable proteins rich in hydrophobic amino acids and also other amino acids like arginine, cysteine

and threonine. Established feather meal production technology is a hydrothermal process. However, in this process there is every possibility for the reduction of amino acids like, methionine, lysine, tyrosine and tryptophan with further disadvantage of low digestibility and low nutritional value. As to work on the disadvantages, now microbes are being utilized for degradation of feathers into feather meal. Many microbes have been identified so far for efficient degradation of feathers. Some of the thermophilic and mesophilic microbes having feather degradation properties are, Bacillus, Streptomyces and Chryseobacterium strains. In addition, different mycotic flora are also responsible for the degradation of keratin by their metabolic product keratinase.

Feather constitutes 5-7% of body weight of the bird that is being disposed off in the massive quantity around the world. Raw feather without processing causes major ailments like chlorosis, mycoplasmosis etc. Because of the positive chemical composition, feather waste can be conveniently processed as valuable protein supplement, adding to the stock of protein rich feed stuffs to the livestock and poultry. Quiet important factor here however is to have cost effective ecofriendly technology to process feather waste into feather meal.

Chicken feather is having enormous tough keratinous materials in the shaft and other places. Disposal of feathers as such in the environment leads to prolific multiplication of mesophilic keratin degrading microorganism especially in keratin rich soil. Such mesophilic keratinolytic organisms act as pathogens to human beings. Increased feather waste accumulation in the environment is a major disturbing ecological factor due to massive growth of the global poultry industry. Existing feather waste management could cause major health problems to the other human beings and animals.

Right now keratinase waste are getting accumulated in the environment as a final waste product from different industries, which are uncared due to lack of proper technology. Attempts are now being made through biotechnological means to hydrolyse these keratinase waste to soluble form by application of keratinase enzymes through microbes. These keratinase enzymes are powerful proteolytic enzymes capable of hydrolysing the insoluble keratins. In addition these enzymes have multiple applications in further processing of waste from poultry industry. Besides these enzymes are also used in detergent production, medicine, cosmetics, feather and feed industries and need areas like prion degradation as a treatment for mad cow disease. This utility enzymes are produced by many insects, microorganisms like bacteria, saprophytic and parasitic fungi and actinomycetes. Bacterial keratinases have special advantages of degradation of insoluble keratin substrates (Lin *et al*. 1995).

Even though feather are considered as a valuable biovalue amino acid resources, still cost effective technology to convert the feather waste into high value feather meal is yet to be established. Bioremediation process is one of the advanced eco-friendly emerging technology to utilize the feather

waste into feather meal, overcoming the limitations in thermo and chemical hydrolysis.

Composition

Chicken feather constitutes around 91% protein, 1% lipids and 8% water. Amino acid sequence of chicken feather is identical to that of other feathers and also mostly similar with reptilian keratins found in the claws.Amino acid composition of the feathers mostly are cysteine, glutamine, proline and serine. On the contrary, histidine, lysine, tryptophan, glutamic acid and glycine are absent. More than 16% serine is present in the feather and its hydroxyl group is responsible for chicken feather stability (Kannappan and Bharathi, 2012).

Keratins are basically insoluble protein commonly seen in wool, hooves, scales, hair, nails (hard keratins) and also in stratum corneum (soft keratins). This specific proteins come under the classification of sclera protein groups which are highly resistant to the action of physical, chemical and biological agents. Disulphide bonds, hydrogen bonds, salt linkages and cross linkages present in the keratin are responsible for the mechanical stability and high resistance to proteolytic degradation of keratin.

Table 1: Amino acid content in keratin fiber from chicken feather

Functional groups	Amino acid	% Contents
Positively charged	Arginine	4.30
Negatively charged	Aspartic acid	6.00
	Glutamine	7.62
Hygroscopic	Threonine	4.00
	Serine	16.0
Hydrophobic	Tyrosine	1.00
	Leucine	2.62
	Isoleucine	3.32
	Valine	1.61
	Cystine	8.85
	Alanine	3.44
	Phenylalanine	0.86
	Methionine	1.02
Special	Proline	12.0
	Aspargine	4.00

Chicken feather fibres are basically having α- helical and some β sheet confirmations. However its outer quil is different by having higher β –sheet conformations and lesser α- helical conformations. Higher cysteine content is

found in hard β –sheet keratins than soft α- helical keratins with greater possibility for higher disulphide chemical bonds responsible for linkage between keratin proteins. Strong covalent bonds present in the feathers are the main factors for the stability of the three dimensional structure which are difficult to break.

Feather Bioconversion

Existing application of technology limits the conversion of feather waste into feather meal in major way. Existing processing technique for feather waste requires steam cooking or chemical digestion to make it more degradable form constantly with more energy requirement. Bioconversion utilizing the micro-organism is an alternative cost effective technology to convert the feather waste into high nutritional value feather meal.

Advantages

Application of bioprocess technology involving keratinolytic organism gives three major advantages like ecological, economical and high nutritional values due to better digestibility.

Keratinolytic activity of several microbial species, such as, *Chrysosporium, Aspergillus, Alternaria, Trichurus, Curvularia, Cladosporium, Fusarium, Geomyces, Gleomastis, Monodictys, Myrothecium, Paecilomyces, Stachybotrys, Urocladium, Scopulariopsis, Sepedonium, Penicillium, Doratomyces (fungi), Streptomyces, Vibrio, Microbacterium* and *Bacillus* (bacteria), has been reported in the literature.

Source of keratinolytic organisms

Most of the keratinolytic organisms isolated from the soil collected near feather processing units are Bacteria, *Bacillus* Sp. FK46, *B. licheniformis*, *B. pumilus* sps, *Vibrio* sps strain Kr2, Actinobacteria, *Streptomyces pactum, S. albus*, and Saprophytic and Dermatophlilic fungi, *Aspergillus* sps, *Rhizomucor* sps, *Trichophyton mentagrophytes, T. rubrum, T. gallinae, Microsporum canis* and *M. gypseum.*Here under, findings in different research trials for bioconversion of feather waste utilizing the microbes are presented.

Bacillus subtilis, B.pumilis & B.cereus

As per investigations carried out by Kim *et al.* (2001) it is found that feather degrading bacteria are ubiquitously present in feather waste. Among the isolates, *B.subtilis, B.pumilis & B.cereus* were commonly present. All these three isolates had degrading ability to the extent of 142, 96 and 109 units of keratinolytic activities respectively. It is stated that *B.pumilis* & *B.cereus* were inducible for production of keratinolytic proteases by feathers, whereas *B.subtilis* responded for the enzyme production with casein, feather and BSA. The optimum condition required for the maximum enzyme production activity were 40°C and pH 5-9 for *B.subtilis* , 40°C and pH 5-6 in respect of *B.pumilis* and 30°C and pH 7.0 for *B.cereus*. Time required for maximum keratinolytic activities for *B.subtilis* and *B.pumilis* were 84 and 72 hrs of incubation respectively with 161 and 149 units/ml enzyme production. *B.cereus* showed maximum enzyme activity of 117 units after 60 hrs of cultivation. It is also

reported that maximum enzyme activities were recorded in the late logarithmic growth phase or the beginning of the stationary phase. Similar trend was observed in production of soluble protein as that of keratinase.

Bacillus sp. MPTK6

According to the studies of Mukesh *et al.* (2012) *Bacillus sp. MPTK6* was found to degrade 30g/L raw feathers into feather protein hydrolysate under optimum pH 10.0 for 72 hrs of fermentation. Also the free radical- scavenging activity of feather protein hydrolysate was analysed using DPPH assay. Hydrolysed feather was found to have improved invitro digestibility than raw feathers.

Bacillus altitudinis

The importance of feather waste degradation by keratinolytic organisms was reported by Vijay Kumar *et al.* (2011) to supplement livestock feed and for production of protein hydrolysates. The keratin degrading bacterium *Bacillus altitudinis GVC11* was isolated and identified by him through morphological, biochemical and 16s RNA studies. The organism degraded white and dark chicken feathers in 48 and 96 hours respectively. Optimal production of keratinase enzyme by feather degradation was observed at 37°C, pH-9 and 200 rpm. During fermentation process, essential amino acids like phenyl alanine, valine, leucine, isoleucine and threonine were observed in 100 ml culture supernatant at 124, 88, 19, 17 and 11 µmols concentrations.

Bacillus licheniformis

B.licheniformis was the first bacterium to be identified for feather degradation and named as poultry waste digester-1 (PWD-1) by William *et al*. 1990. It is a non-pathogenic, gram positive, motile, spore producing, facultative anaerobe mainly present in soils and can be easily isolated due to highly resistant endospores. (Ponnusamy Konar Poovendran *et al.* 2011).

Brutt and Ichida , (1999) reported the presence of keratin hydrolyzing bacteria *B.licheniformis* in the plumage of living birds. They also demonstrated that inoculation of the bacterium intensified keratin degradation in poultry compost due to secretion of keratinase enzyme.

Ekta Tiwary and Rani Gupta, (2012) studied the keratinolytic property of *B.licheniformis* which produced the dimeric keratinase having the ability to degrade chicken feather. 25g of boiled chicken feather was converted into feather meal within 8 hours at 50°C, pH-8 and 150 rpm. 1200U of keratinase was required to degrade 2.5g of chicken feather. Feather meal produced after soaking and boiling was dried at 80°C and ground to fine powder. It contained 14% nitrogen and 44% carbon with all essential amino acids and showed 73% *in-vitro* digestibility.

Bacillus subtilis S1-4

Yong Bin *et al.* (2013) isolated a keratin degrading bacterial strain *Bacillus subtilis S1-4* from chicken feather and identified it by comparative genome analysis. 5% simple basal salt cultures at a narrow pH caused complete degradation of

chicken feathers resulting in the production of 11.61 mg/ml soluble peptides and 6.86 mg/ml amino acids respectively. During this process the organism secreted several proteases and keratinases along with disulphite reductase activity indicating their involvement in feather degradation.

Pseudomonas sp. MS21

As per investigations studies carried out by Tork *et al.* (2010) a keratinase producing organism *Kera MS21* was identified by morphological, physiological and biochemical characteristics as belonging to the genus *Pseudomonas.* The identification was confirmed by 16S rDNA studies. Optimal activity of the organism was observed at 37°C and pH-8.

Leuconostoc sp. and Pseudomonas microphilus

Tamil Kani *et al.* (2012) screened the ability of *Leuconostoc* and *Pseudomonas microphilus* to degrade feather keratin into feather meal. Among the two organisms maximum keratinase activity was 0.884 IU/ml for *Pseudomonas microphilus* in 30 days. 20% of the feather was degraded in 10 days and 70% in 30 days by *P.microphilus*.

Thermophiles

Complex nature of chicken feathers and pathogenicity mesophilic organisms has limited the use of mesophilic keratinolytic organisms. Some obligate thermophiles are also capable of degrading feather. One advantage of using thermophiles is that hard insoluble proteins like keratins gain plasticity at higher temperatures, so that they become more vulnerable to thermal proteases. Anaerobic thermophiles such as *Thermoanaerobacter keratinophilus*, *Fervidobacterium pennavorans* and *Fervidobacterium islandicum AW-1* have been reported so far to have keratinophilic activity (Anupam Bhagat and Smitha Lele, 2012).

Thermus aquaticus

Thermus aquaticus, a non-spore forming, obligate aerobic thermophile has been analysed for keratinase production by Anupam Bhagat and Smitha Lele, (2012) . *Thermus aquaticus* produced 479U/ml of keratinolytic protease by digesting 0.4g/dl chicken feather in 48 hours. Hence *Thermus aquaticus* was found to have remarkable feather-degrading properties and could be used for protease enzyme production for industrial detergent applications. Vitamin K2 rich cells of *T.aquaticus* grown on chicken feather waste along with carotenoids can be used as feather meal for poultry.

Fervidobacterium pennavorans

This bacteria is novel thermophilic bacteria belonging to *Thermotogales* order isolated by Friedrich and Antranikian, (1996) from the hot spring of Azores islands. This is the first known extreme thermophile having ability to digest native feathers at high temperatures. It grows optimally at 70°C and pH 6.5.Based on the physiology, morphology and 16S rDNA studies, the new isolate was considered to be a member of the *Thermotogales* order and was referred as *Fervidobacterium pennavorans*.This strain was mostly related to *Fervidobacterium islandicum* and *Fervidobacterium pullulanolyticum.*

Chemical constituent of the keratinolytic enzyme produced by this organism is a serine protease with a molecular mass of 130 kDa and an isoelectric point of 3.8 along with optimal activity at 80°C and pH 10.0.

Fungi

In addition to several bacteria, certain fungi have been isolated having keratinolytic properties. Some of keratinolytic fungi types are reviewed here. Anbu *et al.* (2011), isolated a total of 20 fungal strains and 5 dermatophytes. He isolated another 20 strains of fungi from poultry farm soil of Namakkal, India. The organisms were screened for the production of extracellular enzymes like amylase, protease, cellulase, lipase and keratinase using plate assay. All the sporulating organisms were found to produce atleast one keratinolytic enzyme. *Chrysosporium keratinophilum*, *Aspergillus flavus*, *A. niger*, *A. terreus*, *Penicillium citrinum*, *P. frequentans* and *P. purpurogenum* were able to produce all types of examined enzymes. *A. versicolor* and *A. niger* produced the highest amount of enzymes amylase and cellulase. *P.citrinum and Trichoderma viride* secreted lipase enzyme and *Scopulariopsis brevicaulis* secreted the highest amounts of protease and keratinase. *Trichophyton mentagrophytes* and *A.niger* produced keratinase in greater amounts.

Feather associated Fungi

Sanjana and Geetha, (1999) isolated fourteen species of keratinophilic fungi namely *Chrysosporium, Malbranchea, Chaetomium, Sepedonium, Microascus, Scopulariopsis, Curvularia, Fusarium, Aspergillus and Penicillium* from 100 poultry birds. The fungi were grown on basal salt solution containing natural keratin from human hair as a source of carbon and nitrogen and in another medium containing minor amount of readily utilisable carbon and natural keratin. All the isolated fungal species released sulphydryl containing compounds cysteine and extracellular keratinase. Improved enzyme production was observed with addition of glucose and vitamins in the broth which also indicated the correlation between the mycelia biomass and proteolytic keratinases.

Filamentous fungi

Nadir *et al.* (2008) analysed 106 filamentous fungal strains from poultry farm waste mainly litter in Brazil. Among those, 13 species belonging to different genera namely *Aspergillus, Acremonium, Alternaria, Beauvaria, Curvularia, Paecilomyces and Penicillium* were identified as feather keratin degrading organisms and were able to produce keratinase in the stationary phase of culture. A comparitive study on four highest keratinase producing organisms was carried out in submerged and stationary conditions. The highest keratinase production was observed in 4 to 6 days in submerged conditions with 53.8± 6.1 U/mL in *Alternaria tenuissima*, 51.2± 5.4 U/mL in *Acremonium hyalinulum*, 55.4± 5.2 U/mL in *Curvularia brachyspora* , and 62.8± 4.8 U/mL in *Beauveria bassiana*.

Non dermatophytic filamentous keratinolytic fungi

Ingle *et al.* (2012) undertook systemic studies to investigate keratinolytic fungi in

poultry feather waste. During this study, 22 fungal species namely *Aspergillus niger, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Myrothecium roridum, Penicillium sp., Trichoderma hamatum* including certain non- spore forming fungi were recovered from poultry farm soil samples as keratin degrading organisms. Optimum growth was observed at 45°C within pH range of 5.0 to 6.5. These organisms degraded feathers completely in 11 days by producing keratinase enzyme.

Aspergillus

Aspergillus is a highly aerobic spore-forming structure, which grows as molds on the surface of its substrates. It has been reported to produce several proteolytic keratinases. Kim, (2003) isolated 14 species of feather associated fungi from soil samples collected from 10 different poultry farms. Fungi belonging to 10 different genera namely *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Monascus*, *Mucor*, *Penicillum*, and *Verticillium* were identified. Especially Aspergillus sp. like *A.flavus*, *A. fumigatus*, *A. niger*, *A. nidulans*, and *A. terreus* degraded chicken feather keratin, producing sulphydryl compounds which were detected as keratinase and cysteine. Among the five species *A.flavus* was reported to have higher keratinase activity of 12.9 KU/ml and *A.fumigatus* had the lowest enzyme production of 10.4 KU/ml.

Knachana and Divakar, (2013) also attempted to find a way to mitigate the increased output of keratin containing wastes from poultry industries. They reported that keratinous wastes can be readily fermented and made into useful products like animal nutritional feed additives. A novel feather-degrading *Aspergillus sp.* was isolated from poultry farm soils of Goa, India. Maximum keratinase production was obtained when *Aspergillus sp.* was cultured at 37°C for 72 hours with pH 7-9.

Chrysosporium tropicum

Avasn Maruthi, (2011) assessed the degradation activity of feather and hair wastes using the fungus *Chrysosporium tropicum*. The fungi was highly keratinophilic and was cultured in mineral medium along with defatted feather. Maximum protein and Keratinase production of 6.9mg/ml and 8.56KU/ml was observed on 40th day sample with increase in alkalinity of the broth to pH-9.

Malbranchea aurantiaca and Fusarium culmoram

Piyusha, (2012) analyzed the feather degradation of hen feathers by 82 fungi isolated from Sagar, Dhar, Bhopal and Jabalpur districts of India by calculating percentage weight loss and growth of fungi on substrate. Among the isolated fungi 64 species were identified as keratinophilic fungi and *Malbranchea sp. S010* had the maximum keratinolytic activity of 37.81% feather degradation followed by *Malbranchea auranticum G012* of 35.57% and *Fusarium culmoram P010* of 28.66% degradation. Hence all these fungal isolates can be used for processing keratinous waste in large scale.

CONCLUSION

Owing to the fast growth of poultry industry with a CAGR of 8% in layer and 12% in broiler, there is enormous increase in poultry waste generation. There is a definite need to find an apt method to utilize the solid waste. Poultry feather contains valuable amino acids like cysteine and methionine. It was processed into feather meal using thermal and chemical methods. These methods had many disadvantages like loss of essential amino acids and energy expense. Bioremediation is a promising eco-friendly approach to utilize the poultry feather waste into value added products. This review highlights the use of bacteria and fungi as efficient waste utilization tools to process poultry feather waste. A number of keratinolytic bacteria and fungi in bioremediation of the feather are reviewed. Among the bacteria and fungi reported in this review, *Thermus aquaticus* a thermophile had the highest keratinolytic activity of 479U/ml. Thus Bioremediation can be used for best utilization of poultry waste to maintain the biodiversity.

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Full Length Research Articles

PLASMA TAURINE ESTIMATION IN DILATED CARDIOMYOPATHY OF LABRADOR RETRIEVERS

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ABSTRACT

The objective of this study was to quantify the concentrations of taurine in the plasma of Labrador retrievers with clinical disease of Dilated Cardiomyopathy (DCM) and to compare with the healthy Labrador retrievers. The study was conducted in thirty clinical cases of Dilated cardiomyopathy (DCM) in Labrador Retrievers and in thirty healthy Labradors. The plasma taurine estimation by HPLC showed a highly significant decrease in taurine levels (18.712 ± 3.53 nmol/mL) in dogs with Dilated cardiomyopathy compared to healthy dogs (51.022 ± 5.55 nmol/mL). Therefore, it was concluded that there was a significant role of plasma taurine in the pathogenesis of Dilated cardiomopathy in Labrador Retrievers . This pilot study justifies the supplementation of taurine in DCM of Labradors and also it opened up a new area for extensive research at a larger scale involving multi centres. In addition a commercially viable method for estimation of taurine was developed.

INTRODUCTION

Idiopathic, Dilated Cardiomyopathy (DCM) is a condition of unknown aetiology, characterized by progressive dilatation of one or both ventricles with severe impairment of systolic function in the absence of congenital, coronary arterial, hypertensive, vascular, pulmonary parenchymal, valvular, or other cardiovascular disorders (Cobb 1992). Although it is a disease of uncertain aetiology, various factors such as genetic and nutritional were proposed. Among nutritional, low L-carnitine in certain Boxers and low taurine in Cocker Spaniels and Newfouland were reported. Therefore, it is possible that Labrador Retrievers affected with DCM may have low taurine levels as this breed had originated from Newfounland.

Kramer *et al*. (1995) in a study of 76 dogs with DCM reported that plasma taurine concentration was low (< 25 nmol/mL) in 17% (13/76) of the dogs. Seven of the 13 dogs with low plasma taurine

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concentrations were Cocker Spaniels and Golden Retrievers. Belanger *et al*. (2005) reported taurine deficiency in a family of five Golden Retrievers. Backus *et al*. (2006) in a study of 216 Newfoundland a giant dog breed with high incidence of idiopathic DCM (1.3-2.5%), reported low plasma taurine in 8% of dogs. Of the nine taurine-deficient, clinically evaluated dogs, three had DCM that was reversed by taurine supplementation.

Taurine, an amino acid that exhibits anti-angiotensin II and osmoregulatory activity, is found in very high concentration in the heart. When the intracellular content of taurine is dramatically reduced, the heart develops contractile defects and undergoes an eccentric form of hypertrophy. The development of myocyte hypertrophy has been largely attributed to angiotensin II, whose growth properties are antagonized by taurine. Overt heart failure is usually associated with myocyte death, including death due to angiotensin II-induced apoptosis (Schaffer *et al*.2003).

The proposed research will estimate the prevalence of a possible unrecognized, widespread, taurine deficiency in the Labrador retriever breed leading to Dilated cardiomyopathy. The objective of the project is to quantify the concentrations of taurine in the plasma of Labrador retrievers with clinical disease of Dilated Cardiomyopathy (DCM) and to compare with the healthy Labrador Retrievers.

MATERIALS AND METHODS

The clinical cases of Dilated Cardiomyopathy was identified by the clinical findings, radiography, electrocardiography and confirmed with echocardiography. Laboratory investigations were done to rule out other coexisting diseases. The blood samples from the confirmed cases of Dilated Cardiomyopathy were collected in a heparinised polyurethane tubes and the plasma separated by centrifugation and stored at -20ºc for estimation of taurine. The control group includes healthy Labrador Retrievers attending hospital for regular health check up and vaccination. Plasma samples from healthy Labradors were collected and submitted for taurine estimation.

GROUPS OF CLINICAL STUDY

Group I: Thirty apparently healthy Labrador Retrievers acting as Control group

Group II: Thirty confirmed cases(twenty males and ten females) of Labrador Retrievers with Dilated

Cardiomyopathy

Physical examination was carried out as suggested by McCurin and Poffenbarger (1991). The animals under study were subjected to thoracic radiography to record the changes and Vertebral Heart Score (Plate-1) was calculated as suggested by Buchanan and Bucheler (1995). Electrocardiography was recorded as per the standard procedure described by Tilley and Smith (1997) usingWellchAllyn ECG monitor. Echocardiographic examinations were performed as suggested by Boon (1998)using ALOKA SSD 3500 ultra sound system with a Phased array Transducer of 3.0 – 6.0 MHz to obtain Two dimensional, M- mode, Pulsed wave and color flow Doppler echocardiography images of heart.

The following guidelines for the diagnosis of DCM as proposed by McEwan *et al.*(2003) were used with little modification for clinical cases of Dilated cardiomyopathy to be included in the study group:

1. Left ventricular dilation (especially in systole but also in diastole).
2. Depressed systolic function.
3. Altered geometry of the left ventricle (increased sphericity).
4. Left or bi-atrial enlargement
5. M-mode fractional shortening of < 25%.
6. Left ventricular ejection fraction less than 40%.
7. Increased mitral valve M-mode E point to septal separation (EPSS).
8. No co-existing diseases

Procedure of Plasma Taurine estimation by High Performance Liquid Chromatography (HPLC)

The mobile phase composition of HPLC consists of 0.02M phosphate buffer and the acetonitrile in the ratio of 84:16 and the PH 6 was maintained using 0.1% acetic acid. C18 column of 250 X 4.6mm and particle size of 5µ was used in this method. The temperature was set at 30° C throughout the analysis. RF fluorescence detector was used at the excitation wavelength of 350nm and emission wavelength of 570nm. The injection volume of 10ul is injected in to the HPLC system. The flow rate was maintained at 1.2ml/minute.

Preparation of Standard Solutions

Standard taurine solutions were made as close to 10, 20 and 50 ppm as possible. A solid taurine sample (Sigma-Aldrich) of 0.2519 g was dissolved in DI water in a 500-mL volumetric flask and diluted to the mark. Three 50-mL volumetric flasks were obtained and 1.0, 2.0 and 5.0 mL of the previous solution were added to each, respectively. The flasks were then diluted to volume with DI water. The intra- and the inter-day coefficients of variation for the method were 5.3% and 7.7%, respectively. The calibration curve was linear from 0.1 µmol/L to 30.0 µmol/L with a correlation coefficient of 0.9995. A results summary for each of the three standards. Sample Conc (ppm) Run 1 Area (mAU·s) Run 2 Area (mAU·s) Run 3 Area (mAU·s) Run 4 Area (mAU·s) Standard 1 9.968 54.5 54.3 51.3 52.4 Standard 2 19.94 113.4 114.1 125.7 125.9 Standard 3 49.84 307.0 306.9 339.9 339.1.LOD 50 ug/Kg, LOQ75 ug/Kg2. Recovery percentage 85-91%3. Linearity range The intra- and the inter-day coefficients of variation for the method were 5.3% and 7.7%, respectively. The calibration curve was linear from 0.1 µmol/L to 30.0 µmol/L with a correlation coefficient of 0.9995.

RESULTS AND DISCUSSION

The average age of affected dogs was 6.68 ± 0.47years, and the incidence in male dogs were 67 per cent (20/30) and female dogs were 33 per cent (10/30).The physical examination findings recorded

were dyspnoea in 100 per cent (30/30) of dogs, ascites in 97 per cent (29/30) of dogs, gallop rhythm in 87 per cent (26/30) of dogs, systolic murmur in 73 per cent (22/30) of dogs, limb oedema in 53 per cent (16/30) of dogsand weak femoral pulse in 50 per cent (15/30) of dogs. In ECG, normal sinus rhythm was appreciated in 63 per cent (19/30) of dogs andST depression in 70 per cent (21/30) of dogs that were affected with DCM. The abnormal rhythm includes atrial fibrillation in(fig 1) 27 per cent (8/30) of dogs and ventricular premature contraction in 10 per cent (3/30) of dogs.

In radiography,(fig2) highly significant($p \leq 0.01$) increase in the vertebral heart score was noticed in DCM (12.51 ± 0.14) dogs compared to control (10.78 ± 0.03). The other major radiographic abnormalities in the DCM affected dogs were cardiomegaly in 100 per cent (30/30) of dogs, pulmonary edema in 80 per cent (24/30) of dogs and pleural effusion in 20 per cent (6/30) of dogs.

In 2-D echocardiography, highly significant ($p \leq 0.01$) increase in the LA (4.09±0.05cm), Ao (2.08±0.05cm), LA/Ao (2.01±0.06), EDV(95.16±1.97ml) and ESV(67.30±2.36ml) were noticed in DCM dogs compared to control. Highly significant decrease in the EF (29.11±2.13 per cent) was noticed in DCM dogs compared to control. In M-Mode, highly significant increase in the LVIDd (5.84±0.13cm), LVIDs (5.05±0.12cm), RVIDs (1.24±0.09cm) and EPSS (1.92±0.09cm) were noticed in DCM dogs compared to control whereas highly significant decrease in the IVSs (0.81±0.04cm), LVPWs (0.97±0.03cm), FS (13.45±0.73per cent) and IVS FT (15.99±2.38per cent) were noticed in DCM dogs compared to control (fig 3).

The present physical findings, electrocardiography, radiography and echocardiography findings in dilated cardiomyopathy affected dogs are consistent with the findings of previous reports byCalvert *et al*. (1982); Atkins and Snyder (1992); Monnet *et al*. (1995); Tidholm and Jonsson (1996); McEwan *et al.*(2003)and Borgarelli *et al*. (2006)

The plasma taurine estimation by HPLC showed a highly significant decrease in taurine levels (18.712 ± 3.53 nmol/mL) in dogs with Dilated cardiomyopathy compared to healthy dogs (51.022 ± 5.55 nmol/mL). Delaney *et al* (2003) reported plasma taurine levels of less than 40 nmol/mL as critically low. In the present study the mean plasma taurine levels in DCM dogs were well below the critical levels reported Delaney *et al* .2003.When compared, no significant changes in ECG, radiograph, echocardiography and plasma taurine levels were recorded in between the males and female Labrador retrievers with Dilated Cardiomyopathy.

Schaffer *et al*. (2003) reported a very high concentration of taurine in the heart and exhibits anti-angiotensin II and osmoregulatory activity. The authors proposed that when the intracellular content of taurine is dramatically reduced, the heart develops contractile defects and undergoes an eccentric form of hypertrophy. The development of myocyte hypertrophy has been largely attributed to angiotensin II, whose growth properties are antagonized by taurine. Overt heart failure is usually

associated with myocyte death, including death due to angiotensin II-induced apoptosis.

CONCLUSION

From the study, it was concluded that there was a significant ($p<0.01$) decrease in plasma taurine levels in DCM Labrador retrievers compared to normal Labradors and therefore, taurine deficiency has a significant role in the pathogenesis of DCM in Labrador retriever. This pilot study justifies the supplementation of taurine in DCM of Labradors and also it opened up a new area for extensive research at a larger scale involving multi centres. In addition a commercially viable method for estimation of taurine was developed.

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ACKNOWLEDGEMENT

The authors thank Tamil Nadu Veterinary and Animal Sciences University for providing their corpus funds to carry out this research work.

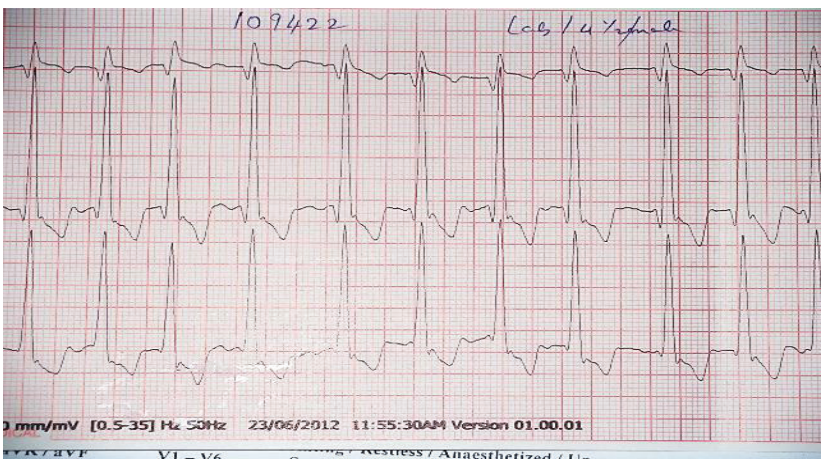


Figure 1 – Electrocardiographic findings in DCM-Atrial Fibrillation

Figure 2- cardiomegaly with pulmonary edema

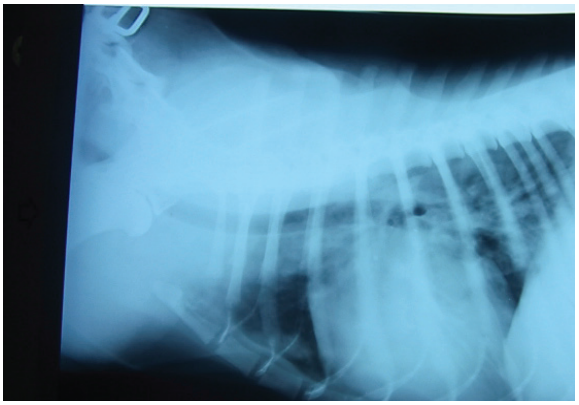
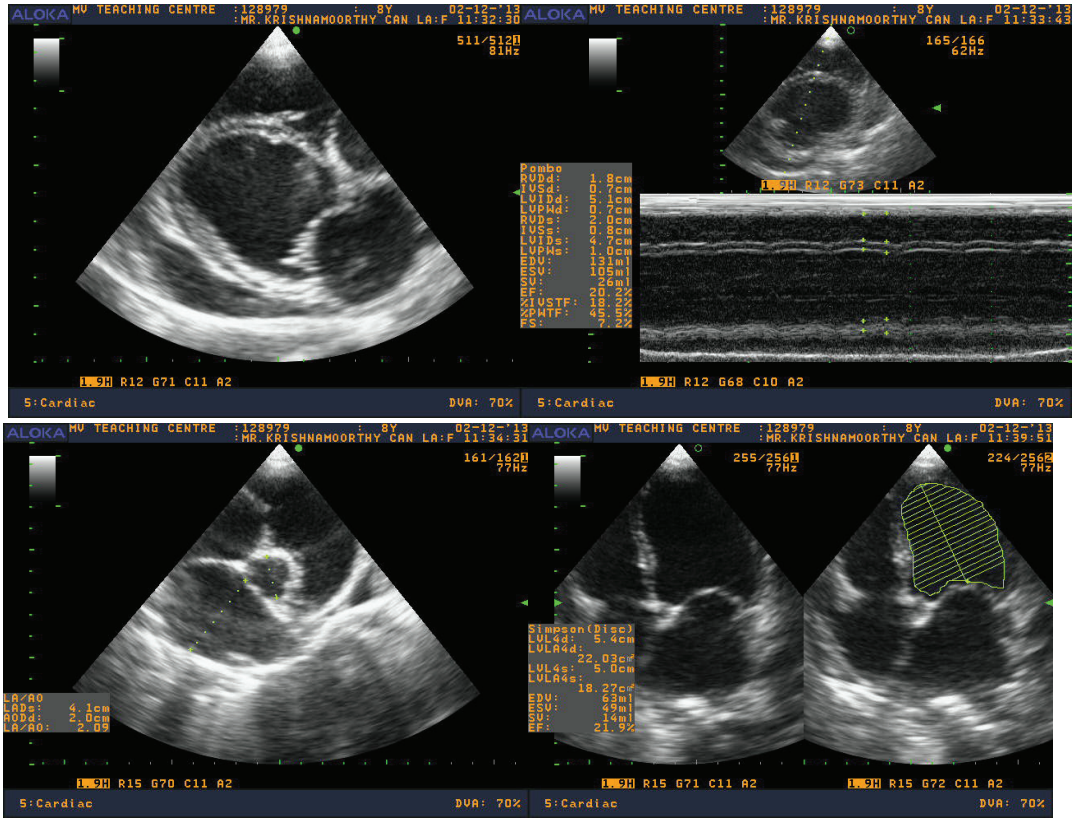


Figure 3 – Echocardiographic findings in DCM



Echocardiograph showing dilated heart chambers and reduced contractility

EFFECT OF FARMYARD MANURE ON BIOMASS YIELD, CARBON ASSIMILATION POTENTIAL AND CORRELATION COEFFICIENT IN FODDER MAIZE (*Zea mays* L.) IN DIFFERENT AGROCLIMATIC ZONES

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ABSTRACT

A field experiment was conducted to study the effect of inorganic fertilizer and the synergistic effect of farmyard manure (organic) with inorganic fertilizer on the Green Fodder Yield (GFY), Dry Matter Yield (DMY), Carbon Assimilation Potential (CAP) and Correlation Coefficient between soil organic carbon and plant organic carbon in Fodder Maize (*Zea mays L.*) crop field in North Eastern and Western Zones of Tamil Nadu, India during summer season of 2012. For this study, in Western zone two districts viz., Coimbatore and Erode districts and in North Eastern Zone Tiruvannamalai and Vellore districts were selected for the field experiments. From the selected district, two villages were randomly selected (2 village/ district) for field experiments totaling to eight experimental sites for the study. Green fodder yield for T1 (Recommended dose of NPK) and T2 (Recommended dose of organic and inorganic fertilizer) on 60th day ranged between 36.15 to 39.07 t/ha and 36.92 to 40.23 t/ha for all villages. On the other hand, Dry Matter Yield for T1 and T2 on 60th day varied between 6.37 to 6.91 t/ha and 6.51 to 7.09 t/ha for all villages. Carbon Assimilation Potential for T1, T2 on 60th day varied between 3.17 to 3.82 t/ha and 3.32 to 3.99 t/ha for all villages. A positive correlation ($P<0.01$) existed between soil and plant organic carbon in Fodder Maize for both treatments (T1 and T2) during the study period. This study recommended the use of farmyard manure along with inorganic fertilizer as the best option for increased biomass yield which also had positive effect on carbon sequestration potential

Key Words: Green fodder yield, Dry matter yield, Farm yard manure, Fodder Maize,

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Inorganic fertilizer

INTRODUCTION

In India, modern agriculture based on chemicals is not sustainable because of problems associated with loss of soil productivity. Excessive soil erosion, plant nutrient losses, surface and ground water pollution as a result of pesticides and fertilizers are the factors that are responsible for loss of soil productivity. Intensive agriculture has also a negative effect on the soil environment over the past decades. Chemical fertilizers play a crucial role to meet the nutrient requirement of the crop, persistent nutrient depletion poses a greater threat to sustainable agriculture. Nowadays, consumer prefers organically grown produce as they are free of toxic residues and are grown with a concern for environment. Therefore, there is an urgent need to reduce the usage of chemical fertilizers and in turn increase the usage of organic manure. Use of organic manures alone or in combination with chemical fertilizers, helps in improving physico-chemical properties of the soil and improves the efficient utilization of applied fertilizers and results in higher fodder yield and quality. Judicious use of combinations of organic and inorganic resources is a feasible approach to overcome soil fertility constraints (Abedi *et al*., 2010). Combined organic and inorganic fertilization could enhance carbon storage in soils and reduce emission from N fertilizer use, while contributing to high productivity in agriculture (Pan *et al*., 2009). Sustaining soil health through inclusion of manure in the fertilization schedule is important since it can improve the organic carbon status and available N, P, K and S in soil (Tiwari *et al*., 2002). To improve soil physical properties, addition of various organic materials could be undertaken and combined use of NPK and FYM increases soil organic matter compared to application of NPK through inorganic fertilizers (Bhattacharya *et al*., 2008). Organic manures *viz*., farm yard manure, vermicompost, poultry manure and oilcakes help in the improvement of soil structure, aeration and water holding capacity of soil. Further, it stimulates the activity of microorganisms that makes the plant to get the macro and micro-nutrients through enhanced biological processes, increase nutrient solubility, alter soil salinity, sodicity and pH (Alabadan *et al*., 2009). Hence the present study was undertaken to determine the effect of inorganic fertilizer and combined effect of inorganic fertilizer with organic fertilizer (farm yard manure) on biomass yield, carbon assimilation potential and correlation coefficient between soil and plant organic carbon in Fodder Maize *(Zea mays* L.*)* in two agro climatic zones of Tamil Nadu.

MATERIALS AND METHODS

The field experiment was carried out using the Annual fodder crop, Fodder Maize (*Zea mays* L.) in two agro climatic zones of Tamil Nadu, India viz., Western and North Eastern zone during the summer season of 2012. In each zone two districts viz., Coimbatore and Erode districts (Western Zone) and Tiruvannamalai and Vellore districts (North Eastern zone) were selected. From each district, two villages were randomly selected, totaling to eight experimental sites for the study. In Coimbatore and Erode district the experimental sites were located at

Kondaiyampalayam (V1), Idigarai (V2), Velankattuvalasu (V3) and Velliyampalayam (V4). In Tiruvannamalai and Vellore district, the selected experimental sites were located at Vannankulam (V5), Kolathur village (V6), Saduperi (V7) and Thirumani (V8).

In all the selected sites the land was ploughed twice by a tractor with chisel ploughing followed by harrowing. The fields were brought to fine tilth, leveled with a wooden plank and laid out in to the proper plot size (6 x 4 m).The experiment was laid out with six replications per treatment in all the study fields. Fodder maize was planted at 60 x 30 cm intervals on either side of the ridges. The experiment consisted of two treatments viz., Treatment 1 (T1) which was control with recommended dose of NPK fertilizers (60 N, 40 P_2O_5 and 20 K_2O kg/ha) alone and Treatment 2 (T2) which included Farmyard Manure (Organic – Recommended dose - 12.5 t/ha) along with NPK fertilizer (inorganic – Recommended dose). The fertilizers were applied in the form of urea (N), Di-ammonium Phosphate (P_2O_5) and Muriate of Potash (K_2O). In all, 50 per cent of nitrogen and entire dose of P_2O_5 and K_2O were applied at the time of sowing and remaining 50 per cent of nitrogen was top dressed in the form of urea at 30 days after sowing (DAS). All the cultural practices were followed as per the recommended package of practices for the Fodder Maize crop. The necessary after care operations such as hand weeding were done as per the requirement. The plant protection measures have been adopted to control the pest and disease. Irrigation was carried out immediately after sowing (0^{th} day), on 3^{rd} day and there after once in 7 days. Green fodder yield was recorded from each net plot in one square meter area and expressed in tonnes/ha. For dry matter determination, aluminium containers were oven dried and weighed using electric balance. Ten grams of plant sample was weighed in each container and placed in an oven at 105 °C till constant weight was attained using the following formula.

$$\text{Dry Matter (\%)} = \frac{\text{wt. of oven dry sample}}{\text{wt. of sample before drying}} \times 100$$

$$\text{Dry matter yield (t/ha)} = \frac{\text{Fresh fodder yield} \times \text{Dry Matter (\%)}}{100}$$

The carbon sequestration (assimilation) by the plant was calculated using the following formula (Negi *et al*., 2003).

Carbon sequestered (Assimilation) = Biomass x Carbon (%)

Statistical analysis

The data collected were subjected to‘t’ test to find out the significant difference between treatments for all villages. In addition, One-Way ANOVA was performed using SPSS 13.0 to evaluate the significant difference between districts and zones. Also interpretation of data was done as per the procedure described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Green Fodder Yield in Fodder Maize

The Mean values of Green Fodder Yield (GFY) for Fodder Maize in Western and North Eastern zone of Tamil Nadu are presented in Table 1. Green fodder yield for T1 and T2 in 60th day ranged between 36.15 to 39.07 t/ha and 36.92 to 40.23 t/ha for all villages. Statistical analyses revealed highly significant (P<0.01) difference in fodder yield between treatments for the villages, V1, V2, V4 and significant (P<0.05) difference for other villages on 60th day of the trial period. The green fodder yield increase was due to the result of higher plant height, stem diameter and more dry matter production per plant. This was due to the regulatory role of nitrogen in production of amino acids and plant hormones responsible for cell division and enlargement and higher nitrogen facilitated optimum development of photosynthetic apparatus which captured the incident light more efficiently (Tariq *et al*., 2011). It could be observed from Table 1 that T2 values pertaining to green fodder yield were significantly (P < 0.05 or 0.01) higher than T1 at harvest stage (60th day). This could be due to the benefits of organic matter providing N, P, and K supply which resulted in improvement of microbial activity, better supply of macro and micro nutrients such as S, Zn, Cu and B which were not supplied by inorganic fertilizers and due to the lower losses of nutrients from the soil (Bhattacharya *et al*., 2008) . Moreover, farmyard manure with inorganic fertilizers not only increased the availability of nutrients and improved the soil fertility, but also enhanced fodder production significantly (Ahmad *et al*., 2011). Farmyard manure contained readily metabolizable carbon and N which increased the root biomass and root exudates and contributed to its biomass increase (Liu *et al*., 2010). Moreover, the beneficial effect of farm yard manure on yield might be due to increased organic matter present that has improved the soil structure conditions which encouraged the plant for good root development by improving the aeration of the soil. This was in agreement with the findings of Ouda and Mahadeen (2008) and Salam and Salem (2012). Moreover farm yard manure provided abounding organic matter for the growth of microorganisms which favored increased yield (Gong *et al*., 2009).

Dry Matter Yield in Fodder Maize

The Mean values of Dry matter yield (DMY) for Fodder Maize in Western and North Eastern zone of Tamil Nadu are presented in Table 2. Dry Matter Yield for T1 and T2 on 60th day ranged between 6.37 to 6.91 t/ha and 6.51 to 7.09 t/ha for all villages. Statistical analyses revealed highly significant (P<0.01) difference between treatments in DMY for Fodder Maize in the villages, V1, V2, V4 and significant (P<0.05) difference for other villages on 60th day of the trial period. The Dry matter yield was associated with the green fodder yield which in turn depends on fodder production. This was in concurrence with the findings of Ali *et al*. (2012). It could be observed from the results that T2 values were significantly (P < 0.05 or 0.01) higher than T1 at harvest stage (60th day). This might be due to the incorporation of farm yard manure which provided essential nutrients for growth of plant which significantly enhanced the fodder production and in turn on the dry

matter yield. This was in agreement with the findings of Sharma *et al.* (2012).

Carbon Assimilation Potential in Fodder Maize

The Mean values of Carbon Assimilation Potential (CAP) for Fodder Maize in Western and North Eastern zone of Tamil Nadu are presented in Table 3. CAP in Fodder Maize for T1 and T2 on 60th day ranged between 3.17 to 3.82 t/ha and 3.32 to 3.99 respectively for all villages. Statistical analyses revealed highly significant ($P<0.01$) difference between treatments in CAP for all the villages on 60th day of the trial period. The carbon assimilation potential depends mainly on the plant organic carbon as well as dry matter yield. With the increase of dry matter yield and plant organic carbon, the carbon assimilation potential of Fodder Maize increased. The results were in accordance with the findings of Montagnini and Nair (2004) and Yadava (2010). In general, fertilization stimulates biomass production and enhances carbon accumulation. This was in agreement with the findings of Schuman *et al.* (2002). Montagu *et al.* (2005) reported that biomass was an important indicator in carbon sequestration. Ground biomass of plants definitely has greater influence on the carbon sequestration potential in energetic crops (Walker *et al*., 2008). As far as villages are concerned, it could be observed that on 60th day, the carbon assimilation potential in Fodder Maize was significantly higher in V3 followed by V4, V2, V1, V6, V5, V8 and V7 in descending order. This might be due to significant increase in green fodder yield observed in V3 than V4, V2, V1, V6, V5, V8 and V7. This was in agreement with the findings of Ahmad *et al.* (2011) who stated that higher SOC content and combination of farm yard manure with inorganic fertilizers increased the availability of nutrients in soil resulting in higher fodder yield and enhanced carbon assimilation potential.

Correlation Coefficient

The correlation coefficient between Soil Organic Carbon and Plant Organic Carbon for Fodder Maize in Western zone and North Eastern zone of Tamil Nadu are presented in Table 4. The results revealed significant ($P < 0.01$) positive correlation between soil and plant organic carbon in Fodder Maize for both treatments (T1 and T2) during the experimental period. This might be due to plant growth which absorbed carbon-dioxide from the atmosphere and in turn stored in leaves, stems and root biomass (Tariq *et al.,* 2011). This was in agreement with the findings of Ingram and Fernandes (2001) who stated that abundant carbon accumulation was observed during plant harvest and root biomass acted as a sink in increasing soil organic carbon. Hence, with increased plant organic carbon there would be enhanced accumulation of soil organic carbon which was positively correlated.

It is concluded from the results that farmyard manure along with inorganic fertilizer resulted in significantly higher green fodder yield as well as dry matter yield of the fodder crop than individual. This increased green fodder yield resulted in higher carbon assimilation potential of the fodder crops which in turn could increase the soil fertility and productivity. Thus this study recommended the use of farmyard manure along with inorganic fertilizer as the best option for increased biomass yield

which also had positive effect on carbon sequestration potential.

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Table – 1. Green Fodder Yield (t/ha) in Fodder Maize (60th day) of Western and North Eastern zone of Tamil Nadu

Zone	District	Villages	Green Fodder Yield (t/ha)		
			T1	T2	t value
			Mean ± S.E	Mean ± S.E	
Western Zone	Coimbatore	Kondaiyampalayam (V1)	37.27 ± 0.12^{cd}	38.18 ± 0.17^{cd}	4.40**
		Idigarai (V2)	37.70 ± 0.13^{de}	38.82 ± 0.32^{de}	3.27**
	Erode	Velankattuvalasu (V3)	39.07 ± 0.26^{f}	40.23 ± 0.34^{f}	2.71*
		Velliyampalayam (V4)	38.08 ± 0.21^{e}	39.42 ± 0.31^{e}	3.59**
North Eastern Zone	Tiruvannamalai	Vannankulam (V5)	36.65 ± 0.20^{ab}	37.38 ± 0.17^{ab}	2.76*
		Kolathur (V6)	36.95 ± 0.21^{bc}	37.77 ± 0.19^{bc}	2.86*
	Vellore	Saduperi (V7)	36.15 ± 0.19^{a}	36.92 ± 0.17^{a}	2.88*
		Thirumani (V8)	36.47 ± 0.26^{ab}	37.38 ± 0.22^{ab}	2.67*
F value			21.96**	21.37**	

T1 (Recommended dose of NPK) and T2 (Recommended dose of organic and inorganic fertilizer)

Means bearing different superscripts between columns (T1 and T2) do not differ significantly

* - Significant (P<0.05) ** - Highly Significant (P<0.01)

Table – 2. Dry Matter Yield (t/ha) in Fodder Maize (60th day) of Western and North Eastern zone of Tamil Nadu

Zone	District	Villages	Dry Matter Yield (t/ha)		
			T1	T2	t value
			Mean ± S.E	Mean ± S.E	
Western Zone	Coimbatore	Kondaiyampalayam (V1)	6.58 ± 0.02^{cd}	6.73 ± 0.03^{c}	4.09**
		Idigarai (V2)	6.64 ± 0.02^{de}	6.85 ± 0.05^{d}	3.58**
	Erode	Velankattuvalasu (V3)	6.91 ± 0.05^{f}	7.09 ± 0.06^{e}	2.34*
		Velliyampalayam (V4)	6.70 ± 0.04^{e}	6.96 ± 0.05^{de}	4.00**
North Eastern Zone	Tiruvannamalai	Vannankulam (V5)	6.45 ± 0.03^{ab}	6.59 ± 0.03^{ab}	2.91*
		Kolathur (V6)	6.52 ± 0.04^{bc}	6.67 ± 0.03^{bc}	3.01*
	Vellore	Saduperi (V7)	6.37 ± 0.03^{a}	6.51 ± 0.02^{a}	2.95*
		Thirumani (V8)	6.44 ± 0.05^{ab}	6.61 ± 0.04^{abc}	2.79*
F value			22.68**	20.87**	

T1 (Recommended dose of NPK) and T2 (Recommended dose of organic and inorganic fertilizer)

Means bearing different superscripts between columns (T1 and T2) do not differ significantly

* - Significant (P<0.05) ** - Highly Significant (P<0.01)

Table – 3. Carbon Assimilation Potential (t/ha) in Fodder Maize (60th day) of Western and North Eastern zone of Tamil Nadu

Zone	District	Villages	Carbon Assimilation Potential (t/ha)		
			T1	T2	t value
			Mean ± S.E	Mean ± S.E	
Western Zone	Coimbatore	Kondaiyampalayam (V1)	3.47 ± 0.01^{de}	3.63 ± 0.03^{d}	5.73**
		Idigarai (V2)	3.53 ± 0.02^{e}	3.74 ± 0.04^{e}	4.81**
	Erode	Velankattuvalasu (V3)	3.82 ± 0.03^{g}	3.99 ± 0.04^{g}	3.49**
		Velliyampalayam (V4)	3.64 ± 0.03^{f}	3.86 ± 0.03^{f}	5.51**
North Eastern Zone	Tiruvannamalai	Vannankulam (V5)	3.35 ± 0.02^{c}	3.50 ± 0.02^{bc}	5.11**
		Kolathur (V6)	3.40 ± 0.02^{cd}	3.57 ± 0.03^{cd}	4.14**
	Vellore	Saduperi (V7)	3.17 ± 0.02^{a}	3.32 ± 0.02^{a}	4.98**
		Thirumani (V8)	3.27 ± 0.03^{b}	3.43 ± 0.03^{b}	3.31**
F value			68.02**	53.62**	

T1 (Recommended dose of NPK) and T2 (Recommended dose of organic and inorganic fertilizer)

Means bearing different superscripts between columns (T1 and T2) do not differ significantly

* - Significant (P<0.05) ** - Highly Significant (P<0.01)

Table – 4. Correlation Coefficient between Soil Organic Carbon and Plant Organic Carbon for Fodder Maize in Western and North Eastern zone of Tamil Nadu

Soil Vs fodder	Correlation Coefficient	
Treatments	Fodder Maize	
	30 days	60 days
T1	0.76**	0.73**
T2	0.78**	0.76**

** - Highly Significant (P<0.01)

GROWTH PERFORMANCE OF LAMBS UNDER INTENSIVE, CONTINUOUS AND ROTATIONAL GRAZING SYSTEMS OF MANAGEMENT

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ABSTRACT

A trial was conducted to assess the growth performance of lambs under intensive, continuous and rotational grazing systems of management in indigenous ewe lambs, aged 4-5 months. They were randomly selected and allotted to three treatment groups: T_1 (Intensive system – control), T_2 (Rotational grazing) and T_3 (Continuous grazing). The three treatment groups were compared for fortnightly bodyweight, average daily gain, fecal egg count (EPG). An artificial pasture was developed with *Panicum maximum* (Guinea grass), *Stylosanthus*sp., *Desmanthus*sp., and *Cenchrusciliaris*(Buffel grass) for the lambs under grazing systems. The Lambs under T_1 were raised under stall fed system of management, the lambs under T_2 were grazed under rotational grazing strategy in the four paddocks (2500 sq. ft. each) of plot-A while the lambs under T_3 were continuously grazed in plot-B (10000 sq. ft.). At the end of the study period, statistical analysis revealed no significant difference between the treatment groups in fortnightly body weight as well as average daily gain (except for the fourth fortnight). However lambs under T_2 had better mean fortnightly body weight and average daily gain. Significant differences were noticed in EPG (for Strongyle eggs) and biomass yield of fodder. Lambs under T_2 had significantly ($P \leq 0.01$) lower E.P.G. than T_3. Plot-A under rotational grazing had significantly ($P \leq 0.01$) higher fodder biomass yield and comparatively higher crude protein content than plot-B under continuous grazing. The results of this study pointed towards benefits in terms of lower EPG and better quantity and quality of biomass in rotational grazing compared to continuous grazing.

Keywords: Rotational grazing, Continuous grazing, Ewe lambs, Growth Performance

India possesses 65.06 million sheep (19th Livestock census- All India report, 2012). The multi-faceted sheep husbandry forms an integral part of rural agrarian economy of arid and semi-arid zones of India. Sheep is mainly raised

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under extensive system of management and grazing in natural range lands is the predominant source of nutrition.

The poor productivity of Indian pasture has always been a saddening fact. Shankar and Gupta (1992) classified the Indian grazing lands as fragile eco-systems and ranked them as class IV and V in their land capability classification. The carrying capacity of our native rangeland is 0.20 to 1.47 adult cattle unit (A.C.U) / ha.,but the present stocking rate happens to be much higher.

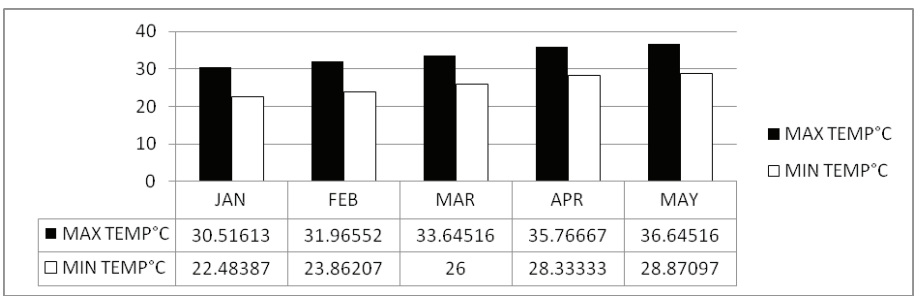
The extensive system involving grazing also suffers from the worm nemeses. The parasites play a major part in contributing to the production losses of sheep husbandry in India. Further, overuse of anthelmintics has raised the problem of drug resistance in field conditions.

The concept of creating artificial pasture for animals has been cropping up lately in few countries like Turkey and China. These pasture promise better biomass and nutrient availability than the native rangeland (Acar*et al.,* 2010). Moreover, the system of grazing has been found to affect the quantity and quality of biomass yield from developed pastures. Grazing management with pasture rotation plays a major role in sustainable helminth control in livestock as reported by Waller (1997), Bukhari and Sanyal (2011) and Kumar *et al*. (2013). In the present study the concept of developed pasture for sheep rearing was investigated. The seasonal pasture biomass and herbage dynamics of the artificial pastures were also observed. The production performance of indigenous ewe lambs and exposure to helminth infection under different systems of grazing in developed pasture was studied.

MATERIALS AND METHODS

Experimental location: The experiment was carried out at University Research Farm, TANUVAS, Chennai-51. It lies between latitudes 12º 9’ and 13º 9’ and longitudes 80º 12’ and 80º 19’ E with an altitude of 22 m above MSL.The experiment was conducted for a period of five months from January 2016 to May 2016. The maximum and minimum temperature recorded during the experimental period was 36.64ºC and 30.51ºC respectively. Rainfall was observed in the month of January (0.95 mm) and May 2016 (185.55 mm). The maximum and minimum temperature during the experimental period is given in Fig.-1.

Figure-1 Maximum and minimum temperature of the experimental location



	JAN	FEB	MAR	APR	MAY
■ MAX TEMP°C	30.51613	31.96552	33.64516	35.76667	36.64516
□ MIN TEMP°C	22.48387	23.86207	26	28.33333	28.87097

Animals and pasture management: The trial was conducted with thirty indigenous ewe lambs in the age group of 4-5 months maintained at the University Research Farm, Madhavaram. The experimental animals were dewormed before the start of the experiment.

Ten animals were randomly allotted based on body weight to the following three treatment groups: T_1 (Intensive system – control), T_2 (Rotational grazing) and T_3 (Continuous grazing).The Lambs under T_1 were raised under stall fed system of management and was sheltered in pens on slatted floormade of wooden slats of 4 cm width. The gap between slats was 18 mm. The slatted floor was elevated 2 feet from the ground level. The lambs under intensive system were fed with cereal and leguminous fodder*ad-libitum* in the ratio of 1:1.

An artificial pasture of 0.18 hectare was developed with *Panicum maximum* (Guinea grass), *Stylosanthus*sp., *Desmanthus*sp., and *Cenchrusciliaris*(Buffel grass) for the lambs under grazing systems. The lambs after grazing were sheltered in night shelters. All the experimental animals were supplemented with 100 g of concentrate per day.

Grazing management: The Lambs under T_2 were grazed under rotational grazing strategy in the four paddocks (2500 sq. ft. each) of plot-A while the lambs under T_3 were continuously grazed in plot-B (10000 sq. ft.). The grazing period was 4-5 days in each paddock since the infective stage of *Haemonchuscontortus*L_3 takes 7 days to develop (Colvin *et al.,* 2009).

Rest period was 15 days per paddock since the fodder biomass was optimum at this stage. A rest period, more than 15 days was not practically applicable as the biomass was surplus at this stage. Further, Guinea grass after 15 days of rest period had a sward length more than 15 cm which makes parasite prevalence less (Kumar *et al.,* 2013) in the top surface of the sward to be consumed by the lambs.

The animals under treatment 3 were allowed for continuous grazing in the plot- B. Animals under both the systems of grazing were allowed to graze for a period of 6-8 hours.

*Experimental sampling and analysis:*A wooden quadrate of 1 m² was made and was swung from the center of the paddock to attain randomness in selection of area to be harvested for biomass estimation (Onatibiaand Aguiar 2016).The herbage in area under wooden quadratewas clipped and weighed and then extrapolated to calculate fodder biomass of the plot in terms of dry matter yield expressed in Tons per hectare. Analysis of dry matter, crude protein, crude fibre, ether extract and total ash of the fodder samples were carried out as per AOAC, (2005).

The body weight and average daily gain (g) of all the experimental animals were recorded fortnightly with an electronic digital weighing balance with 50 g accuracy. The parasitic burden was assessed by fecal examination-direct floatation method and the eggs were enumerated using the McMaster technique following standard protocol.

The carrying capacity of the developed pasture involved in this experiment was calculated in terms of A.C.U(1 A.C.U = 0.2 Sheep) irrespective of the biomass availability of the pasture.

Carrying capacity / hectare = Animal units involved in this experiment X 10.8

Statistical Analysis: The data collected were subjected to statistical analysis as per the methodssuggested by Snedecor and Cochran (1994).

RESULTS

The fortnightly body weights pf lambs pertaining to the different treatment groups are on Table-1. Statistical analysis revealed no significant difference in fortnightly body weights of lambs under different system of management. However lambs, under rotational grazing system had comparatively better body weights, than those under intensive system and continuous grazing system of management.

Table-1: The fortnightly mean ± S.E and analysis of variance of body weights (kg) of lambs under different treatment groups

Fortnights	T_1 (Intensive system)	T_2 (Rotational Grazing)	T_3 (Continuous Grazing)	P value
1	13.03 ± 0.64	12.10 ± 0.68	11.94 ± 0.80	0.55 ^{NS}
2	13.48 ± 0.59	12.38 ± 0.72	12.64 ± 0.75	0.55 ^{NS}
3	13.81 ± 0.63	12.92 ± 0.83	12.79 ± 0.63	0.58 ^{NS}
4	14.30 ± 0.52	12.99 ± 0.90	14.01 ± 0.83	0.49 ^{NS}
5	15.13 ± 0.52	13.70 ± 0.93	14.04 ± 0.89	0.47 ^{NS}
6	16.02 ± 0.48	14.90 ± 1.06	14.71 ± 0.90	0.55 ^{NS}
7	15.79 ± 0.74	16.07 ± 1.15	15.70 ± 0.65	0.94 ^{NS}
8	16.46 ± 0.68	16.97 ± 1.16	16.12 ± 0.92	0.83 ^{NS}
9	16.59 ± 0.60	16.95 ± 1.07	16.00 ± 0.93	0.85 ^{NS}
10	16.67 ± 0.64	17.91 ± 1.2	16.10 ± 0.90	0.78 ^{NS}

The fortnightly average daily weight gain in lambs pertaining to different treatment groups are given in Table-2.

Table-2: The fortnightly mean± S.E of average daily weight gain (g) and analysis of variance of lambs under different treatment groups

Fortnights	T_1 (Intensive system)	T_2 (Rotational Grazing)	T_3 (Continuous Grazing)	P value
1	52.8 ± 16.1	30.1 ± 26.4	17.8 ± 15.3	0.46^{NS}
2	32.1 ± 08.7	20.0 ± 14.8	49.3 ± 23.5	0.47^{NS}
3	23.5 ± 14.2	38.0 ± 18.3	10.7 ± 16.1	0.49^{NS}
4	35.0^{b} ± 16.5	05.0^{b} ± 10.1	90.0^{a} ± 18.8	0.00^{**}
5	59.2 ± 07.9	50.0 ± 33.1	10.7 ± 16.2	0.26^{NS}
6	63.5 ± 20.9	85.7 ± 37.3	36.4 ± 26.5	0.49^{NS}
7	15.0 ± 15.9	83.5 ± 25.2	70.7 ± 32.3	0.14^{NS}
8	47.8 ± 11.5	64.0 ± 17.1	30.0 ± 35.3	0.59^{NS}
9	09.2 ± 07.9	07.8 ± 04.4	02.8 ± 03.8	0.70^{NS}
10	05.7 ± 04.1	07.8 ± 9.37	07.1 ± 10.4	0.82^{NS}
Overall Weight Gain	25.5 ± 2.87	36.3 ± 5.11	29.6 ± 5.16	0.28^{NS}

NS – Not Significant

** Significant at one per cent level (P<0.01)

The overall average daily gain recorded was 25.5 ± 2.87, 36.3 ± 4.84 and 29.6 ± 4.90 grams for the animals in T_1, T_2 and T_3 respectively. There weren't any significant difference in all the fortnightly average daily weight gains belonging to different treatment groups except the fourth fortnight (P<0.01).

The fortnight Strongyle E.P.G in lambs pertaining to various treatment groups are in Table-3.

Table-3: The mean ± S.E and analysis of variance of fortnightly Strongyle E.P.G in lambs of three treatment groups

Fortnights	T_1 (Intensive system)	T_2 (Rotational Grazing)	T_3 (Continuous Grazing)	F value
1	$1860^{c} \pm 0155.00$	$4310^{b} \pm 1173.0$	$7170^{a} \pm 701.9$	0.000^{**}
2	$2700^{b} \pm 0614.00$	$3500^{b} \pm 967.0$	$7480^{a} \pm 788.0$	0.001^{*}
3	$2270^{b} \pm 1004.00$	$3020^{b} \pm 710.0$	$7280^{a} \pm 617.0$	0.000^{**}
4	$2840^{b} \pm 0854.00$	$3260^{b} \pm 791.0$	$6970^{a} \pm 817.0$	0.001^{**}
5	$2810^{b} \pm 0838.70$	$3390^{b} \pm 797.1$	$6943^{a} \pm 817.0$	0.003^{**}
6	$1760^{b} \pm 0337.00$	$3850^{a} \pm 645.0$	$4570^{a} \pm 508.0$	0.002^{**}
7	$1230^{b} \pm 0289.00$	$3130^{a} \pm 459.0$	$4380^{a} \pm 789.1$	0.002^{**}
8	$1250^{b} \pm 0216.10$	$2220^{b} \pm 562.8$	$4150^{a} \pm 897.0$	0.009^{**}
9	$1120^{a} \pm 0191.30$	$1980^{ab} \pm 246.0$	$3760^{a} \pm 1098.0$	0.026^{*}
10	$1260^{a} \pm 0224.00$	$1960^{ab} \pm 317.0$	$3700^{a} \pm 983.0$	0.025^{*}

* Significant at five per cent level (P<0.05)

** Significant at one per cent level (P<0.01)

Means bearing different superscript in the same column differ significantly

There was a highly significant difference (P<0.01) in the fortnightly Strongyle E.P.G in lambs pertaining to the three treatment groups. The lambs in T_3 had a significantly higher Strongyle E.P.G compared to T_2 and T_1.

The monthly fodder biomass yield (tones/hectare) of pasture plots A (Rotational grazing) and B (continuous grazing) are given in Table- 4. The monthly fodder dry matter dry matter yield in the plot-A was significant higher (P<0.01) when compared to plot-B.

Table-4: Mean±S.E of fodder biomass (DM) yield (tons/hectare) and Independent t-test of the experimental plots

Experimental Plots	Months				
	January	February	March	April	May
Rotational Grazing-PLOT A	3.50±0.013	6.13±0.008	5.55±0.006	4.38±0.003	3.22±0.009
Continuous Grazing-PLOT B	3.24±0.005	1.46±0.005	1.30±0.005	1.70±0.05	1.72±0.05
'P' Value	0.34	0.00*	0.00**	0.00**	0.00**

** Significant at one per cent level (P<0.01)

Table-5: Proximate composition (in percentage) of fodder samples pertaining to the experimental Plot A and B

Plot-A	Crude protein	Ether Extract	Total Ash	Acid Insoluble Ash	Crude Fibre
Start of the trial (January)	7.92±0.03	2.93±0.03	9.02±0.05	6.5±0.03	19.03±0.03
Mid trial (March)	6.95±0.49	2.55±0.02	7.97±0.03	6.4±0.02	20.15±0.08
End of the trial (May)	5.97±0.59	2.67±0.01	7.31±0.12	5.6±0.02	22.16±0.08
Plot-B					
Start of the trial (January)	7.42±0.01	2.14±0.01	7.8±0.10	5.5±0.02	24.47±0.03
Mid trial (March)	5.21±0.24	2.43±0.01	9.9±0.04	5.5±0.03	22.85±0.08
End of the trial (May)	5.24±0.01	2.67±0.02	9.9±0.03	5.8±0.02	21.89±0.04

The proximate composition of fodder samples taken during the start of the trial, mid period of the trial and during the end of the trial pertaining to plots A and B are given in Table-5.

There weren't any difference in the proximate principles observed at various intervals between plots A and B. But a seasonal decrease in the crude protein content of the fodder samples was observed from winter to summer seasons in both the experimental plots.

DISCUSSION

Growth performance of lambs: The statistical analysis of the body weights (kg) and average daily gain (g) of lambs under intensive, continuous and rotational systems of grazing management in general revealed no difference.In a similar study with Barbari goats Mahantha*et al*. (2012) observed no statistical difference in body weight between continuous, rotational and deferred rotational systems of grazing. In contrast, Sharrow and Krueger (1979) observed a 10 per cent increase in the weight gain of lambs under rotational grazing in a study on performance of Romney ewes under rotational and continuous system of grazing.

Though the difference in body weight of lambs reared under different systems of management was not significant statistically, lambs reared under rotational system of grazing had comparatively better weight gain than the other two treatments groups. This was in accordance with the findings of Bozkurt*et al*. (2015) who observed that there was a tendency of beef cattle to perform better in the rotational system of grazing management. The final body weight and the average daily gain of the lambs in all the three treatment groups were lower since the lambs subjected to the trial was 5 months of age and has passed the active growth phase period during the experimental period.

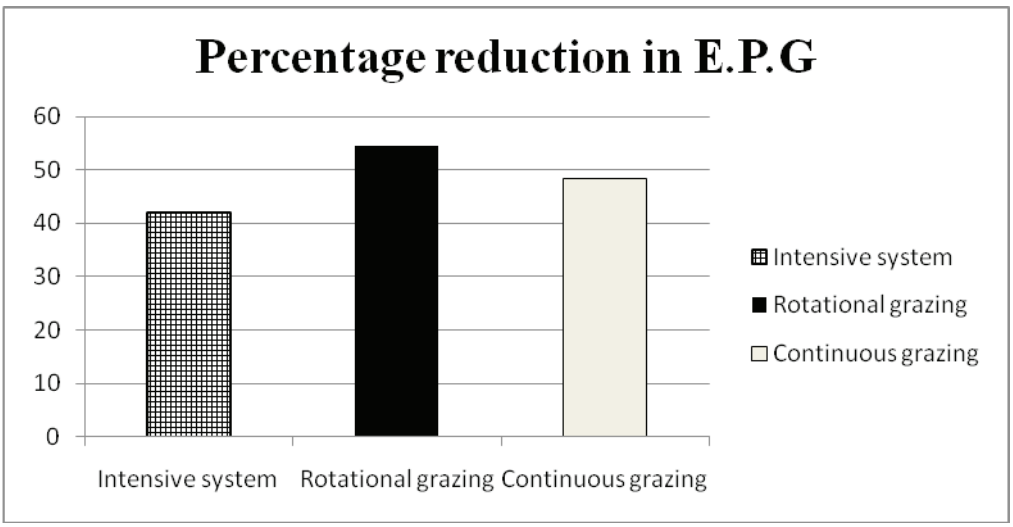
Parasitic load: In our present study the Strongyle E.P.G of feces differed significantly ($P \leq 0.01$) between the lambs pertaining to different treatment groups. The lambs under rotational grazing system of management had significantly lower E.P.G than those under continuous grazing system of management. The lowest E.P.G was observed in lambs under intensive system of management. Majority of the parasite larvae (80%) lives in the first 5 cm of the vegetation. The rest period of 15 days ensured that the sward height of grasses in the rotational system of grazing paddocks remained more than 20 cm at any given point of time. However, the sward height of grasses in the continuously grazed paddocks remained well below 10 cm. This might have influenced the E.P.G. resulting in reduced E.P.G count in lambs under rotationally grazed paddocks.

The overall percentage of reduction in E.P.G was higher in T_2, which was 54 per cent.Irrespective of the system of grazing management, a general tendency in decline of E.P.G. was observed from the month of January to May i.e. from winter to summer season. The increase in average monthly temperature from winter to summer which was not conducive for the survival of infective larval stages, might have contributed to the lower E.P.G count

towards summer season. The seasonal influence in E.P.G. of lambs observed in the present study was in accordance with the findings of Soundarajan (2001).

Fodder biomass and herbage dynamics: In this study the rotational grazing plot-A had a significantly (P≤0.01) higher fodder biomass yield than the continuous grazing plot-B as observed in Table-4. This was in agreement with the observation of Onatibia and Aguiar 2016, Upadhyay*et al.* (1971) and Das and Paroda (1980).The prevalence of green biomass, a lesser standing or dead biomass and absence of bare-ground area were observed in the rotationally grazed paddocks when compared to the continuously grazed plot. A similar trend was observed by Kurtz *et al.* (2015) and Teague *et al*. (2011).

Figure-2: Overall percentage of reduction in different treatment groups



Further, comparatively more weed infestation was observed in the continuously grazed paddocks. Similar observations were reported by Virostek*et al.* (2015). It can be observed from the results that rotationally grazed plots had better crude protein content than the continuously grazed plots. Moreover, a seasonal decrease in the crude protein content from winter to summer seasons was observed in both the experimental plots.

The higher crude protein percentage content in the rotational grazing paddocks shall be attributed to the accentuating nitrogen content in Plot-A under rotational grazing due to uniform and extensive footfalls of lambs. These observations are in line with the findings of Shinde (1997), Walton *et al*. (1981), Turk *et al*. (2014) and Mahantha*et al.* (2012).In concurrence with results of the present study, Shinde*et al.*(1997) also observed seasonal variation in the proximate composition of fodder grasses.

The developed pasture of 10,000 sq. ft. involved in this experiment has supported 10 lambs so the carrying capacity of pasture irrespective of biomass availability throughout the experimental period and hence the approximate carrying capacity is 21.6 A.C.U.,whereas, it is only 0.2-1.47 A.C.U / hectare under conventional grazing system of management.

Results of this study indicate advantages of rotational grazing in terms of better biomass yield from pasture (quality and quantity) and lesser infestation of weeds. The lambs raised under rotational grazing system had a significantly ($P \leq 0.01$) lower E.P.G than those under continuous system of grazing thereby assuring reduced cost on deworming and better health of lambs. This study explores the first baseline of sheep rearing in artificial pasture under rotational grazing; further investigation on this area may yield more favorable and reliable results.

ACKNOWLEDGEMENT

The authors thankfully acknowledge the logistic support provided by the University Research Farm and Directorate of Center for Animal Production Studies, Tamil Nadu Veterinary and Animal Sciences University, Chennai-51.

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ASSESSMENT OF MICROBIAL LOAD IN RAW MEAT SAMPLES FROM OPEN MARKETS OF PRODDATUR, Y.S.R. KADAPA DISTRICT, ANDHRA PRADESH

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ABSTRACT

The present investigation was aimed at assessing the microbial load in raw meat samples procured from open markets of Proddatur city, YSR Kadapa District, Andhra Pradesh. Thirty chevon and thirty chicken samples were collected separately, from different local open markets of the city and were subjected for Total Aerobic Plate count, E. *coli* count, *Staphylococcus aureus* count, detection of Salmonella and Yeast and Mould counts, following standard microbiological procedures. The range of Total Aerobic Plate count of chevon samples varied from 5.97 – 7.67 $\log_{10}$CFU/cm^2, whereas the E. *coli* counts varied from 1.86 to 2.28$\log_{10}$CFU/cm^2, the Staph. *aureus* counts ranged from 2.39 – 4.73 $\log_{10}$CFU/cm^{2}and Yeast and Mould counts fluctuated from 0 to 5.92 $\log_{10}$CFU/cm^2. Salmonella was detected in 10% of chevon samples. Chicken samples had a mean + S.E. Total Aerobic Plate count of 7.03 ± 1.65 $\log_{10}$CFU/cm^2, E. coli counts of 2.98 ± 0.42 $\log_{10}$CFU/cm^2, Staph. *aureus* counts of 3.58 ± 0.16$\log_{10}$CFU/cm^{2}and Yeast and Mould counts of 2.71 ± 0.22 $\log_{10}$CFU/cm^2 .20 % of chicken samples were Positive for Salmonella detection. Meat consumers should be cautious about the meat handlers while purchasing the meat and also should be well aware about the proper measures like cleaning and cooking the meat to overcome microbial contamination.

Key Words : Microbial load, Total Plate count, E. *coli* count, *Staph.aureus* count, Salmonellae, Yeast and Mould counts.

INTRODUCTION

Meat is an excellent source of protein in human diet, also rich in fat, vitamins and minerals, low in carbohydrate content, and is delicious, appetizing and easily digestible food item. Thewhole nutritional requisite can be met easily and efficiently if reasonable amount of meat is included in the diet. However, Meat, which is rich in protein is a good medium for growth of microbes, and with sufficient water activity, is highly susceptible to microbial contaminations, which supports the growth

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of both spoilage and pathogenic bacteria and can cause its spoilage and food borne infections in human, ensuing economic and health losses (Komba*et al.,* 2012).

Muscles of healthy animals are essentially sterile, do not contain microorganisms, however, meat tissues get contaminated during the various stages of pre and post slaughter (Warriss, 2000; Alvarez *et al.,* 2009;Adzitey, 2011;Adzitey and Huda, 2012;Adzitey and Huda, 2011) and transportation (Ercolini*et al.,* 2006).A great diversity of microbes inhabits fresh meat generally. However, only certain types may become dominant depending on pH, composition, texture, storage temperature, and the modus of transportation of raw meat (Ercolini*et al.,* 2006; Li *et al.,* 2006; Adu-Gyamfi*et al.,* 2012). Raw meat harbors many important pathogenic microbes i.e. Salmonella spp., Campylobacter jejuni/coli, Yersinia enterocolitica, E. coli, S. aureus and, to some extent, Listeria monocytogenes. Improper handling and control of these pathogens, may lead to occurrence of food borne ill-nesses, making the meat a risk for human health ((Nørrung*et al.,* 2009). Meat provides an ideal condition for the proliferation of different spoilage bacteria (Mayr*et al.,* 2003) thus making meat highly perishable.

Contaminated raw or undercooked poultry and red meats are particularly important in transmitting food-borne pathogens (Zhao *et al.,* 2001). Retail meat harbors all the bacteria that are already present in meat as inherent contamination through infection and also that are introduced during handling, improper dressing, cleaning, insanitary condition and retailing. Meat can also be contaminated at several points throughout the processing procedures. Higher microbial load in retail cuts could be a consequence of increase in exposed surface area, more readily available water, nutrient and greater oxygen penetration which leads to spoilage of meat. Meat borne zoonotic diseases such as Salmonellosis, Campylobacteriosis, E.coli enteritis and food poisoning by Clostridium, Staphylococcus, etc. are the major problems encountered by the consumers eating contaminated meat. Foodborne infections, which still remain one of the major problems of public health worldwide, have been linked to the consumption of meat. Meat infected with microorganisms is the cause of many food-borne diseases (WHO,1997).

Slaughtering of livestock continue to escalate as a result of the enhanced demand for meat and its products (Warris, 2010). Meat has been and continues to be an important constituent of our daily repasts, since it provides us with proteins and serves as source of energy (Stufflebeam, 1983).

The sanitary conditions of abattoirs and its surrounding environment are chief facets contributing to bacterial contamination of meat (Gill *et al.,* 2000). Contaminations can be compounded during transportation, storage and handling of meat at the butcher shops (Adzitey, 2011; Adzitey*et al.,* 2011). In developing countries the abattoir environment, its sanitary level, transportation and storage conditions not only contaminate but also enhance the growth of different types of spoilage (Adzitey*et al.,* 2011; Adzitey*et al.,* 2014)as well as pathogenic bacteria in meat. Mukhopadhyay*et al.,*(2009) reported

that, increased total aerobic counts on meat may be due to the impact of hot and humid climate areas. To control the food-borne illnesses and to keep the microbial load of raw meat under check, the food safety requirements should be followed stringently in accordance with HACCP (Hazard analysis critical control point).

Consumers in developed countries acquired an escalated consciousness for microbiologically safe food.There is a need to produce better quality and disease free meat, especially in most developing countries. It is an illustrious fact that meat coming in contact with microbial populationduring production, processing, transportation and distribution, presents a challenging hazard to meat industry,which poses problems of infection, spoilage and intoxications (Ramasastry*et al.,* 1999; Dhanze*et al.,* 2012). To increase meat quality, assurance in accordance with microbial load assessment is deemed necessary.

Hence the present study has been carried out with an objective to "Assess the Microbial Load in Raw Meat Samples from Open Markets of Proddatur, Y.S.R. Kadapa District, Andhra Pradesh", to create awareness among the consumers regarding the safety levels of meat they consume.

MATERIALS AND METHODS

Sample collection and Processing :Goat and chicken meat samples were collected from thirty different open market meat outlets in Proddatur town, Y.S.R. Kadapa district, Andhra Pradesh, India. Sampling was carried out by swabbing the muscular surface of fore and hind quarter of each goat carcass as well as from total chicken carcass surface, after flaying and washing. An area marked within a sterile frame of 10 cm X 10 cm on each site of the carcass was identified and a sterile cotton swab was rotated on the surface of carcass for 30 seconds and swabs were transferred to a screw-capped test tube containing 10 ml of sterile maintenance medium (0.85% NaCl and 0.1% peptone) (Bell, 1997). The tubes were transported to lab at 4^{O}C and processed for further analysis within four hours.

Serial decimal dilution was then carried out with one (1) ml of each samples in nine (9) ml of 0.1% peptone water (pH 7.0, sterilized at 121^{O}C for 15 mins.) to obtain the neat(dilution of 10^{-1}).Tenfold serial dilutions were prepared as $10^{-3,}$ $10^{-4,}$ $10^{-5,}$ 10^{-6}, 10^{-7},by transferring 1 ml of aliquot from diluted tube to dilution blank and inoculated onto petri dishes with appropriate media.

Total Plate Count : Total Aerobic plate count was carried out on total plate count agar as described by (Obeng*et al*., 2013). The medium was autoclaved and maintained at 46^{O} C. Samples were serially diluted decimally and an aliquot of 1 ml of each of serial dilution was transferred to the petri dishes (4 inch diameter) and molten agar (15-20 ml) was poured on it. Plates were gently swirled to uniformly mix the sample and incubated at 37^{O} C for 24 hours, before the colonies were counted and reported as CFU/cm^2, using a digital colony counter (Deep Vision Colony Counter, Model : 362).After 24hrs of incubation, plates with countable colonies (30-300cfu) wereidentified, and TPC was determined from appropriate plates as CFU/cm^2.

Enumeration of *Escherichia coli:* *Escherichia coli* were enumerated on Eosin Methylene Blue agar (Himedia Laboratories, Mumbai) by plating an appropriate dilution on plates followed by aerobic incubation at 37^{O} C for 24hrs. After incubation E. coli were counted as colonies with distinct metallic sheen (Bhandare*et al.,* 2007).

Enumeration of *Staphylococcus aureus* : Baird Parker agar (Himedia Laboratories, Mumbai), a selective medium for the isolation and counting of coagulase positive staphylococci was used for the enumeration of *Staphylococcus aureus* as described by (Bhandare*et al.,* 2007). Enumeration of *S.aureus* was done by spreading an appropriate dilution of sample on agar plates followed by aerobic incubation at 37^{O} C for 48hrs. Further confirmation of S.aureus was carried out by grams staining and catalase testing.

Isolation and identification of *Salmonella*: Presence of salmonella in meat sample was established by pre-enrichment of meat sample in lactose broth followed by enrichment in tetra-thionate broth and final detection on Bismuth Sulphite agar as recommended by WHO procedures.

Enumeration of Yeastand Mould Count: Yeast and Mould counts load was estimated by incubating the appropriate dilution of the sample on Potato Dextrose agar(Himedia Laboratories, Mumbai), followed by incubation at 37^{O}C for 5 days.

Statistical Analysis: Microbial counts (CFU/cm^2) were represented as $\log_{10}$ CFU/cm^2 and means were calculated. Microbial counts were compared by ANOVA using SPSS software 19.0.

RESULTS AND DISCUSSION

Total Plate Counts : In the present study, the mean Total Plate Count of chevon samples collected from local market of Proddatur were $6.79 \pm 1.02\log_{10}$ CFU/cm^2, with a range of 5.97 – 7.67. The higher prevalence of microorganisms may possibly be ascribed to unhygienic and inapt handling of meat during slaughtering, dressing and evisceration of goat by the local butchers (Mukhopadhyay*et al*., 1998). In general,Meat is transported to the markets in unhygienic meat vans, motor cycles, and often on bicycles. It is also a familiar practice to see people carrying carcasses on their bare shoulders (Obeng*et al*., 2013). As per the observations ofBhandare*et al*.,(2007), contamination of meat with microorganisms has been a consequence of the unhygienic practices of meat processing in developing countries. Introduction of saliva on the meat, from meat sellers who were busily conversing, coughing, and sneezing also might result in contamination. Mukhopadhyay*et al*., (2009) opined that, hot and humid climate areas contribute to escalating total aerobic counts on meat; and that also could be a contributing factor for the high total aerobic counts of the meat in this study.

The observations in the present study were in consonance with the findings of Keshab Prasad Sharma and Chattopadhyay (2015), who observed that the raw mutton samples sold in the open markets of Kolkata had a General Viable Count,ranging from 2×10^6 CFU/gm to 1.1×10^7 CFU/gm in

different (Thigh, Neck, Groin) meat parts of mutton.Singh *et al*., (2014)recorded a SPC of 6.96 ± 0.78 (log_{10}CFU/gm) in the raw chevon procured from local markets of Agra. Similar findings were reported by Ahmad*et al*., (2013), who recorded Aerobic Plate Count of 6.92±2.16 log_{10} CFU/cm^2 in mutton samples and 6.62± 1.12 log_{10} CFU/cm^2 in chevon samples collected from various abattoirs of Lahore city. Analogous interpretations were also reported by Bhandare*et al.*, (2007) in Sheep/Goat carcasses; Haque*et al.*, (2008)in goat meat and Lambey*et al.* (2010) in chevon.

The mean Total Plate Count of microbes in chicken samples collected from local market of Proddatur were 7.03 ± 1.65log_{10} CFU/cm^2, with a range of 6.48 – 7.69.The higher counts might be due to the contamination from butcher's practices. Anachinaba*et al*., (2015) analyzed that butchers,who handle meat did not pay appropriateinterestto their personal hygiene and carried the meat with unclean hands and clothing. Meats are sold in the open markets sometimes in sieves (or) without sieves. Meats were put on tables which are not well cleaned before and after the day's work and also in the open, exposing the meat to houseflies. Scanty sanitation was observed in the immediate environment where meats are sold. Adziteye*t al*. (2014) observed similar unhygienic practices in meat handling. Food can be infected with microorganisms as a consequence of "coughing" and "sneezing" from those who handle and process these foods (Okonko*et al*. 2008, Koffi-Nevry*et al*. (2011)also declared that, "careless sneezing and coughing among butchers can cause contamination of the products". The afore-mentioned practices add to the high microbial load.

The findings in the present study corroborate with the results of Ahmad *et al*., (2013), who found out that the Aerobic Plate Counts of Chicken meat samples collected from differentretail outlets of Lahore city were 7.22± 2.11 log_{10} CFU/cm^2, while Singh *et al*., (2014) observed that the SPC of poultry meat samples obtained from local markets of Agra were, 6.75±0.04 (log_{10}CFU/gm). Keshab Prasad Sharma and Chattopadhyay (2015), recorded that the General Viable Count of poultry varied from 2.4 X 10^6 CFU/gmto 4.1 X 10^7 CFU/ gm in different (Thigh, Neck, Groin) meat parts of poultry gathered from the open markets of Kolkata. The present findings are also comparable to the findings of Alvarez-Astorga*et al.*, (2002) fromretail chicken by-products in Spain, Hassan *et al*.,(2010) from retail meat shops in Karachi, Pakistan and Obeng*et al*., (2013) in the Northern Region of Ghana.

***E. coli*Counts :**The mean *E. coli* Count in chevon samples collected from local market of Proddatur were 2.02 ± 0.48log_{10} CFU/cm^2, with a range of 1.86 – 2.28.*E. coli*existence in several outlets is a sign of fecal contamination of the meat, which may be owed to unhygienic handling of meat starting, from slaughtering, butchering equipments, handling, transportation, and processing (Warris, 2010).

The findings in the present study were congruent with the findings ofAhmad *et al*., (2013), who found out that the *E.*

coli Counts of mutton samples collected from various retail outlets of Lahore city were 2.78± 1.10$\log_{10}$ CFU/cm^{2}and of the same counts in chevon samples were 1.94±1.12$\log_{10}$ CFU/cm^2. Similar findings were reported by Bhandare*et al*., (2007) and Doyle (2007).

The mean *E. coli*Count in Chicken meat samples collected from local market of Proddatur were 2.98 ± 0.42$\log_{10}$ CFU/cm^2, with a range of 2.76 – 3.28. The usual practice of washing the carcass with the same water in which intestines and offal had been sluiced, was considered as one of the principal reasons for enhanced microbial counts of the carcasses (Mukhopadhyay*et al*., 2009).

The findings in the present study were harmonious with the findings of Ahmad *et al*., (2013), who found out that the *E. coli* Counts of chicken meat samples collected from differentretail outlets of Lahore city were 2.74± 1.13 $\log_{10}$ CFU/cm^2. Correspondingly parallel findings were reported by of Alvarez-Astorga*et al.*, (2002) fromretail chicken by-products in Spain and Adu-Gyamfi*et al*., (2012) in chicken sold in Accra, Ghana.

Stap.aureus* Counts :**Postgate (2000) reported that staphylococcus spp. is an ingredient of the normal flora on the skin of humans and animals, which can be passed on from person to meat and meat products through unhygienic practices. The mean ***Stap.aureusCount in chevon samples collected from local market of Proddatur were 3.47 ± 0.21$\log_{10}$CFU/cm^2, with a range of 2.39 – 4.73.Animals are slaughtered in abattoirs and sometimes in backyards without observing strict hygienic practices. Suchmeat isfurther contaminated by butchers, producing unhygienic meat (Norrung. *et al*.,(2009).

The findings in the present study were in agreement with the findings of Singh *et al*., (2014), who reported that the Staphylococcus Counts raw chevon meat procured from local markets of Agra were 3.84 ±0.12 $\log_{10}$ CFU/gm. Whereas, Ahmad *et al*., (2013), recorded *Staph. aureus*Count of 2.96±1.66 $\log_{10}$ CFU/cm^2 in mutton samples and 3.07± 1.45 $\log_{10}$ CFU/cm^2 in chevon samples collected from various retail outlets of Lahore city. Similar findings were reported by Haque.*et al.*, (2008)in goat meat and of Tassew*et al*., (2010)in minced meat.

Chicken samples collected from local market of Proddatur, had a mean ***Stap.aureus***count of3.58 ± 0.16 $\log_{10}$ CFU/cm^{2}with a range of 2.46 – 4.92. Anabsoluteunawareness on the part of the meat handlers/butchers in hygienic treatment of carcasses during slaughter and retailing processes might be the corefactor for turning out meat with elevated microbial load (Mukhopadhyay*et al*., 2009).

The results obtained from the present study correlate well with the findings of Ahmad *et al*., (2013), and the author statedthat, the mean ***Stap.aureus***Counts of chicken meat samples collected from different retail outlets of Lahore city were 3.80±1.34 $\log_{10}$ CFU/cm^2.Besides, Singh *et al*., (2014) observed that the Staphylococcus Counts of poultry meat samples obtained from local markets of Agra were, 3.35± 0.10 ($\log_{10}$CFU/gm). Collateral findings

were also reported by VoidarouC.D.*et al.,* (2011)in poultry meat.

Salmonella detection : Meat and poultry carcasses and their parts are recurrently contaminated with pathogens, which get in contact with the carcasses from the intestinal tract or from fecalmaterial on feet and feathers. Cross contaminations is a specific problem and have been frequently published to control pathogens in the chain right through from hatcheries to the home made preparations. Animals with certain illness may lead to higher possibility of mistakes, such as gastrointestinal ruptures, in the processing plant, which would lead to increased microbial contamination and cross-contamination (Singer *et al.,*2007).

In the present investigation, Salmonella contamination has been recognized in 10% of the chevon samples (3 samples) collected from local market of Proddatur. The high prevalence of Salmonella can be attributed to the usage of contaminated water for washing of carcasses.Ahmad *et al*., (2013), assessed the microbial load of chevon samples collected from different retail outlets of Lahore city, and reported that 10 % of the samples procured from goat abattoirs and 10 % of the samples obtained from goat retail outlets were positive for Salmonellae. However, Keshab Prasad Sharma and Chattopadhyay (2015) detected a prevalence of Salmonella in 2 % of the raw mutton samples sold in the open markets of Kolkata.

Salmonella is one of the recurrently isolated bacteria from the abattoir environment and gastrointestinal tract of all farm and wild animals, particularlyin poultry (Norrung*et al.* 2009, EFSA, 2007). The chicken samples collected from local market of Proddaturrevealed pervasiveness of Salmonella up to 20 % (5 samples). Ahmad *et al*., (2013), observed that 25 % of chicken samples collected from different retail outlets of Lahore city were positive for Salmonellae.Maharjan*et al*., (2006)also noticed Salmonella Species in raw chicken samples of a Local Market in Kathmandu, Nepal.

Yeast and Mould Counts : The mean Yeast and Mould Count in chevon samples collected from local market of Proddatur were $2.94 \pm 0.15 \log_{10}$ CFU/ cm^2, with a range of 0 – 5.92. The present study inferences were covenant with the interpretations ofMukhopadhyay*et al*., (1998), who testified that the Yeast and MouldCount of mutton samples collected from different retail shops of Namakkal city, Tamil Nadu,were $\log_{10}$ 2.9 CFU/gm, and also informed a Count of $\log_{10}$6.90 CFU/gmin chevon samples collected from different retail outlets of Pondicherry(2009). Similar conclusions were reported by Duffy *et al.*, (2001) in lamb carcasses processed in United States.

The mean Yeast and MouldCount of chicken samples collected from local market of Proddatur were $2.71 \pm 0.22 \log_{10}$ CFU/cm^2.The findings in the present study concurs with the outcomes of Santosh Kumar *et al*., (2014), that the Yeast and Mould Counts of market slaughtered chicken were 2.91 ± 0.22 $\log_{10}$ CFU/gm. Similar findings were reported by Anand*et al.*, (1989) in dressed chicken.

CONCLUSION

A microbial check must be carried out periodically and meat hygiene practices are essential for public health point of view with objectives to safeguard the diseased free meat to the consumers, to arrest the spread of disease among meat consumers, to trace the source of disease by proper examination of animals prior and after the slaughter of meat, to check the spread of diseases which have a definite life cycle, to encourage the honest butchers and retailers, to promote the trade of quality meat and meat products across the country and eliminating the risk of rejections of foreign consignments of meat.

The present study reveals the poor quality of meat sold in the open markets of Proddatur, by increase in the microbial load, which might be attributed to unhygienic production, processing, transportation and storage measures. Raw meat should be properly handled with suitable hygienic measures to safeguard the consumer health. Consumers also should be aware of the microbial quality of meat available from the market and simple measures like cleaning and proper cooking of meat should be followed before consuming.

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Table 1 : Mean and Range of Total Plate, *E.coli, Staph. aureus*, Salmonella and Yeast &Mould counts of chevon and chicken samplescollected from open market meat outlets in Proddatur town, Y.S.R. Kadapa district, Andhra Pradesh, India

S. No.	Sample		Total Plate Counts (log 10 cfu /cm^2)	*E. coli* Counts (log 10 cfu /cm^2)	*Stap. aureus* Counts (log 10 cfu /cm^2)	Detection of Salmonella	Yeast &Mould count (log 10 cfu /cm^2)
1.	**Chevon**	Mean ± S.E.	$6.79^a \pm 1.02$	$2.02^a \pm 0.48$	$3.47^a \pm 0.21$	10 % Positive	$2.94^a \pm 0.15$
		Range	5.97 – 7.67	1.86 – 2.28	2.39 – 4.73		0 – 5.92
2.	**Chicken**	Mean ± S.E.	$7.03^b \pm 1.65$	$2.98^b \pm 0.42$	$3.58^a \pm 0.16$	20 % Positive	$2.71^a \pm 0.22$
		Range	6.48 – 7.69	2.76 – 3.28	2.46 – 4.92		0 – 6.02

n = 30

Short Communications

BIODEGRADABLE DUNKS TO CONTROL CULICINE LARVAE

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Culicine mosquitoes generally prefer stagnant water to lay their eggs. They most commonly infest ponds, marshes, swamps, wetland habitats, a week old water body, stagnant puddles, streams etc. However, they are capable of thriving in a variety of locations and can successfully grow in numbers even when not in their natural habitat. Many species of mosquitoes use containers of water as egg-deposit sites (Tusting et al. 2013)

Mosquito control is a vital public-health practice throughout the world and especially in the tropics because mosquitoes spread many diseases, such as malaria, yellow fever, west nile fever, dengue fever, filariasis (Zaim, 2008).

Utilizing conventional insecticides in targeting anthropogenic mosquito habitats prove to be very expensive in control programs. Moreover, various environmental related concerns arise due to the application of most conventional insecticides (Curtis, 2010). Hence, there is a need for alternative methods, which are effective, environment friendly and less expensive. Use of dunks to release herbal formulations in a sustained manner could be a viable alternate control strategy (Alouani et al. 2009). The current study envisaged the use of commonly available neem (*Azadirachta indica*) and sweet cane (*Acorus calamus*) components in producing a cheap and efficient mosquito dunk, whose benefits could be exploited even by the common man.

Fresh neem leaves were washed, dried and crushed in a motor to form fine powder. Fresh pieces of stem bark of neem tree were also dried, powdered and sieved to separate finer particle from granules and fibres. Roots from sweet cane (*Acorus calamus*) were collected, dried and powdered. Binding agent, gum powder (3g) was added to the neem leaf powder, neem bark powder and acorus powder to prepare neem leaf dunk, neem chip dunk and acorus dunk. Juvenile stages of culicine mosquitoes were collected from a pool of stagnant water using a larval collection net. The culicine larvae were kept in a beaker with a netted enclosure. In vitro trials were conducted to evaluate the efficacy of the dunks at different concentrations (2.5%, 3%, 4% and 5%) on the developmental stages of culicines. The effect of 1% and 1.5% was almost nil, so 2.5% was selected as base line concentration for all trials. Each trial was conducted with 100 mosquito larvae in a beaker with 200 ml distilled water and covered with a netted

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enclosure. The dunk was suspended into the beaker using a permeable cloth (Howard *et al.* 2009).

Effect of *Acorus calamus* dunk on culicine larvae

The effect of dif ferent concentrations of *Acorus calamus* dunk on the juvenile stage of culicines was depicted in table- 1. After 6 hours, acorus dunk at the concentration of 2.5% resulted in 27% larval mortality, while 24%, 22% and 20% larval mortality were noticed with 3%, 4% and 5% acorus dunk respectively. About 95% mortality was observed with 2.5% dunk after 24 hours. No further development was observed in the remaining 5% of larvae upto 24 hours.

Effect of neem leaf dunk on culicine larvae

The effect of different concentrations of neem leaf dunk on the juvenile stage of culicines was depicted in table- 2. After 12 hours, the larval mortality observed was 20%, 16%, 13% and 10% with 2.5%, 3%, 4% and 5% neem leaf dunk respectively. 95% mortality was observed with 2.5% dunk after 72 hours. The remaining 5% of larvae failed to show further development upto 24 hours.

Effect of neem chip dunk on culicine larvae

The effect of different concentrations of neem chip dunk on the juvenile stage of culicines was depicted in table- 3. A larval mortality of 20%, 15%, 12% and 10% was observed with 2.5%, 3%, 4% and 5% neem chip dunk respectively, 12 hours post exposure. After 72 hours, 95% mortality was observed with 2.5% dunk. The remaining 5% of larvae failed to develop into the next stage.

Comparative analysis of the culicine dunks

Larval mortality of 95% could be obtained with 2.5% of *Acorus calamus* dunk within 24 hours and a similar level of mortality was obtained with 2.5% neem leaf dunk and 2.5% neem chipping dunk only after 72 hours.

The active ingredient of acorus is beta-asarone while that of neem is azadirachtin (Chavan, 2005). They are one of the widely used insect growth regulators. Because of its structural resemblance to the natural insect molting hormone ecdysone, azadirachtin and beta-asarone interrupts molting, metamorphosis, and development of the female reproductive systems (Sharook et al. 2010). Immature mosquitoes exposed to azadirachtin and beta-asarone (mainly by ingestion) may molt prematurely or die before they can complete a properly timed molt . Those that survive the acorus and neem dunk treatment are likely to develop into deformed adult incapable of feeding, dispersing, or reproducing (Kalyanasundaram and Dos, 2011). It was concluded that further investigation would explore their use as agents for culicine larval control.

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Table 1. Effect of *Acorus calamus* dunk on culicine larvae

Concentration of the dunk	Per cent larval mortality			
	6 hours post exposure	12 hours post exposure	18 hours post exposure	24 hours post exposure
2.5% *Acorus calamus* dunk	27	55	75	95
3% *Acorus calamus* dunk	24	40	63	85
4% *Acorus calamus* dunk	22	38	60	85
5% *Acorus calamus* dunk	20	38	58	83
Control 1 & 2	-	-	-	-

Control 1 (3g binder in 200ml water) = No mortality.
Control 2 (200 ml water) = No mortality.

Table 2. Effect of neem leaf dunk on culicine larvae

Conc. of the dunk	Per cent larval mortality					
	12 hours post exposure	24 hours post exposure	36 hours post exposure	48 hours post exposure	60 hours post exposure	72 hours post exposure
2.5% neem leaf dunk	20	38	55	73	79	95
3% neem leaf dunk	16	30	46	62	77	82
4% neem leaf dunk	13	26	43	59	77	80
5% neem leaf dunk	10	22	41	57	76	80
Control 1& 2	-	-	-	-	-	-

Table 3. Effect of neem chip dunk on culicine larvae

Conc. of the dunk	Per cent larval mortality					
	12 hours post exposure	**24 hours post exposure**	**36 hours post exposure**	**48 hours post exposure**	**60 hours post exposure**	**72 hours post exposure**
2.5% neem chip dunk	20	40	56	73	79	95
3% neem chip dunk	15	30	48	63	78	84
4% neem chip dunk	12	25	43	58	76	82
5% neem chip dunk	10	22	41	57	74	80
Control 1 & 2	-	-	-	-	-	-

EFFECT OF SEASON ON REPRODUCTIVE PERFORMANCE OF OSTRICH (*Struthio Camelus*)

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Effect of season on reproductive performance of ostrich was carried out at the Post Graduate Research Institute in Animal Sciences, Tamil Nadu Veterinary and Animal Sciences University, Kattupakkam, Kanchipuram district in Tamil Nadu during 2014 - 2015. *A total of 860 eggs were utilized for this study. The influence of season on reproductive performances viz., fertility, hatchability and embryonic mortality were studied.* Season had a highly significant ($P \leq 0.01$) influence on fertility performance in Ostrich and higher percentage of fertility were observed during northeast monsoon (34.18 ± 4.80) and winter (30.76 ± 5.51) than summer (12.78 ± 1.86) and southwest monsoon (10.13 ± 1.43) seasons. Similarly, higher total hatchability percentage was observed during northeast monsoon (12.29 ± 2.14) and winter (8.48 ± 1.44) followed by summer (4.85 ± 1.02) and southeast monsoon (3.69 ± 0.69). However, no significant difference was observed for fertile hatchability and embryonic mortality among different seasons. Study concluded that the reproductive performances in ostrich are seasonal dependent in tropical climate.

Ostrich is the largest living bird found on earth and is a member of ratite family which includes Emu, Rhea, Cassowary and Kiwi. Nowadays Ostrich is becoming fundamentally attractive for production of leather, meat, oil and feather (Malecki *et al.* 2008). They are polygamous and a male will breed with one major female and two or more secondary females thereby limiting their reproductive performance. In addition Ostrich are seasonal breeders and primarily breed between December and June in tropical climate. Average annual egg production in farm-raised Ostrich vary between 30 and 50 eggs per hen (Deeming, 1999) or even less (More, 1996). Although some females are capable of producing 60 eggs or more per breeding period, variation between individual hens is large. This indicates that egg production performance can still be improved in Ostrich. Unpredictable egg production, unstable fertility, poor hatchability, embryonic mortality, poor chick survival are some of the major constraints in viable Ostrich farming. Further, hatching performance

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of Ostrich is influenced by many factors such as breed, age, egg qualities, storage time and environmental conditions. In this study the effects of seasonal variations on reproductive performance of ostrich have been studied and this research will be fruitful in planning the breeding programme of Ostrich.

The effect of season on reproductive performance of ostrich was carried out at the Post Graduate Research Institute in Animal Sciences, Tamil Nadu Veterinary and Animal Sciences University, Kattupakkam, Kanchipuram district in Tamil Nadu during 2014 - 2015. The station is situated approximately at 12.5°N latitude and 80° to 81°E longitudes and at the height of 48 meter above mean sea level. Being nearer to East coast of India it enjoys a tropical maritime monsoon climate. During this study period, the average high and low temperature, relative humidity and the annual rainfall were recorded as 34.2°C, 23.2°C, 87.3 per cent and 1391 mm, respectively. This station gets most of its seasonal rainfall from the northeast monsoon i.e. during October to December. To study the seasonal effect, the year was divided in to four seasons namely, winter (January and February), summer (March, April and May), southwest monsoon (June, July, August and September) and northeast monsoon (October, November and December (Indian Metrological Department, Pune). A total of 860 eggs were utilized for this study. Eggs were collected immediately after laying and fumigated with 3 X concentrations. Eggs were set in incubator with constant temperature of 97.9°F and relative humidity of 30-40 per cent for 38 days. The eggs were turned once in four hours by automatic turner at 90° angle up to 38 days. The eggs were transferred to hatcher on the 39th day for hatching into the hatcher. After hatching, the remaining unhatched eggs were broke opened to record infertile eggs and embryonic mortalities if any. Fertility was calculated after deducting infertile eggs from the total number of eggs set. Hatchability on total eggs set and on fertile eggs set was calculated. Embryonic mortalities were calculated based on fertile eggs set. All the fertility parameters were expressed in percentage. The data were analyzed by One-way ANOVA as per the procedure of Duncan's multiple comparison test (Duncan, 1955) after arc-sine transformation.

Influence of season on reproductive performance of ostrich is presented in table 1.

The season had a highly significant ($P \leq 0.01$) influence on fertility performance in ostrich, and higher percentage of fertility was observed during northeast monsoon (34.18) and winter (30.76) than summer (12.78) and southwest monsoon (10.13). The mean per cent fertility observed in this study (22.07) is accordance with Hariharan (2013) in seven year-old ostrich. Similarly, Ipek and Umran (2006), Dzama (2009), Dzama (2010), Elobied *et al.* (2010) and Kontecka *et al.* (2011) observed fertility ranged from 50 to 80 per cent in ostrich. Further, seasonal effects on fertility parameter have not been studied adequately in ratites for discussion. However, similar studies were carried out in other avian

species by Pruthi and Aggarwal (1987) in ducks, Prabakaran *et al.* (1992) in Japanese quails, Hossain *et al.* (2002) in broiler and Mahiye *et al.* (2005) in turkeys and they observed good fertility in monsoon and winter season than summer season.

The effect of season on per cent total hatchability varied significantly ($P \leq 0.01$) with an overall mean value of 7.47. Significantly ($P \leq 0.01$) higher total hatchability percentage was observed during northeast monsoon (12.29) and winter (8.48) followed by summer (4.85) and southeast monsoon (3.69). However, no significant difference was observed for fertile hatchability among different seasons. Earlier study on effect of season on total and fertile hatchability in ostrich was not traceable for comparison. However, Sundaresan (2014) observed comparable percentage of total and fertile hatchability in emu during artificial insemination. Similarly, higher percentage of fertile hatchability than our present study was reported by Malecki *et al.* (1995) in emu, Hariharan (2013) and Dzama (2010) in ostrich observed wide range of hatchability percentage (30-70 per cent) in ostrich.

The per cent dead germ, dead in shell and total embryonic mortality of ostrich hatching eggs among different seasons showed no significant difference. The overall mean per cent dead germ, dead in shell and total embryonic mortality among different seasons were 41.80, 24.00 and 65.80 respectively. Comparatively lower percentage of embryonic mortality were reported by Cloete *et al.* (1998), Majewska (2001), Sahan (2003) and Kontecka *et al.* (2011) in ostrich. However, Brand *et al.* (2007) and Hariharan (2013) have observed higher embryonic mortality in ostrich. From the above results, it becomes clearly evident that the reproductive performances, mainly fertility and hatchability are seasonal dependent in ostrich and it might be due to hormonal effects.

The above study concluded that the seasonal influence was more pronounced in reproductive performance of ostrich and considered as a seasonal breeder in tropical climate, which is evident from that significantly better fertility and hatchability were observed during northeast monsoon and winter than summer and southeast monsoon seasons.

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Table 1 Influence of season on reproductive performance of ostrich (Mean ± SE)

Parameters	Fertility **	Hatchability		Dead germ^{NS}	Dead in shell ^{NS}	Total embryonic mortality^{NS}
		Total egg set**	Fertile egg set^{NS}			
Winter (Jan-Feb) (n= 189)	30.76^{a} ± 5.51	8.48^{a} ± 1.44	28.36 ± 2.36	37.46 ± 3.60	34.16 ± 3.70	71.63 ± 2.36
Summer (March-May) (n= 191)	12.78^{b} ± 1.86	4.85^{ab} ± 1.02	35.00 ± 7.16	51.66 ± 9.82	13.33 ± 5.95	65.00 ± 7.16
Southwest monsoon (June-Sep) (n= 263)	10.13^{b} ± 1.43	3.69^{b} ± 0.69	35.00 ± 6.30	35.83 ± 3.67	29.16 ± 6.94	65.00 ± 6.30
Northeast monsoon (Oct-Dec) (n= 217)	34.18^{a} ± 4.80	12.29^{a} ± 2.14	37.06 ± 5.24	42.30 ± 7.12	20.62 ± 4.89	62.93 ± 5.24
Overall mean (n=860)	22.07 ± 2.54	7.47 ± 0.92	34.20 ± 2.79	41.80 ± 4.55	24.00 ± 2.98	65.80 ± 2.79
F value	10.67	7.16	0.40	0.57	2.47	0.40

n=No. of egg set / season; Means bearing different superscripts within the same column differ significantly; **Highly significant (P≤ 0.01); NS-Not significant.

A RARE CASE REPORT OF PEROSOMUS ELUMBIS WITH CONCURRENT VISCERAL ABNORMALITIES IN A JERSEY CROSS BRED CALF

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Perosomus elumbis (PE) is an occasional congenital anomaly of cattle, swine, sheep, and dogs with unknown aetiology. This congenital anomaly occurs in both the sexes (Jones, 1999). Perosomus elumbis, which occurs in ruminants and swines, is characterized by hypoplasia or aplasia of the spinal cord, which ends in the thoracic region. The regions of the body including the hindlimbs, which are normally supplied by the lumbar and sacral nerves, exhibit muscular atrophy, and joint movement does not develop (Noakes et al, 2009). This abnormality is a fairly common congenital defect in cattle (Roberts, 1986). It usually includes arthrogryposis of the hind limbs, characterized by ankylosis of the joints, with associated malformations of the musculature (Roberts, 1986). Perosomus elumbis in a calf was first reported in the veterinary literature in 1832 by Ernst Gurlt, and since then cases have been reported (Jones, 1999). This paper describes the clinical and radiographic evaluation of perosomus elumbis concurrent with a lot of visceral abnormalities in a Jersey cross-bred calf.

A day old calf was presented to Large Animal Clinic-Out Patient (LAC-OP) unit of Madras Veterinary College Teaching Hospital with the history of constipation and unable to defecate since birth. On the physical examination there was atresia ani, recto-vaginal fistula (Fig: 1) and angular limb deformities, and distended abdomen. Plain radiography of abdomen lateral view was taken, which was confirmed as Perosomus Elumbis (PE) anomaly.

Radiographic analysis revealed agenesis of sacral and coccygeal vertebrae (Fig: 2). The femurs were malformed. There was fusion of both hock joints. The hind limbs were hypoplastic with bilateral symmetric arthrogryposis and muscular atrophy (Fig: 3,4). Thoracic vertebrae and ribs were normal.

Scientific publication on Perosomus Elumbis (PE) in cattle have only consisted

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of case reports focusing on morphology (Jones, 1999; Williams, 1931; Greene et al, 1973) although a recent publication suggested the fetal infection with bovine viral diarrhoea virus (BVDV) may contribute to the development of PE (Karakaya et al, 2013). There is also a report of ovine PE case following feeding of pregnant sheep with Veratrum californicum on gestation days 16 and 17 was mentioned by Dennis et al, 1975. Although the spinal lesions are the most striking and the cause of hind limb dysplasia, visceral defects were present as well in PE. Visceral defects may occur due to disturbed development of the embryonic back. Similar lesions have been found in other bovine syndromes with widespread disturbed segmentation of the embryonic back such as the brachyspina syndrome (Agerhome et al, 2004) but such abnormalities seem also to be rather frequent in cases with spinal lesions restricted to the coccygeal vertebrae, i.e. the caudo-recto-urogenital syndrome (Vitellozzi et al, 1988). Perosomus Elumbis cases are often reported to have caused dystocia requiring cesarean section and the syndrome is therefore associated with maternal welfare concerns and economic losses beyond those of the lost offspring (Williams, 1931). In the above case there was history of dystocia which was relieved by local veterinarian. Caudal presentation is usually present in only 5% of bovine deliveries (Noakes, 2009) and is apparently much more common in PE affected calves. Due to the hind limb arthrogryposis, calves will thus be presented in breech position, which is a life threatening condition for the dam as such calvings may remain unnoticed thus leading to fetal death, emphysema and maternal intoxication. A majority of bovine fetuses are in caudal presentation during gestation months between 4 – 6½ after with most of them (95%) reposition to cranial presentation (Noakes, 2009). The underlying mechanisms for these fetal movements seem to be disturbed in PE affected fetuses.

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Fig: 1 Atresia ani and rectovaginal fistula

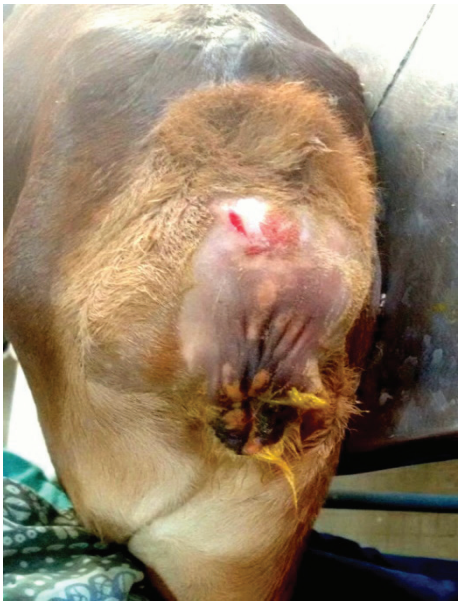


Fig: 2 Plain radiograph- Abdomen lateral view Agenesis of sacrum and coccygeal vertebrae

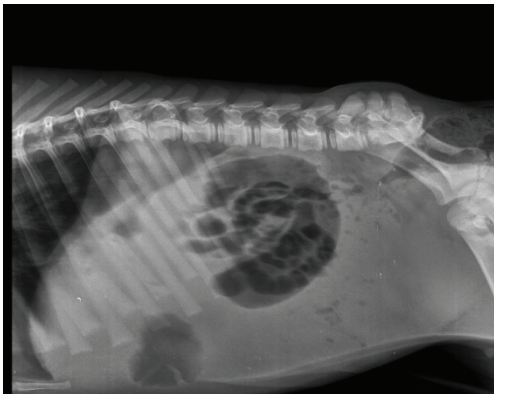


Fig :3 Stifle lateral view- Arthrogryposis

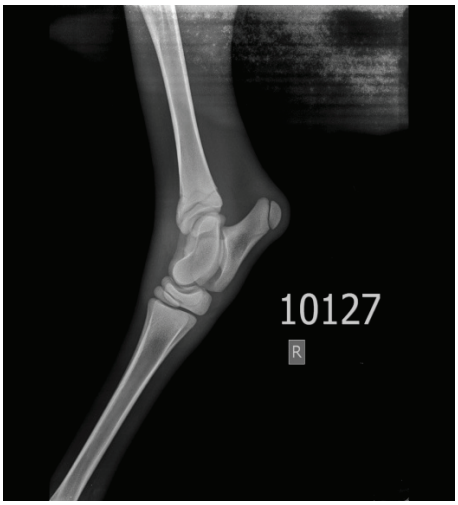
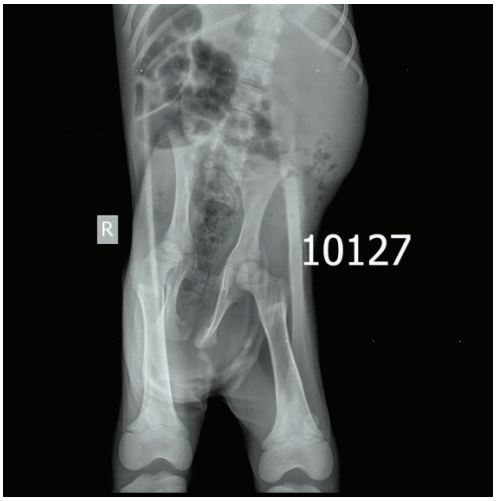


Fig: 4 Pelvis V-D view



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