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Effect of Different Concentrations of Areg on Cumulus Expansion of Cumulus Oocyte Complexes Isolated from *In-Vitro* Grown Sheep Preantral Follicles*

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ABSTRACT

The present study was aimed to assess effects of different concentrations of amphiregulin (AREG) on cumulus expansion of cumulus oocyte complexes (COCs) isolated from *in-vitro* grown sheep prenantral follicles (PFs). PFs were isolated and cultured in media containing different concentrations of AREG i.e., 5 ng, 10 ng, 25 ng, 50 ng, 100 ng and 150 ng for 7 days. At the end of the culture period COCs were dissected out under a stereozoom microscope from follicles and transferred in groups (3-5/group) into 50µl droplets of in-vitro maturation (IVM) medium consisting of tissue culture media-199 (TCM-199), 10% fetal bovine serum, 0.05 IU/ml of follicle stimulating hormone (FSH) and 50µg/ml of gentamycin. The droplets containing oocytes were then covered with warm (38.5 °C) mineral oil and the petridishes were placed in a CO_2 incubator for 24 h. Cumulus expansion was observed by stereozoom microscope and was assessed according to the visual assessment of the degree of expansion (Nandi *et al.,* 2006). Cumulus expansion (Degree-2) of oocyte was significantly ($P<0.05$) increased in the groups consisting of 100ng and 150ng of AREG compared to all other groups. There was no significant difference ($P>0.05$) in cumulus expansion (Degree-2) of oocytes cultured in the control and groups treated with 5ng and 10ng of AREG.

Keywords: Preantral follicles, cumulus oocyte complex, amphiregulin, cumulus expansion

Cumulus expansion is necessary for the release of oocyte into the abdominal cavity (Dragovic *et al*., 2005) and impaired expansion leads to sterility (Fulop *et al*., 2003). COCs expansion depends on gonadotropins or epidermal growth factors (EGFs) and also paracrine signals from the oocyte called cumulus expansion-enabling factors (CEEFs) (Dragovic *et al*., 2007). Cumulus expansion due to FSH or EGF and CEEFs is associated with increased expression levels of extracellular matrix (ECM) genes (*Has2*, *Ptgs2*, *Ptx3*, and *Tnfaip6*) in cumulus cells (Salustri *et al*., 2004). Prostaglandin synthase 2 produced from cumulus cells and granulosa cells is required for maximum cumulus expansion and ovulation (Lim *et al*., 1997). In addition to extracellular matrix, intracellular signalling cascades also control growth factors-induced cumulus expansion. The key mediators of LH (Luteinizing hormone) are the EGF like growth factors, ampiregulin (AREG), epiregulin (EREG) and betacellulin (BTC) which are transiently and sequentially expressed in the ovarian follicle to induce oocyte maturation and stimulate cumulus expansion. Initially expression of EGF like peptides is in the mural granulosa cells and subsequently within the cumulus cells. Cumulus oocyte complexes (COCs) coordinates signals from mural granulosa cells but also from thecal cells and the oocyte itself.Treatment

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with AREG causes cumulus expansion of COCs leading to oocyte maturation (Park *et al*., 2004). Hence, AREG was chosen to study its effect at different concentrations on cumulus expansion of COCs isolated from *in-vitro* grown sheep preantral follicles (PFs).

MATERIAL AND METHODS

Isolation and culture of preantral follicles

Thin and small ovarian sections were made from the ovarian cortex collected from slaughter house. PFs of 100 to 250 µm size were isolated from collected specimens by micro-dissection method using 26G disposable needle and scalpel blade. PFs were cultured in 100ml droplets of PFs culture medium supplemented with different concentrations of amphiregulin (AREG) i.e., 5 ng, 10 ng, 25 ng, 50 ng, 100 ng and 150 ng. They were cultured under mineral oil in 35 mm petri dish placed in CO_2 incubator (38 ^{0}C, 5 % CO_2 in air, 90-95 % relative humidity) for 7 days. The culture medium was replaced every alternate day.

Retrieval and culture of cumulus oocyte complexes from *in-vitro* grown preantral follicles

At the end of the culture period, follicular vitality was assessed by trypan blue staining technique. COCs were dissected out with fine needles (26G) under a stereozoom microscope from all the surviving follicles. Oocytes with homogenous ooplasm and atleast one layer of compact cumulus cell layer were considered as acceptable for *in-vitro* maturation. The recovered COCs were checked for viability using trypan blue staining technique. COCs which do not take stain were considered viable and were washed thrice in TCM-199 supplemented with 0.3% BSA and were transferred in groups (3-5/group) into 50µl droplets of IVM culture medium consisting of TCM 199, 10% fetal bovine serum, 50µg/ml of gentamycin and 0.05 IU/ml of FSH. The droplets containing COCs were then covered with warm (38.5 ^{0}C) mineral oil and the petridishes were placed in a CO_2 incubator (38 ^{0}C, 5 % CO_2 in air, 90-95 % relative humidity) for 24 h.

Assessment of cumulus expansion

Cumulus expansion was assessed at 24 h of IVM according to the visual assessment of the degree of expansion (cumulus expansion score). Degree 0- no expansion; Degree 1- moderate expansion, cumulus cells were non-homogeneously spread and clustered cells were still observed; and Degree 2- fully expanded, cumulus cells were homogeneously spread and clustered cells were no longer present (Nandi *et al*., 2006).

Statistical analysis

Expansion were analysed by ANOVA and the respective means were compared using Bonneferoni multiple comparison test (Graph Pad Prism, Graph Pad Software Inc., San Deigo, USA). Differences between the mean values were considered significant when the P values were less than 0.05.

RESULTS AND DISCUSSION

Cumulus expansion (Degree-2) was significantly ($p<0.05$) increased in the groups containing 25 ng and 50ng of AREG compared to all other groups. There was no significant difference ($p>0.05$) in cumulus expansion (Degree-2) of COCs cultured in the control and groups treated with 5 ng and 10 ng of AREG. Cumulus expansion (Degree-2) was significantly ($p<0.05$) increased in the groups containing 100 ng and 150 ng of AREG compared to all other groups, but significant increase was observed in the group treated with 25 ng of AREG (Table 1). This might be due to transactivation of the EGF like factors through signalling network and AREG is an EGF like growth factor that has been shown to be important for normal ovarian physiology in rodent model systems (Su *et al*., 2002). The results indicated that cumulus cell expansion level might be considered as a good measure of oocyte maturation. Cumulus cell expansion during *in-vitro* COCs maturation was beneficial for completion of the maturation process. Cumulus cell expansion leads to synthesis of pyruvate which provides energy during this period (Prochazka *et al*., 2011). Previous studies have shown that cumulus expansion (Eppig 1979) and oocyte maturation (Kalous *et al*., 2003) were stimulated by EGF like growth factors i.e., epiregulin and

Table 1: Effect of different doses of AREG on cumulus expansion of COCs from *invitro* grown PFs

Groups	PFs	COCs expansion		
		Degree-0	Degree-1	Degree-2
Control	59	$1.8^{a} \pm 0.6$	$0.7^{a} \pm 0.1$	$0.4^{a} \pm 0.1$
AREG (5 ng)	56	$2.5^{b} \pm 0.2$	$1.7^{b} \pm 0.2$	$0.5^{a} \pm 0.2$
AREG (10 ng)	56	$2.7^{b} \pm 0.6$	$1.7^{b} \pm 0.2$	$0.5^{a} \pm 0.2$
AREG (25 ng)	56	$2.2^{b} \pm 0.2$	$3.7^{c} \pm 0.2$	$1.7^{b} \pm 0.6$
AREG (50 ng)	56	$1.9^{a} \pm 0.2$	$3.0^{c} \pm 0.7$	$2.0^{b} \pm 0.4$
AREG (100 ng)	60	$1.0^{c} \pm 0.4$	$2.1^{b} \pm 0.2$	$4.5^{c} \pm 1.2$
AREG (150 ng)	56	$0.7^{c} \pm 0.2$	$1.0^{a} \pm 0.2$	$4.0^{c} \pm 0.8$

Cumulus expansion of COCs (Based on 5 replicates per treatment with 2-3 oocytes per plot) from *invitro* grown PFs (mean SEM) during *in vitro* culture.
Values with different superscript letters differ significantly ($p<0.05$) within column.

amphiregulin which share common receptors with EGF (Jones *et al.*,1999). Therefore AREG serves as signal for cumulus expansion and ultimately causes oocyte maturation.

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Effect of Sub-chronic Exoposure to Arsenic on Aortic Recativity to Noradrenaline in Rats

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ABSTRACT

Arsenic is a groundwater pollutant and can cause various cardiovascular disorders in the exposed population. The aim of present study was to examine whether arsenic exposure can alter the aortic reactivity to adrenergic agonists. Rats were exposed to arsenic as 25, 50 and 100 ppm of sodium arsenite through drinking water for 90 consecutive days. On day 91st day, animals were euthanized by bleeding from the posterior vena cava after intraperitoneal injection of urethane. Lungs and heart were taken out *en block* along with the thoracic aorta and immediately placed into cold (4^{0}C) Modified Krebs Henseleit Solution (MKHS). Thoracic aortas were isolated and the adhering tissues were cleared off and 3-4 mm long aortic rings were mounted in organ bath for vascular reactivity studies. Aortic rings of the control animals showed dose-dependent contraction to noradrenaline and the E_{max} and pD_2 were 0.84±0.09 g and 6.50±0.09, respectively. The 25 and 50 ppm concentrations of SA did not cause any significant alteration in the efficacy, and the E_{max} values were 1.03±0.12 g and 1.03±0.10 g, respectively. But 100 ppm significantly increased the efficacy (E_{max}: 1.28±0.06 g) of noradrenaline. However, SA at 25, 50 and 100 ppm significantly increased the pD_2 of noradrenaline to 6.98±0.08, 8.03±0.09 and 8.01±0.07, respectively. Thus, sub-chronic exposure to sodium arsenite caused concentration-dependent enhancement of noradrenaline induced contractile response in aorta of male wistar rats.

Key words: Arsenic, aorta, noradrenaline, contractile response and rats

Arsenic is widely distributed in the environment and its exposure occurs primarily through contaminated water, food and soil. Arsenic contamination in groundwater has been reported across several countries, such as Bangladesh, India, Taiwan, Chile, Argentina and the USA (Argos *et al*., 2010; Cheng*et al*., 2011). What is worse is that arsenic contamination in groundwater is slowly spreading to states of India like Bihar, Jharkhand, Uttar Pradesh, Assam, Chhattisgarh and Chandigarh (Mukherjee *et al*., 2006). According to the World Health Organization guidelines, the maximum permissible limit of arsenic in drinking water is 0.01 ppm (WHO, 2011). However, the range of arsenic concentrations found in natural waters around the world varied from <0.0005 to >5 ppm (Rahaman *et al*., 2013). In certain areas in the Indian subcontinent, the maximum arsenic concentration in ground water was found to be around 3.7 ppm to 4.7 ppm, leading to several heath problems (Chatterjee and Chatterji, 2010). But there is report that in West Bengal, India, people were exposed to arsenic-contaminated water even in the range of 0.05-14.2 ppm (Guha Mazumder and Dasgupta, 2011).

The chronic poisoning caused by high levels of arsenic in well waters led to public health emergency in Bangladesh (Alam *et al*., 2002; Chen *et al*., 2009). Long-term arsenic exposure was linked to both

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carcinogenic and non-carcinogenic diseases. Several studies have associated high-level of arsenic exposure from drinking water with elevated risk of vascular diseases, including peripheral vascular disease, hypertension, ischemic heart disease and carotid atherosclerosis (Stea *et al*., 2014). It appears from the literature that no *in vivo* studies were carried out to investigate the role of noradrenaline signaling in arsenic-induced cardiovascular disorders. Our particular interest is to examine in an *in vivo* model the mechanistic association between arsenic-induced vascular dysfunction and alteration in noradrenaline action. We, therefore, evaluated in a rat model whether sub-chronic arsenic exposure through drinking water can induce vascular dysfunction and whether the dysfunction could relate to inflection of noradrenaline.

MATERIAL AND METHODS

The study was conducted in adult male Wistar rats (200-230 g) procured from the Laboratory Animals Resource Section of the ICAR - Indian Veterinary Research Institute, Izatnagar. Rats were housed in polypropylene cages with chopped wheat straw as the bedding material. Animals were maintained under standard management conditions and handled as per the Institute Animal Ethics Guidelines. Rats were given standard rat chow (Amrut Feeds, Pranav AgroIndustries, New Delhi) and water *ad libitum* throughout the study. All the animals were kept in the laboratory conditions for 7 days or more before initiation of the experiment.

Selection of arsenic concentration

Arsenic concentration was selected based on the reported contamination levels of arsenic in West Bengal, India, where people were exposed to arsenic-contaminated water in the range of 0.05-14.2 ppm (Guha Mazumder and Dasgupta, 2011). Based on this, we selected 14.2 ppm concentration so as to know the effects of an environmentally relevant high arsenic contamination level. Since we exposed the animals to arsenic as sodium arsenite (SA), we gave 25 ppm of SA to provide almost an equivalent amount of elemental arsenic. Thus, 25 ppm of SA formed the basal concentration. As per the standard protocol for hazard identification and risk characterization, a minimum of three doses of the test chemical are used to understand the dose-response relationship. Accordingly, we selected two more concentrations, *i.e.*, 50 and 100 ppm of SA in the current study.

Experimental design and tissue collection

Rats were divided randomly into 4 groups consisting of 6 each. Animals of Group I received only drinking water, while of groups II, III and IV received 25, 50 and 100 ppm of SA, respectively, through drinking water for 90 consecutive days. On the 91st day, animals were euthanized by bleeding from the posterior vena cava under intraperitoneal injection of urethane (1.2 g/kg body wt) anesthesia. Lungs and heart were taken out *en block* along with thoracic aorta and immediately placed into cold (4 ^{O}C) Modified Krebs Henseleit Solution [MKHS; composition (mmol/L): 118.0 NaCl, 4.7 KCl, 2.5 $CaCl_2.2H_2O$, 1.2 $MgSO_4$. $7H_2O$, 1.2 KH_2PO_4, 11.9 $NaHCO_3$ and 11.1Glucose]. Thoracic aortas were isolated and the adhering tissues were cleared off. They were cut into 3-4 mm long rings and mounted for vascular reactivity studies using organ bath.

Tissue preparation and isometric recording

The aortic rings were held between two hooks made from 25 G stainless steel wire and mounted under a resting tension of 1.5 g in a thermostatically controlled (37.0±0.1 ^{O}C) Organ Bath (UGO Basile, Italy) of 10 ml capacity containing MKHS and was continuously aerated with medical gas (74% N_2 + 21% O_2 + 5% CO_2). The rings were equilibrated for 60-80 min in the organ bath filled with MKHS before recording of tension. During equilibration period, the bath fluid was repeatedly changed once in every 15 min. The change in tension was measured by a high-sensitivity isometric force transducer and recorded in a PC using Lab Chart 7 software programme (Powerlab, AD Instruments, Australia). At the beginning of each experiment, the tissue viability was checked by eliciting contraction with high K^+ (80 mM)-depolarizing solution. On attaining contraction-plateau, high potassium solution was replaced by normal MKHS to restore baseline resting tension.

Following a lapse of 50 min after reaching the baseline and 2-3 washes with MKHS, the rings were again contracted with cumulative addition of noradrenaline [-10 M to -5.5 M] to the bath solution at an increment of 0.5 log unit for obtaining concentration-related response.

RESULTS

The representative tracings in Figure 2 illustrate the noradrenaline-induced contractile response in the control and SA-exposed rat thoracic aorta. Aortic rings of the control animals showed dose-dependent contraction to noradrenaline and the E_{max} and pD_2 were 0.84±0.09 g and 6.50±0.09, respectively. As shown in Fig. 1, the 25 and 50 ppm concentrations of SA did not cause any significant alteration in the efficacy. The respective E_{max} values were 1.03±0.12 g and 1.03±0.10 g. But 100 ppm significantly increased the efficacy (E_{max}: 1.28±0.06 g) of noradrenaline. However, SA at 25, 50 and 100 ppm significantly increased the pD2 of noradrenaline to 6.98±0.08, 8.03±0.09 and 8.01±0.07, respectively.

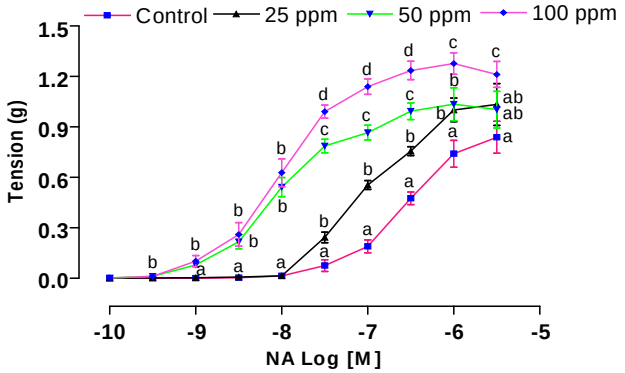

Figure 1: Dose-response relationship of noradrenaline in the sodium arsenite-exposed rat aorta. Values are mean of 6 rats and vertical bars represent standard errors. Values of the same concentration point bearing no superscripts common vary significantly (P<0.05) in Duncan's multiple comparison post-hoc test.

DISCUSSION

We examined whether arsenic exposure can alter the aortic reactivity to adrenergic agonists. The contractile response to norepinephrine was enhanced in the arsenic-treated aortic rings, indicating enhancement of vascular response due to increase in the sympathetic activity. The augmented contraction in hypertension was attributed to increased phosphor inositide hydrolysis, release of intracellular Ca^{2+}, activation of PKC or alterations in the number and affinity of sarcoplasmic reticular IP_3 receptors (Khalil, 2013). Further, in the hypertensive rats, the impaired

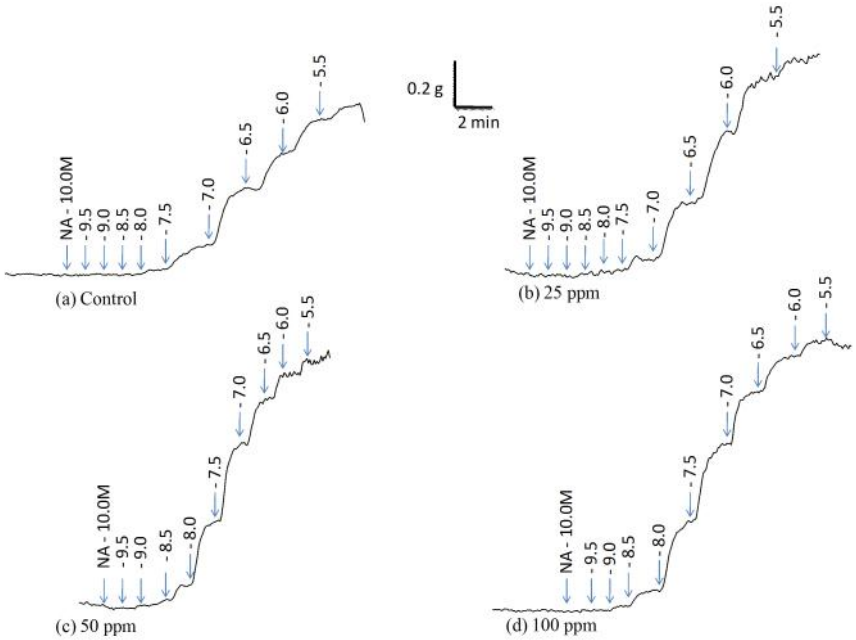

Figure 2: Representative tracings of noradrenaline-induced contractions in control (a) and sodium arsenite-exposed rat aorta [25 ppm (b); 50 ppm (c); 100 ppm (d)].

endothelial modulation of vascular contractions induced by norepinephrine could relate to the decreased NO production, increased NO inactivation and/or altered NO-cGMP signaling (Choi *et al*., 2014). Several of these processes may be responsible for the increased vascular sensitivity to norepinephrine in the arsenic-induced hypertension. It is beyond the scope of the current study to provide a mechanistic basis for the increased sensitivity.

CONCLUSION

The current study revealed that sub-chronic exposure to sodium arsenite in Wistar rats cause a concentration-dependent enhancement in contractile response in aorta in *vitro.* Thus, there exists a vast scope to explore the mechanistic basis role of sympathetic nervous system in arsenic induced alterations in vascular sensitivity.

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Cytoarchitecture of Gracile Nucleus in the Buffalo (*Bubalus bubalis*)*

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ABSTRACT

The gracile nucleus constitute first order relay centers in the large spinal afferent pathway, which subserve touch, pressure and joint proprioceptive sensory inputs to the cerebral cortex. In the buffalo it was located in the fasciculus gracilis adjacent to the dorsal median septum, dorsal to the central canal and rostral to the obex. Nucleus was composed of small and medium sized neurons. Majority of the medium sized neurons were multipolar, triangular and fusiform in shape. The nucleus of these neurons was central or eccentric and the position of the nucleolus was variably central and darkly stained. The average true diameter of cell body and nucleus of medium sized neurons in the gracile nucleus of buffalo were 33.57 ±0.6 µm and 12.11 ±0.23µm, respectively. The average true diameter of the cell body and nucleus of small sized neurons were 19.69 ±0.37µm and 9.54 ±0.17µm, respectively. Small sized neurons were concentrated in the caudal and rostral pole of the nucleus. The Nissl substance was fine and moderately stained in both types of neurons. The total neuron population of the gracile nucleus in the buffalo was 11,082 ±145. The neurons in the nucleus were sparingly distributed.

Keywords: Gracile nucleus, morphology, cytoarchitecture, buffalo and brain

The nuclei of the dorsal funiculi namely, gracilenucleus, medial cuneate and lateral cuneate function as important relay nuclei in the dorsal funicular system. These nuclei constitute the first order relay centers in the large spinal afferent pathway, which subserve touch, pressure and joint proprioceptive sensory inputs to the cerebral cortex. Lesions of the dorsal funiculus or these nuclei abolish or diminish cutaneous sensation and ipsilateral joint proprioceptive deficits. Though extensive research has been done on the extent and cytoarchitecture of these nuclei in man (Olszewski and Baxter, 1954), cat (Taber, 1961), sheep (Rao, 1964) ox (Goller, 1963), horse (Salam, 1971), dog (King and Lowell, 1999) and camel (Saleh *et al.*, 2012), therefore it was necessary to understand the various conditions that affect the loss of somesthetic sense in animals more particularly in buffaloes as there was meager information on this nucleus in buffalo.

MATERIAL AND METHODS

Brains of eight buffaloes were obtained from the Slaughter House, Bangalore. The heads, as a whole were collected immediately after slaughter and were perfused with 10 percent buffered formalin through the common carotid artery till a clear fluid came out. The perfused heads were kept for two weeks in 10 percent buffered formalin. The cranium was opened carefully and the brain along with the brainstem were removed and preserved in 10 percent buffered formalin for a further period of two weeks.

The brainstem from the level of first cervical to trapezoid body (medulla oblongata) were cut and processed for paraffin technique. The cytoarchitectural description of the nuclei was based on the transverse serial sections of 20 µ thickness stained with toludine blue to study the size and shape of the cell body, Nissl pattern, size and position of

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the nucleus and nucleolus from the transverse sections were stained with neutral red and cresyl fast violet (Keller, 1960).

The true neuron population in the right and left side of the median raphe was determined by counting the neurons in all the serial transverse sections obtained from one animal. Further, for the estimation of total neuron population of a nucleus in other five animals, systematically sampled every 10^{th} section from each of the five animals were used in this study. Only those neurons that had a distinct nucleolus were counted. Neuron counts for the right and left nuclei were recorded separately and were compared statistically using unpaired 't' test (Snedecor and Cochran, 1996).

The total numbers of neurons in these nuclei were determined by using the formula AxB, where A is the number of neurons counted in each sampled section and B is the number of sections up to the next counted section, and by adding the products of AB for all the sections counted. The counting of neurons from the systematically sampled sections was done from caudal to the rostral end of the nucleus.

An ocular micrometer was used to measure the size of the neurons. The neurons were measured at a magnification of X 600. The length and width of a cell was measured and the average was taken to arrive at its diameter. Similarly the size of the nucleus was also determined.

These diameters were considered as the true diameters. The true diameter of the cell body formed the basis for classification of neurons in the nuclei under study. The neurons were classified as large (greater than 50 mm), medium (26 to 50 mm) and small (less than 25 mm in diameter).

RESULTS AND DISCUSSION

The gracile nucleus in the buffalo comprised small to medium sized neurons. The small sized neurons were round, oval or triangular neurons similar to the findings in sheep (Pattison and Holman, 1943) and horse (Salam, 1971). Medium sized neurons were found to be fusiform and multipolar neurons in the present study as described in European bison (Szteyn and Robak, 1989). In man, the neurons of the gracile nucleus exhibited considerable pleomorphism with varied staining qualities (Olszewski and Baxter, 1954). In contrast, in cat there were small, medium and large neurons (Taber, 1961). The small neurons were oval or spindle shaped, the medium and large were multiformed. In camel, the neurons were classified into six types based on soma size and shape (Saleh *et al.*, 2012). However in the buffalo only small and medium sized neurons were observed (Fig.1).

In the buffalo, the caudal pole of the gracile nucleus contained small sized and sparsely distributed neurons. In the middle portion, the neurons were medium and small sized. Majority of the medium sized neurons were multipolar and fusiform with few round, oval and triangular in shape. Whereas in primates, the caudal pole of the gracile nucleus comprised predominantly of small neurons (Gerhard and Olszewski, 1974).

Small sized neurons in the gracile nucleus of the buffalo comprised central or eccentric nuclei and the position of the nucleolus was variably central and darkly stained. In the buffalo, Nissl granules in the neurons of the gracile nucleus were fine and moderately stained (Fig. 2 and 3). Similar observations were noticed in the horse by Salam (1971). Whereas in man, nucleus was centrally placed and in most instances the Nissl granules were indistinct and peripherally arranged (Olszewski and Baxter, 1954). In the cat, the nucleus of medium sized neurons was variably central with fine, evenly dispersed and moderately stained Nissl granules (Taber, 1964). In large neurons the nucleus was central and Nissl granules were coarse and deeply stained and these large neurons were not observed in the buffalo.

The average true diameter of cell body and nucleus of medium sized neurons in the gracile nucleus of buffalo were 33.57 ±0.6 µ and 12.11 ±0.23 µ respectively. The average true diameter of the cell body and nucleus of small sized neurons were 19.69 ±0.37 µ and 9.54 ±0.17 µ (Table1). Where as in the horse, small neurons ranged in size from 13 to 22 µ with an average of 20 µ (Salam, 1971). The stellate

neurons ranged in size from 28 to 41 µ with an average of 35 µ. Thus the average sizes of both small and medium neurons were similar in the horse and buffalo. The estimated population of medium sized neurons for both left and right nuclei together was 4,984, while the average population of small sized neurons was 6,215. Thus the proportions of medium to small sized neurons in the gracile nucleus of buffalo were 4984:6215. The approximate ratio of medium to small neurons were 1:1.25 (Table 2). The neuron population in the right gracile nucleus ranged from 5298 to 5760 with a mean of 5471±76 and in the left from 5430 to 5940 with a mean of 5611±73. Total neuron population of the gracile nucleus in the buffalo (left and right side combined) was 11,082±145 (Table 3). In man, the neuron population in the right side of the gracile nucleus was 4,066 and left side was 4,303 (Olszewski and Baxter, 1954). In monkey, right side was 2,321 and left side was 2,337 (Arthur, 1973). The total neuron population in the gracile nucleus was 5,400 in the cat (Bermejo *et al.*, 2003). From these observations it appears that number of neurons increases with the increase in the size and surface area of the animals, being lowest in the cat and monkey (small animals) to the highest recorded in the buffalo (large animals). This can be functionally correlated to the larger skin surface area to be covered in transmitting somatosensory modality.

TABLES

Table 1. True diameters of the medium and small neurons and the ratio between the diameter of nucleus and cell body in the gracile nucleus

Number of animals	Size of neuron	Mean true diameter in µ (Mean ± SE)		Ratio between diameter of nucleus: cell body
		Cell body	Nucleus	
6	Medium	33.57 ± 0.62	12.11 ± 0.23	1: 2.10
	Small	19.69 ± 0.37	9.54 ± 0.17	1: 2.83

Table 2. Distribution and relative proportion of medium and small neurons in the nuclei

Nucleus	Medium neuron	Small neuron	Relative proportion
Gracile nucleus	4984	6215	1:1.25

Table 3. Comparison of neuron population in the left and right gracile nucleus in the buffalo

Side of Brain	Buffalo number						Mean ± SE	't' (P> 0.05)
	B1	B2	B3	B4	B5	B6		
Right	5298	5760	5460	5370	5310	5628	5471±76	
Left	5480	5940	5658	5570	5430	5590	5611 ±73	
Total	10778	11700	11118	10940	10740	11218	11082±145	1.32

Note: The mean neuron population in the right and left gracile nucleus showed no significant difference (P> 0.05).

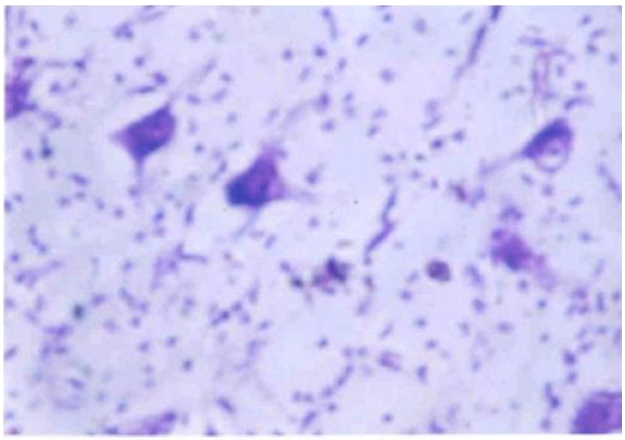

Fig.1:Photomicrograph showing types and distribution of neurons in the nucleus gracilis Cresyl fast violet X200

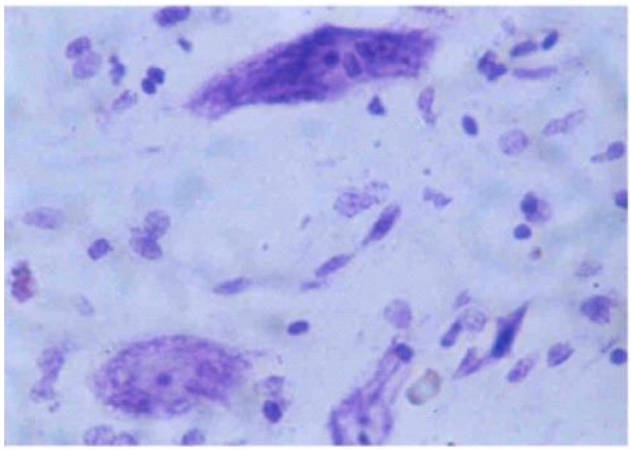

Fig. 2:Photomicrograph showing some characteristic neurons in the nucleus gracilis Cresyl fast violetX400

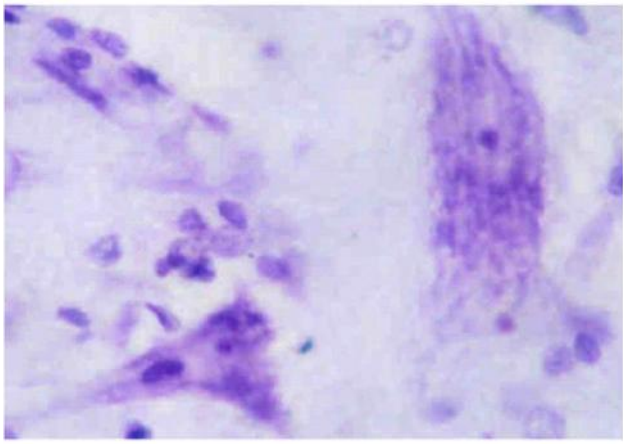

Fig.3:Photomicrograph showing some characteristic neuron in the nucleus gracilis Cresyl fast violet X600

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Effect of Aflatoxin and Mycoplasmal Organisms on the Performance of Broiler Chicken*

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ABSTRACT

To evaluate the individual and interactive effect of the aflatoxin and mycoplasmal infection in broiler chicken, day-old-birds were divided into four equal groups of 36 birds each and subjected to the following treatments. Group I, fed with normal feed thorough out the experiment was considered as control group. Group II was fed with o.5ppm of aflatoxin in feed from Day1 to Day 35. Group III was fed with normal feed and inoculated with $2.4X10^8$ cfu mycoplasmal organisms (MG) on Day 14. Group IV was fed with 0.5 ppm of aflatoxin in feed from Day 1 and inoculated with mycoplasmal organisms on Day 14. Group II birds showed low body weight and relative weights of lymphoid organs, while the relative weights of liver and kidney were increased. Group III showed no significant variation in these parameters except for the slight increase in relative weight of spleen in the last two weeks due to lymphofollicular reaction stimulated by mycoplasmal organisms. Group IV birds showed marked decrease in body weight gain, decrease in the relative weights of lymphoid organs like thymus and bursa, increased relative weights of liver, kidney and spleen.

Key words: Aflatoxin, mycoplasma, broiler chicken

Aflatoxicosis and mycoplasmosis are two important diseases encountered by the poultry industry most commonly in field conditions. These diseases are of economic importance since they involve high mortality and morbidity, high cost of treatment, low weight gains of survived birds and low egg production leading to lower profit margins. The present study was undertaken to study the individual and combined effect of aflatoxin in low dose and mycoplasmal organisms in broiler chickens with an effort to mimic the above conditions similar to that of their natural occurrence in the field which can provide insight of the pathology of the mentioned diseases in concurrence.

MATERIALS AND METHODS

For the production of spores of *Aspegillus parasiticus* culture in sterile rice culture, the method described by Shotwell *et al*. (1966) was adopted. For inoculation of mycoplasmal organisms the stock culture of *Mycoplasma gallisepticum* procured from Veterinary College and Research Institute, Namakkal was subcultured and used.

One hundred and fifty, day-old-broiler chickens were divided into four groups. Group I was kept as control, Group II was fed with AF (0.5 ppm). Group III was inoculated with mycoplasmal organisms (2.4 X 10^8cfu/bird, I/N) on 14^{th} day. Group IV was fed with AF from Day 1 to 35 and on 14^{th} day

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mycoplasmal organisms were inoculated. Six birds from each group were weighed and sacrificed at weekly intervals for five weeks followed by blood collection and detailed PM examination and recording of different organ weights.

RESULTS AND DISCUSSION

Body weight

The body weights were 1563.33±23.05, 1090.00± 67.08, 1405.00±100.59 and 1066.67±60.09 in Group I to IV respectively at the end of fifth week.

The results of the present study (Table1 and Fig. 1) revealed decrease in the body weight of AF fed birds (Group II and IV) when compared to untreated birds (Group I and III). The reduction was statistically significant by the end of third week onwards.

Reduction in the body weight gain in broiler chicken during aflatoxicosis could be attributed to reduced feed consumption and/or inhibition of protein synthesis, as rightly pointed out by Smith and Hamilton (1970). Reduced protein synthesis with altered protein metabolism by AF may also contribute to decreased weight gain in broilers (Thaxton *et al*., 1974).

Reduction in the body weight gain may be attributed to the anorexia, decreased feed consumption, lethargy and respiratory distress of the infected birds.

Concomitant infection of mycoplasmosis with aflatoxicosis in broiler chickens resulted in reduced body weight gain without any marked interaction.

Feed conversion ratio

The FCR recorded at the end of fifth week were 1.64, 2.38, 1.84 and 2.42 in Group I to Group IV respectively. The feed conversion efficiency was increased in AF fed birds as compared to AF untreated birds (Table 2 and Fig. 2).

The decreased feed efficiency can be attributed to the altered protein metabolism and/or inhibition of protein synthesis by AF as described by Thaxton *et al*. (1974).

Mycoplasma challenged birds showed decreased feed efficiency which was also observed by Landman *et al*. (2004).

Feed efficiency was low in concomitant infection of mycoplasmosis and aflatoxicosis without any significant interaction.

Relative organ weights

In the present study, the relative weights of liver, kidney and spleen were increased while the relative weights of bursa of Fabricius and thymus were decreased in the toxin fed groups. The findings of the study are substantiated by the reports of earlier workers (Shivappa, 2005 and Mallikarjuna, 2006).

Liver

The mean (±SE) relative weights of liver of Group I to IV were 2.67±0.09, 3.53±0.30, 2.53±0.18 and 3.43±0.15 respectively at the end of fifth week (Table 3 and Fig. 3).

Increase in the relative weight of liver is attributed to the hepatotoxic effect of aflatoxin. Destruction of hepatic parenchyma leading to impaired lipid metabolism and lipid transport resulting in increased deposition of fatty droplets in the hepatocytes contribute to the increased weights of liver (Channakrishnappa *et al*., 1999).

Kidney

The mean (±SE) relative weights of kidney for Group I to IV were 0.73±0.03, 1.02±0.01, 0.69±0.02 and 1.01±0.01 respectively at the end of fifth week (Table 4 and Fig. 4).

Increased relative weight of kidneys could be attributed to renal disposition of aflatoxin leading to fatty changes and haemorrhages in kidney as described by Dafalla *et al*. (1987).

Lymphoid organs

Spleen

The mean (±SE) relative weights of spleen of Group I to Group IV were 0.11±0.01, 0.15±0.00, 0.13±0.01 and 0.15±0.02 respectively at the end of experiment (Table 5 and Fig. 5).

The relative weight of spleen in toxin fed birds decreased initially and gradually increased to a significant level in the later weeks.

The decrease in the relative weight of spleen in the first and second week could be attributed to the lymphocytolytic actiity of aflatoxin, while increase in the later weeks could be attributed to lymphoid stimulation and repopulation of spleen tissue by proliferating lymphoblast cells.

Thymus and bursa of Fabricius

The mean (±SE) relative thymus weight of Group I to IV were 0.44±0.01, 0.37±0.01, 0.41±0.03 and 0.35±0.02 respectively, at the end of fifth week (Table 6,7 and Fig. 6,7).The mean (±SE) values of BF were 0.24±0.04, 0.18±0.01, 0.23±0.05 and 0.16±0.02 in Group I to Group IV birds respectively at the end of fifth week.

The reduced relative weights of thymus and bursa in aflatoxin fed birds throughout the experiment could be attributed to the lymphocytolytic effects of aflatoxin, as it was evident microscopically in the present study.

Conclusion

It can be concluded that aflatoxicosis considerably reduces the performance of broiler birds by reducing their weight gain and also immunocompetance by affecting the lymphoid organs. Thus, aflatoxicosis favours the rapid progression of opportunistic infectious diseases like mycoplasmosis thereby hindering the profits from poultry industry.

Table 1: Mean (±SE) body weight (g) in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection

Groups	Age in weeks				
	1	2	3	4	5
I (Control)	156.67±8.03	441.67±9.10	715.83±16.65^{a}	1150.00±83.02^{a}	1563.33±23.05^{a}
II (AF)	140.00±10.33	341.67±9.46	496.67±19.78^{b}	805.00±36.31^{b}	1090.00±67.08^{b}
III (MG)	150.83±7.57	441.67±9.08	636.67±15.42^{a}	970.00±43.05^{c}	1405.00±100.6^{c}
IV (AF and MG)	140.00±6.32	343.33±9.88	425.0±54.69^{bd}	720.0±34.54^{bd}	1066.67±60.09^{bd}

The means bearing any one common superscript within the column do not vary significantly ($p<0.05$).

Table 2: Mean FCR in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection

Groups	Age in weeks				
	1	2	3	4	5
I (Control)	1.20	1.40	1.45	1.49	1.64
II (AF)	1.42	1.99	2.58	2.32	2.38
III (MG)	1.28	1.37	2.05	1.94	1.84
IV (AF and MG)	1.42	1.97	3.14	2.6	2.42

Table 3: Mean (±SE) relative weight of liver (%BW) in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection

Groups	Age in weeks				
	1	2	3	4	5
I (Control)	3.91±0.12	4.14±0.03[a]	3.54±0.18[a]	2.63±0.09[a]	2.67±0.09[a]
II (AF)	4.26±0.26	6.02±0.20[b]	6.08±0.35[b]	4.32±0.20[b]	3.53±0.30[b]
III (MG)	3.66±0.02	4.21±0.05[a]	3.16±0.07[a]	2.78±0.09[a]	2.53±0.18[a]
IV (AF and MG)	4.04±0.29	5.28±0.22[c]	5.57±0.32b[c]	4.31±0.21b[c]	3.43±0.15b[c]

Means bearing any one common superscript within columns do not vary significantly ($p<0.05$)

Table 4: Mean (±SE) relative weight of kidney (% BW) in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection

Groups	Age in weeks				
	1	2	3	4	5
I (Control)	0.95±0.03	0.79±0.01[a]	0.84±0.05[a]	0.89±0.05[a]	0.73±0.03[a]
II (AF)	0.93±0.07	1.03±0.04[b]	1.06±0.05[b]	1.03±0.00[b]	1.02±0.01[b]
III (MG)	0.98±0.01	0.88±0.03[a]	0.85±0.06[a]	0.82±0.00[a]	0.69±0.02[a]
IV (AF and MG)	0.97±0.05	1.04±0.02b[c]	1.05±0.03b[c]	1.05±0.02b[c]	1.01±0.01b[c]

Means bearing any one common superscript within columns do not vary significantly ($p<0.05$).

Table 5: Mean (±SE) relative weight of thymus (% BW) in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection.

Groups	Age in weeks				
	1	2	3	4	5
I (Control)	0.51±0.01	0.72±0.02a	0.65±0.07a	0.52±0.01	0.44±0.01
II (AF)	0.44±0.04	0.51±0.03b	0.45±0.04b	0.41±0.02	0.37±0.01
III (MG)	0.51±0.01	0.68±0.01a	0.58±0.01a	0.47±0.06	0.41±0.03
IV (AF and MG)	0.45±0.04	0.48±0.02bc	0.46±0.03abc	0.41±0.07	0.35±0.02

Means bearing any one common superscript within columns do not vary significantly ($p<0.05$).

Table 6: Mean (±SE) relative weight of spleen (%BW) in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection

Groups	Age in weeks				
	1	2	3	4	5
I (Control)	0.06±0.00	0.06±0.00	0.11±0.01[a]	0.14±0.01[a]	0.11±0.01
II (AF)	0.07±0.00	0.07±0.00	0.18±0.02[b]	0.19±0.02[a]	0.15±0.00
III (MG)	0.07±0.00	0.06±0.00	0.15±0.03[a]	0.15±0.01[d]	0.13±0.01
IV (AF and MG)	0.07±0.00	0.07±0.00	0.17±0.03b[c]	0.22±0.01[ac]	0.15±0.02

Means bearing any one common superscript within columns do not vary significantly ($p<0.05$).

Table 7: Mean (±SE) relative weight of bursa of Fabricius (% BW) in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection

Groups	Age in weeks				
	1	2	3	4	5
I (Control)	0.51±0.01[a]	0.28±0.01	0.25±0.01[a]	0.19±0.01[a]	0.24±0.04
II (AF)	0.42±0.01[b]	0.33±0.01	0.20±0.00[a]	0.14±0.02[b]	0.18±0.013
III (MG)	0.50±0.01[a]	0.27±0.01	0.29±0.04[d]	0.23±0.03[a]	0.23±0.05
IV (AF and MG)	0.43±50.02[a]	0.27±0.04	0.19±0.01[a]	0.12±0.01[c]	0.16±0.02

Means bearing any one common superscript within columns do not vary significantly ($p<0.05$).

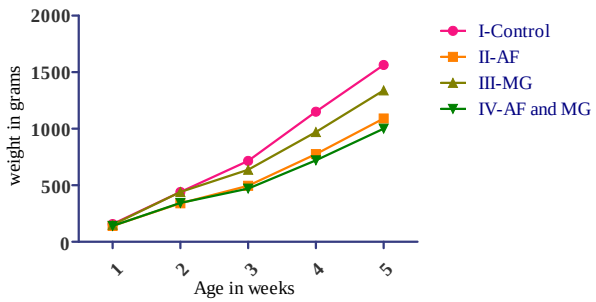

Fig. 1: Mean body weights (g) of broiler chicks in different groups at different age intervals in experimental aflatoxicosis and mycoplasmal infection

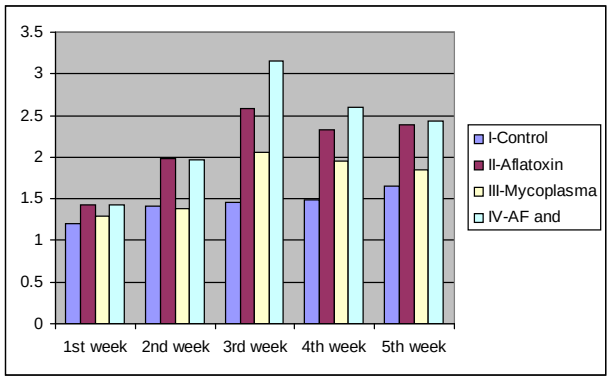

Fig. 2: Mean FCR in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection

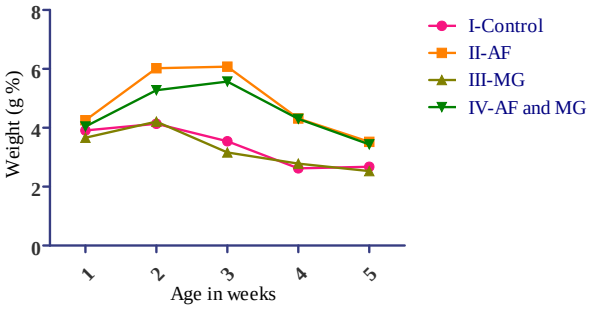

Fig. 3: Mean (±SE) relative weight of liver (g%) in different groups of broiler chicks at different age intervals inexperimental study of aflatoxicosis and mycoplasmal infection.

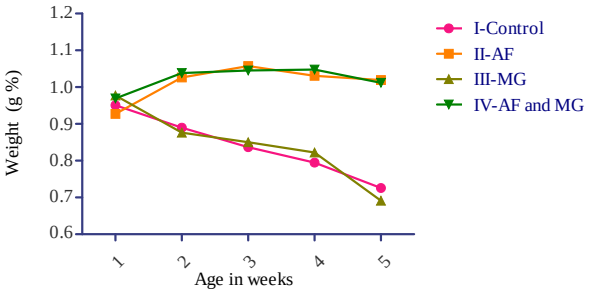

Fig. 4: Mean relative weight of kidney (g %) in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection.

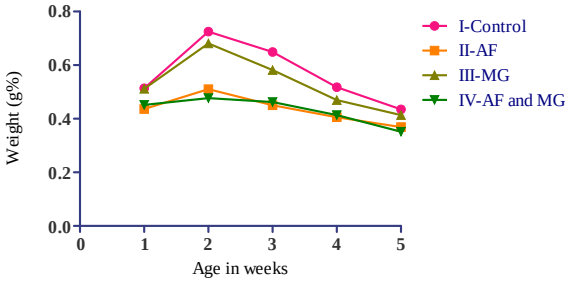

Fig. 5: Mean relative weight of thymus (g %) in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection

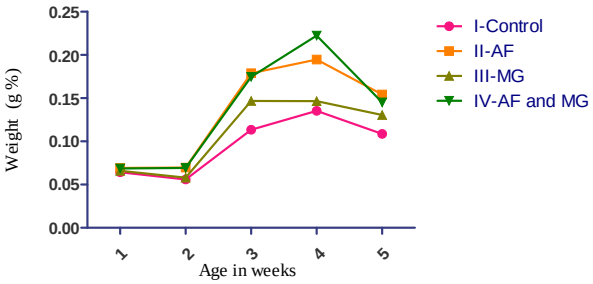

Fig. 6: Mean relative weight of spleen (g %) in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection.

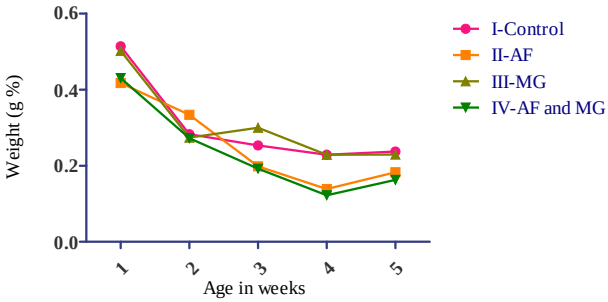

Fig. 7: Mean relative weight of bursa of Fabricius (g %) in different groups of broilerchicks at different age intervals in the experimental study of aflatoxicosis and mycoplasmal infection.

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Screening of *Clerodendrum inerme* (L.) for Pharmacological Activities on Central Nervous System in Mouse

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ABSTRACT

Clerodendrum inerme (L.) belongs to the family *Verbenaceae* and is popular among the traditional practitioners in the treatment of pain, inflammation, skin diseases, topical burns and fever in man. The present study was undertaken to determine the phytochemistry and to screen the hydroalcoholic extract of *Clerodendrum inerme* (L.) (HACI) for pharmacological activities with special reference to central nervous system *viz.,* spontaneous motor activity, forced locomotor activity and analgesic activity in mice. The phytochemical analysis of HACI revealed essentially the presence of alkaloids, triterpenoids, tannins, flavanoids and absence of glycosides, steroids and saponins. HACI at a dose rate of 400, 800 mg/kg significantly (p<0.05) reduced the SMA in mice and results were comparable to diazepam (2 mg.kg^{-1}, i.p). Similarly, HACI showed significant influence on voluntary locomotor activity in all the tested doses as assessed by the rota-rod. Further HACI (400 and 800 mg.kg^{-1}) reduced the pain threshold (Eddy's hot plate), thus devoid of analgesic activity after single *per os* administration. There exists a vast scope to subject the hydroalcoholic extract for further investigations in view of its significant neuropharmacological activities in mice.

Keywords: *Clerodendrum inerme*, hydroalcoholic extract (HACI), mice.

The genus *Clerodendrum* L. [Family: Lamiaceae (Verbenaceae)] is widely distributed in tropical and subtropical regions of the world and is comprised of small trees, shrubs and herbs. *Clerodendrum spp.* used in Indian and Chinese traditional system of medicine for ages. It is widely distributed throughout India; its pharmacological investigation showed stimulant activity (Sharaf *et al*., 1969), insecticidal activity (Patil *et al.*, 2006), ovicidal, growth inhibition and morphogenetic effects against various life stages of a noxious lepidopteron insect-pest (Yankanchi and Patil, 2009), anti-inflammatory and analgesic activity (Yankanchi and Koli, 2010). In Indian tribal medicine, leaves of *Clerodendrum inerme* are used for treating fever, cough, skin rashes and boils, and are used in conjunction with other plants (Pandey *et al.*, 2003). In the Chinese system of medicine *Clerodendrum bungei* is used for treatment of headaches, dizziness, furuncles and hysteroptosis (Zhou *et al*., 1982; Yang *et al*., 2002). However, screening of *Clerodendrum inerme* with respect to its central nervous system activity is limited. Thus the present study was undertaken to evaluate the *Clerodendrum inerme* (L.) for neuropharmacological activities in mice.

MATERIAL AND METHODS

Collection of plant material*:* Fully matured dark green leaves of *Clerodendrum inerme* (L) were collected in- and around the vicinity of Veterinary College campus, Shivamogga during the month of July-August 2012. Plant species was identified by the Department of Botany, Shayadri College,

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Shivamogga, and Karnataka. The plant leaves were gently washed with tap water to remove dust and any other extraneous materials, allowed to dry under shade and, dried leaves were powdered by using electric grinder. Finally, the fine powder obtained through sieving using kitchen strainer for used for extraction.

Hydroalcoholic extraction*:* Fifty grams of grounded plant material was weighed in to a 500 ml conical flask to which mixture of 300 ml of ethanol and 100 ml of water [Ethanol-75:Water-25] were added. The mouth of the flask was covered with aluminum foil and it was continuously kept for agitation for 24 hr. using vortex shaker (50 revolution per min.). The extract was filtered by a muslin cloth, followed by filtration through Whatman filter paper (#1). The filtrate thus collected was subjected for evaporation using hot water bath and the residue (hydroalcoholic extract; HACI) was air dried and stored until further analysis or experimental study.

Phytochemical analysis*:* The phytochemical analysis of HACL was carried out according to standard procedure described (Grover *et. al* 2014).

Experimental animals*:* The study was conducted in the apparently healthy adult Swiss albino mice (26 ±2g) procured from the Small Animals House, Veterinary College, Shivamogga. All the animals were housed in polypropylene cages and maintained as per the guidelines of the CPCSEA with a day: light cycle of 12 hr. each. Animals were fed with standard rodent pellet and provided with *ad libitum* water. Twelve hours before undertaking each of the following experiment the animals had access to water but not feed. Prioir approval of IAEC was obtained before undertaking the current study.

Dosage selection: The 'limit test' was conducted according to Bhusan *et al*. (2014) to determine the acute toxic effects of HACI after per oral administration of test dose of 2000 $mg.kg^{-1}$ in mice. There were no toxic signs and mortality in the test animals, hence in the present studies the three dose levels of HACI were selected *viz*; 200, 400 and 800 $mg.kg^{-1}$ and were employed for assessing the various neuropharmacological activities.

Spontaneous motor activity (SMA): The Spontaneous motor activity (SMA) was measured using actophotometer (Inco Instruments, Ambala) (Kulkarni, 1999). The mice were divided into four groups of six each. Each animal was placed individually in the actophotometer and the basal activity was measured. Animals in group-I were administered (p.o) with normal saline and group - II, III and IV with HACI at the dose rate of 200, 400 and 800 $mg.kg^{-1}$ body weight respectively. To measure spontaneous locomotor activity, each animal was separately positioned in actophotometer, which shows depressant or stimulant effect on central nervous system (CNS). The SMA was recorded for a period of 10 min before and after 30 or 60 min. of administration of HACI.

Forced locomotor activity in mice (FLA)*:* The effect of HACI on forced locomotor activity as a measure of skeletal muscle tone and coordinated movements of animals was studied using rota rod (Rotarod 4C, MKM, Chennai) as described by Dunham and Miya (1957) in mice. The mice which were trained to remain on the horizontal rod rotating at 20 rpm were selected for the study. Only those mice which have demonstrated their ability to remain on the rotating rod for at least 2 min were selected and used for the experiment. FLA was observed at 30, 60 and 90 minute in group-I (control), group- II (diazepam 2 $mg.kg^{-1}$, i.p) and III, IV and V respectively administered (*p.o*) with HACI @ 200, 400 and 800 $mg.kg^{-1}$ body weight. After administration of appropriate treatments, the mice were placed on the rotating rod and the *'fall- off'* time was recorded at regular intervals of 30, 60, and 90. The '*fall off*' time (seconds) after HACI administration in each group was considered for evaluation.

Analgesic activity in mice*:* Eddy's hot plate method described by Turner (1965) was used to determine the analgesic activity in mice. The animals were dropped gently on a plate maintained at 53±0.5°C. Reaction time was taken as the interval between the instant the animal reaches the hot plate till animal shows acute discomfort like licking of forepaws, kicking with hind legs, jumping or frisking of the enclosure. Measurements were carried out

before '0' and after 30, 60 and 90 minutes after oral administration of HACI at the dose rate of 200, 400 and 800 mg.kg^{-1} body weight respectively. The control group was given acetyl salicylic acid (10 mg.kg^{-1}). Analgesic activity was considered to be adequate if the reaction time crossed 15 seconds in the treated mice.

Statistical analysis: The values of the experimental were expressed as mean ± S.E. with 'n' equal to number of animals. The data were analyzed by one way ANOVA followed by Duncan's multiple comparison *post-hoc* test using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Difference when $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical analysis of HACI has been tabulated in Table 1. The phytochemical investigation of *Clerodendrum inerme* (L) showed the presence of alkaloid, flavanoids, triterpenoids, tannins, carbohydrates, and absence of glycosides, sterols and saponins in the hydroalcoholic extracts.

The effect of hydro-alcoholic extract of *Clerodendrum inerme* (L) has showed significant ($p<0.05$) decrease in spontaneous motor activity (Fig. 1) at dose of 400 and 800 mg.kg^{-1} after 30 min and 60 min dosing as compare to control and the animal received the dose of 200 mg.kg^{-1} of hydro-alcoholic extract.

The effect of HACI on forced locomotor activity is presented in Fig. 2. Oral administration of HACI showed significant reduction in fall off time on rotating rod after 60 min at dose of 800 mg.kg^{-1} as compared to control group and diazepam group ($p<0.05$) but there was no significant difference between the 200 and 400 mg.kg^{-1} dose when compare to the control and standard group. The effect of HACI on analgesic activity is depicted in Fig. 3. At dose level of 400 or 800 mg.kg^{-1} the pain threshold was significantly ($p<0.05$) reduced as compared to control group after 60 min or later following HACI treatment. Thus, HACL did not possess analgesic activity at the tested dose levels.

In the present study, the phytochemical analysis of HACI showed the presence of alkaloid, flavanoids, triterpenoids and tannins which may be responsible for various pharmacological activities on central nervous system. Presences of such phytoconstituents were also reported on subjecting the extract of stems of *Clerodendrum multiforum* for phytochemical analysis. HACI significantly ($p<0.05$) influenced voluntary motor activity of CNS in the experimental animals. Further, HACI also significantly inhibited ($p<0.05$) involuntary locomotor activity at a dose level of 800 mg.kg^{-1}, and the effects were comparable to standard reference compound (diazepam) employed. Thus, HACI can influence the muscle relaxant activity or involuntary motor activity. Such CNS related activities were also observed in *Clerodendrum phlomidis* showing tranquillizing, CNS depressant, muscle relaxant and psychopharmacological effects in experimental mice and rats (Murugesan *et al*., 2001).

Further, the CNS related activity observed in the present study may be related to the interaction of the phytoconstituents with central adrenergic, histamine and GABA receptors suggesting synergestic effects as observed with *Clerodendrum mandarinorum* (Zhu *et al.*, 1996).

Pain is an integral part of the inflammation and role of prostaglandins is well established. It has been reported that *Clerodendrum trichotomum* possesses anti inflammatory activity by inhibiting the release of histamine, arachidonic acid and prostaglandin-E_2 *in vitro* and essentially due to the presence of phenyl propanoid glycosides (Lee *et al.*, 2006). Further, the alcoholic extract of roots of *Clerodendrum serratum* showed significant anti-inflammatory activity in carrageenan and cotton pellet model in experimental mice, rats and rabbits (Narayanan *et al*., 1999). However in our study analgesic activity was assessed by Eddy's hot plate method, which essentially derives centrally mediated analgesic activity. However, in our study, HACI did not show analgesic activity *in vivo* at the tested dose levels in the Eddy's hot plate assay. On the contrary it significantly ($p<0.05$) altered the pain threshold, thus there might be alterations in neurotransmitters in the CNS to cause decreased pain threshold.

To conclude, there exists a vast scope for isolation of active principles in *Clerodendrum inerme* (L) and to exploit its activities on central nervous system and to validate folklore claim for various traditional uses.

Table 1: Qualitative analysis of hydroalcoholic extract of *Clerodendrum inerme* (L.)

Test for phytoconstituents	Result
Alkaloid	+
Glycosides	-
Flavonoids	+
Sterols	-
Triterpenoids	+
Tannins	+
Saponins	-

Note: '+' = Present; '-'= Absent

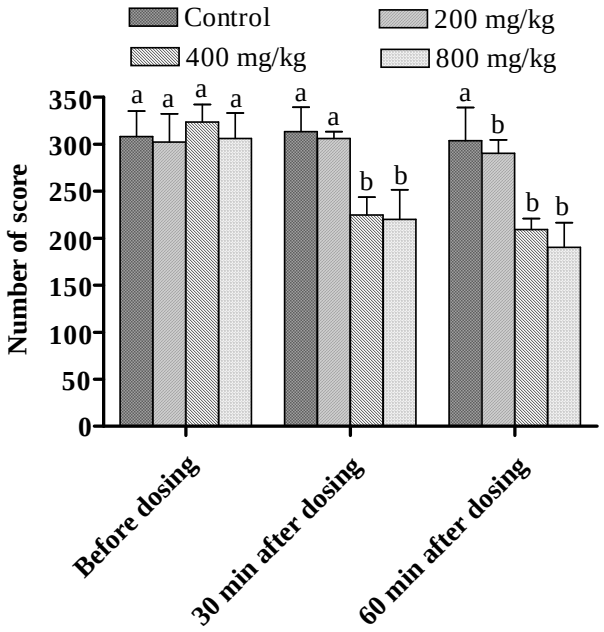

Fig. 1: Effect of hydroalcoholic extracts of *Clerodendrum inerme* (L.) on spontaneous locomotor activity

[Values in the same column (mean± SE; n=6) bearing different superscripts vary significantly (p<0.05) in Duncan's multiple comparison post-hoc test]

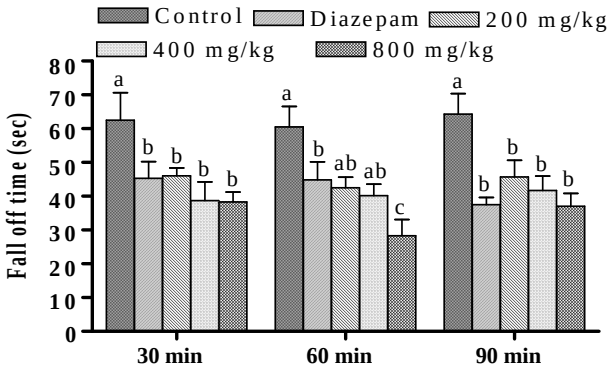

Fig. 2: Effect of hydroalcoholic extracts of *Clerodendrum inerme* (L.) on forced locomotor activity in mice [*Values in the same column (mean± SE; n=6) bearing no common superscripts vary significantly (p<0.05) in Duncan's multiple comparison post-hoc test*]

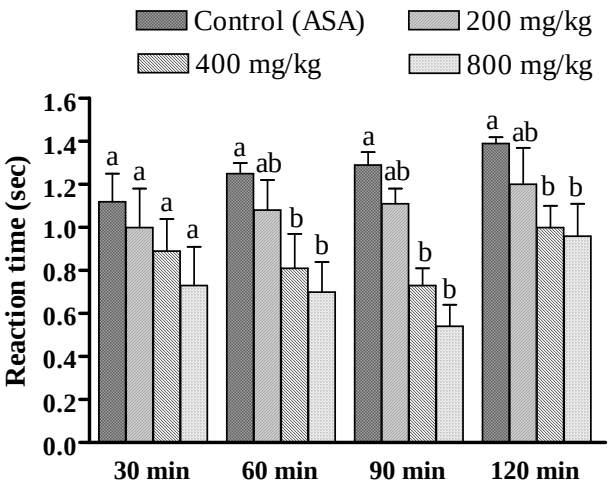

Fig. 3: Effect of hydroalcoholic extracts of *Clerodendrum inerme* on analgesic activity

[*Values in the same column (mean± SE; n=6) bearing no common superscripts vary significantly (p<0.05) in Duncan's multiple comparison post-hoc test*]

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Effect of Aflatoxin and Mycoplasmal Organisms on Serum Biochemical Parameters in Broiler Chicken*

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ABSTRACT

To evaluate the individual and interactive effect of aflatoxin and mycoplasmal infection in broiler chicken, day old birds were divided into four equal groups of 36 birds each and subjected to the following treatments. Group I, fed with normal feed throughout the experiment was considered as control group. Group II was fed with 0.5 ppm of aflatoxin in feed from Day 1 to Day 35. Group III was fed with normal feed and inoculated with $2.4X10^8$ cfu of mycoplasmal organisms (MG) on Day 14. Group IV was fed with 0.5 ppm of aflatoxin in feed from Day 1 and inoculated with mycoplasmal organisms on day 14. In Group II and IV birds biochemical parameters like alanine aminotransferase, aspartate aminotransferase were increased and total protein was decreased due to hepatotoxic effect of aflatoxin. Group III and IV showed gradual decrease in albumin globulin ratio when compared to Group I and II due to production of antibodies (globulins) against mycolasmal organisms. However higher AG ratio in Group IV than Group III suggested decreased immunocompetance of AF fed birds.

Key words: Aflatoxin, mycoplasmal organisms, serum biochemical parameters, broiler chicken

Aflatoxicosis and mycoplasmosis are two important diseases encountered by the poultry industry commonly in field conditions. These diseases are of economic importance since they involve high mortality and morbidity, high cost of treatment, low weight gains of survived birds, low egg production leading to lower profit margins. The present study was undertaken to study the individual and combined effect of aflatoxin in low dose and mycoplasmal organisms in broiler chicken with an effort to mimic the aflatoxicosis and mycoplasmosis similar to that of their natural occurrence in the field and poultry farms thus providing insight of the pathology of the mentioned diseases which in turn can help in formulating better feed and treatment.

MATERIALS AND METHODS

For the production of aflatoxin, spores of *Aspegillus parasiticus* culture in sterile rice the method described by Shotwell *et al*. (1966) was adopted. For inoculation of mycoplasmal organisms the stock culture of *Mycoplasma gallisepticum* procured from Veterinary College and Research Institute, Namakkal was subcultured and utilized.

One hundred and fifty day old broiler chicken were divided into four groups. Group I was kept as control and was fed with normal healthy feed, Group II was fed with feed containing AF (0.5 ppm). Group III was fed with normal feed and inoculated with mycoplasmal organisms at the rate of 2.4 X 10^8 cfu/

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bird through I/N route on 14th day. Group IV was fed with AF contaminated feed at the rate of 0.5ppm from Day 1 to 35 and on 14th day mycoplasmal organisms were inoculated at the rate of 2.4 X 10^8 cfu/bird through I/N route. Six birds from each group were weighed and sacrificed at weekly intervals for five weeks followed by blood collection and detailed PM examination. Serum separated was subjected for analysis of biochemical parameters like AST, ALT, TP and AG ratio.

RESULTS AND DISCUSSION

Serum biochemistry

Alanine amino transferase (ALT)

The mean (±SE) values of ALT for Group I to IV were 28.21±0.38, 41.81±0.89, 30.57±0.48 and 43.93±0.52 respectively at the end of the experimental study.

In the present study (Table 1 and Fig. 1), birds treated with aflatoxin showed a significant increase in the serum ALT activity as compared to AF untreated groups (control and mycoplasma infected).

An increase in the serum ALT levels in aflatoxicosis has been reported by earlier workers (Johri and Sadagopalan, 1989; Shivappa, 2005). The increase in the serum ALT levels can be attributed to hepatic degeneration and subsequent leakage of enzymes into the blood. (Dafalla *et al*., 1987).

Concomitant infection of mycoplasma with aflatoxicosis did not show any significant interaction. This was evident by the serum ALT values which showed no marked variation compared to that of aflatoxin treated group.

Aspartate amino tranaferase (AST)

The mean (±SE) AST values in birds of different groups, at the end of fifth week were 189.33±26.62, 304.9±10.02, 242.48±19.55 and 279.45±25.34 for Group I to IV respectively.

In the present study (Table 2 and Fig. 2), a significant increase in the serum AST values was observed in the AF fed birds (Group II and IV) when compared to control and mycoplasma challenged birds. The findings are well supported by reports of Prashant (2004).

The increase in the serum AST levels is due to hepatocyte degeneration and subsequent leakage of enzyme into the blood stream (Dafalla, 1987)

Serum AST values of mycoplasma infected group revealed that mycoplasmosis alone did not show any effect on liver. Birds with concomitant infection of mycoplasma with aflatoxicosis showed similar mean AST values as that of aflatoxin treated birds which revealed a non significant interaction between these pathological conditions.

Total proteins

The mean (±SE) total serum protein values of Group I to IV birds were 3.77±0.08, 2.31±0.05, 3.75±0.07 and 2.26±0.08 respectively at the end of fifth week (Table 3 and Fig. 3) .

A reduction in serum total proteins in toxin fed birds has been reported to be due to covalent binding of AF metabolites to template DNA and thus inhibition of protein synthesis leading to hypoproteinemia (Hilton *et al*., 1989) and inhibition of protein synthesis in aflatoxicosis as due to disaggregation of polysomes, thus interfering with RNA transcription step of protein synthesis (Godoy *et al*., 1976).

There was no significant difference in the serum total protein in mycoplasma infected birds and control up to the end of fourth week. The minor increase observed at the last week could be attributed to the production of antibodies as a response of host defense mechanism against the mycoplasmal infection. The production of antibodies to mycoplasmal antigen as early as 11 dpi reported by David *et al*. (1997) substantiates the finding of present study.

The serum total protein values of birds with concomitant infection of mycoplasmosis with aflatoxicosis showed no significant interactions while the absence of increase in the values of TSP in the last week compared to mycoplasma infected birds could be attributed to the immunosuppressive effect of aflatoxin by lymphocytolytic activity.

Albumin: globulin ratio

Mean albumin to globulin ratio in birds of Groups I to IV were 2.34±0.17, 2.71±0.18, 1.60±0.05 and 2.21±0.24 respectively at the end of fifth week.

In the present study (Table 4 and Fig. 4) albumin to globulin ratio in the control birds remained unaltered throughout the experiment. The gradual decrease in the albumin to globulin ratio in the mycoplasma challenged birds could be attributed to the increase in globulin portion contributing to the production of antibodies against the antigenic stimulation by mycoplasmal antigen.

The birds treated with AF treatment showed comparatively higher AG ratio than control but remained unaltered significantly throughout the experiment. The increase in the AG ratio might be due to relative decrease in the production of globulin portion attributed to lymphocytolytic activity of AF resulting in immunosupression (Liu *et al*., 2002)

The gradual decrease in the AG ratio of birds with concomitant infection of mycoplasma with aflatoxicosis by the end might be attributed to the production of antibodies due to antigenic stimulation on the 14th day similar to mycoplasma challenged birds. However the ratio remained higher than that of Group III birds suggesting comparatively less immunoglobulin production due to the effect of AF leading to lack of immunocompetance.

CONCLUSION

It can be concluded that aflatoxicosis causes hepatotoxicity leading to considerable reduction in the performance of broiler birds by decreasing the immunocompetence and increasing susceptibility to diseases. Aflatoxicosis thus favours the rapid progression and increased severity of opportunistic infectious diseases like mycoplasmosis leading to unprofitable poultry farming.

Table 1. Mean (±SE) serum ALT in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection

Groups	Age in weeks				
	1	2	3	4	5
I (Control)	24.01±0.77^{a}	25.68±0.52^{a}	26.99±0.52^{a}	27.75±0.43^{a}	28.21±0.38^{a}
II (AF)	26.97±0.50^{b}	30.08±0.58^{b}	31.85±0.91^{b}	36.23±0.81^{b}	41.81±0.89^{b}
III (MG)	23.73±0.49^{a}	25.49±0.48^{a}	27.49±0.42^{a}	28.73±0.24^{a}	30.57±0.48^{c}
IV (AF and MG)	27.04±0.63^{bd}	30.29±0.92^{bd}	33.80±0.60^{bd}	38.32±0.50^{bd}	43.93±0.52^{bd}

Means bearing any one common superscript within columns do not vary significantly ($p<0.05$)

Table 2. Mean (±SE) serum AST in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection

Groups	Age in weeks				
	1	2	3	4	5
I (Control)	174.23±26.61	188.4±28.86^{a}	247.58±10.26^{a}	236.87±22.39^{a}	189.33±26.62^{a}
II (AF)	224.88±15.90	274.23±16.57^{b}	323.02±18.28^{b}	305.53±14.00^{b}	304.9±10.02^{b}
III (MG)	211.13±11.83	188.9±30.61^{a}	244.5±16.13^{d}	263.23±18.62^{a}	242.48±19.55^{a}
IV (AF and MG)	198.47±20.62	267.10±19.69^{cb}	351.30±25.98^{cb}	308±17.74^{cb}	279.45±25.34^{cb}

Means bearing any one common superscript within columns do not vary significantly ($p<0.05$)

Table 3. Mean (±SE) serum total protein in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection

Groups	Age in weeks				
	1	2	3	4	5
I (Control)	3.36±0.10	3.36±0.07^{a}	3.57±0.07^{a}	3.70±0.11^{a}	3.75±0.07^{a}
II (AF)	3.07±0.15	2.83±0.11^{b}	2.74±0.12^{b}	2.39±0.05^{b}	2.26±0.08^{b}
III (MG)	3.29±0.10	3.43±0.07^{a}	3.52±0.11^{a}	3.67±0.16^{a}	3.77±0.08^{a}
IV (AF and MG)	3.10±0.11	2.78±0.05^{c}	2.62±0.12^{c}	2.49±0.12^{c}	2.31±0.05^{c}

Means bearing any one common superscript within columns do not vary significantly ($p<0.05$)

Table 4. Mean (±SE) serum albumin to globulin ratio in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection

Groups	Age in weeks				
	1	2	3	4	5
I (Control)	2.22±0.19	2.26±0.15	2.24±0.13^{a}	2.29±0.11^{a}	2.34±0.17^{a}
II (AF)	2.43±0.17	2.46±0.16	2.59±0.15^{a}	2.64±0.25^{a}	2.71±0.18^{a}
III (MG)	2.07±0.10	2.20±0.06	2.11±0.09^{c}	1.77±0.05^{c}	1.60±0.05^{b}
IV (AF and MG)	2.35±0.13	2.37±0.12	2.43±0.18^{c}	2.26±0.17^{c}	2.21±0.24^{a}

Means bearing any one common superscript within columns do not vary significantly ($p<0.05$)

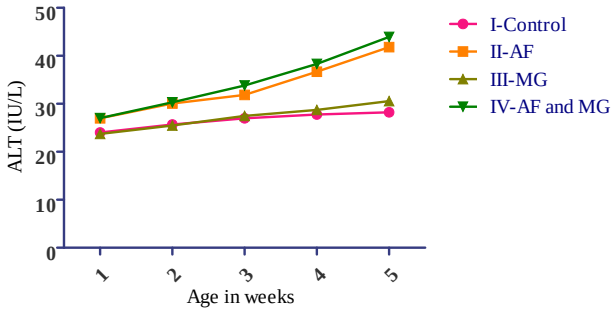

Fig. 1. Mean serum alanine amino transferase (IU/L) in different groups of broiler chicks at different age intervals in the experimental study of aflatoxicosis and mycoplasmal infection.

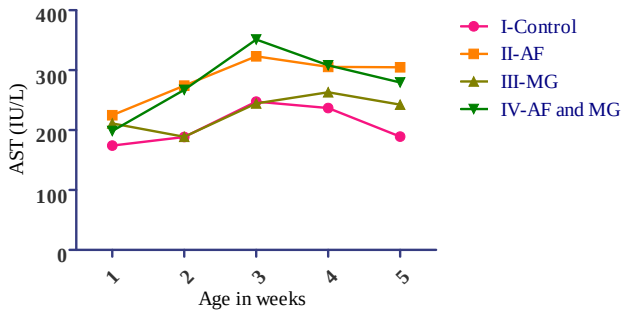

Fig. 2. Mean serum aspartate amino transferase (IU/L) in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection.

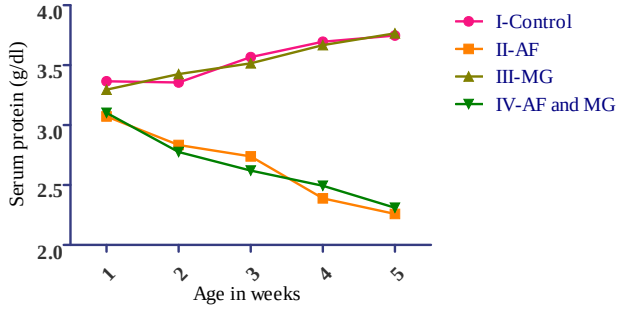

Fig. 3. Mean serum protein (g/dl) in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection.

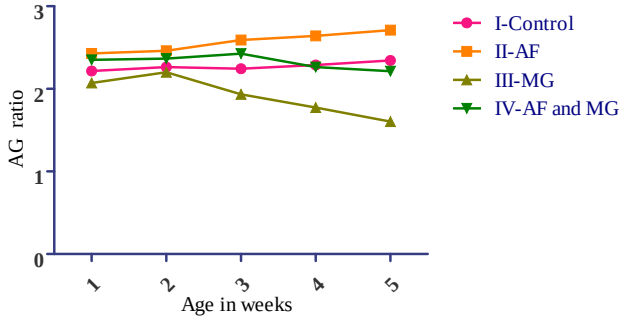

Fig. 4. Mean serum albumin to globulin ratio in different groups of broiler chicks at different age intervals in experimental study of aflatoxicosis and mycoplasmal infection.

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Factors Affecting Peak Yield in Frieswal Crossbred Cattle*

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ABSTRACT

Data on Frieswal cattle of two military dairy farms was utilized to study the influence of various genetic and non-genetic factors on Peak Yield (PY). The overall mean first lactation peak yield obtained in the present study was 13.13±0.21 kg. Farm and period of calving were found to have significant effect on PY-1. Higher mean PY-1 of 13.83±0.23 kg was observed in cows calved at Secunderabad farm, while it was 12.89±0.25 kg at Belgaum farm. The highest mean peak yield values observed in cows calved during P-1 (1994-1999), P-3 (2005- 2009) and P-2 (2000- 2004) periods were 13.73±0.56, 13.50±0.21 and 13.44±0.27 kg, respectively. Differences among these three periods were non-significant. PY-I of cows calved during P-4 period (2010–2013) was significantly lower (11.86±0.20 kg) compared to other three periods. Present investigation revealed that, PY-1 of Frieswal cows varied significantly with the period of calving and farm. PY of recently born Frieswal cows had significantly lower PY than the cows born during the earlier periods.

Key words: PY, Frieswal, least squares, period, season

The Peak Yield (PY) is the maximum quantity of milk produced on any one particular day during the ascending phase of lactation following calving. It is one of the most striking economic traits of dairy cows. It determines the lactation yield, lactation length and shape of lactation curve (Raheja and Balaine, 1982). Although, the farmers in India do not maintain milk records, they most often remember the PY of their cows and rely upon it as a good indicator of cow's milking potential. The research data also indicated that there is very high genetic and phenotypic relationship between PY and total lactation yield of cows. Therefore, it is used as a selection criterion particularly under field conditions (Jahageerdar, 1989). The performance of any organized dairy farm should be studied at regular intervals to breeding and managemental protocols. The present was, thus, undertaken to analyse the records pertaining to two Military Dairy Farms,

MATERIALS AND METHODS

The data for present investigation were collected from history sheets and daily milk yield records of Frieswal Crossbred Cattle maintained at Military Dairy Farms (MF) Belgaum and Secunderabad. The records on production performance of Frieswal Crossbred Cattle at the two farms spread over a period of 20 years (1994-2013) were collected. Frieswal cows calved during the period from 1994 to 2013 were included in the present study. The effect of periods was studied by classifying the data into four periods of approximately five consecutive years duration. The period was classified according to the year of calving as first (1994-1999), second (2000-2004), third (2005-2009) and fourth Periods (2010-2013). Each year was subdivided into three seasons based on meteorological observations *viz*, summer (February to May), rainy (June to September) and winter (October to January) seasons to study the effect of season of calving. The

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data were classified into three genetic groups *viz*, G1 (á50% HF inheritance + ñ50% Sahiwal), G2 (62.50% HF inheritance+ 37.50% Sahiwal) and G3 groups (ñ75% HF inheritance+ á25% Sahiwal), to analyze the differences due to genetic groups.

The influence of various genetic and non-genetic factors on PY was studied by least squares analysis of variance for unequal non-orthogonal data using the technique described by Harvey (1966).

The statistical model assumed was

$$Y_{ijkmn} = \mu + S_i + P_j + F_k + Gm + e_{ijkmn}$$

Where,

Y_{ijkmn} = Dependent trait (PY) of n^{th} cow calved in i^{th} season, j^{th} period at k^{th} Farm and m^{th} genetic grade

μ = Over all mean

S_i = Effect of i^{th} season

P_j = Effect of j^{th} period

F_k = Effect of k^{th} farm

G_m = Effect of m^{th} genetic grade

e_{ijkmn} = Random error, assumed to be normally and independently distributed with mean zero and constant variance *i.e.* NID (0, $ó_e^2$).

Duncan's multiple range test as modified by Kramer (1957) was used for testing differences among least squares means (using the inverse coefficient matrix).

RESULTS AND DISCUSSION

The computed overall least squares mean for PY-1 based on 1298 observations was 13.13±0.21 kg (Table 1). The present estimate is comparable with the reports of Deshpande and Bonde (1981), Tomar and Tomar (1982), Mukherjee (2005) and Gaur *et al.* (2007) who reported values ranging from 12.76±0.19 to 14.05±0.21 kg in HF x Sahiwal crossbreds/ Frieswal herds at different locations of the country. However, the present value is less than the values recorded in Frieswal cattle by Gaur (2001) and Anon, (2013-14; 2014-15) in HF x Sahiwal/ Frieswal cattle and whose values ranged from 15.06±0.28 to 15.18 kg. The estimated mean PY-1 is much higher than the means reported by Kaul *et al.* (1977) in HF x Hariana, Deshpande and Bonde (1981), Tomar and Tomar (1982) in HF lower crosses which were in the range of 8.83±0.04 to 10.44 kg.

Least squares analysis of variance for first lactation peak yield revealed significant (pá0.05) influence of farm and period of calving and non-significant influence of season of calving as well as genetic grades (Table 2).

The mean values for PY-1 of Frieswal cows in first, second and third genetic groups were 13.41±0.46, 13.26±0.26 and 12.73±0.17 kg respectively. However, differences in mean PY-1 values among different genetic grades were non-

Table 1: Least squares means of PY-1 in frieswal crossbred cattle

Factors	PY-1	
	No.	**Mean ± SE**
Overall	1298	13.13±0.21
Farms		
Belgaum	480	12.89±0.25[a]
Secunderabad	818	13.83±0.23[b]
Period of birth		
1994-1999 (P1)	44	13.73±0.56 [b]
2000-2004 (P2)	258	13.44±0.27 [b]
2005-2009 (P3)	527	13.50±0.21 [b]
2010-2013(P4)	469	11.86±0.20 [a]
Season of birth		
Summer (S1)	501	13.24±0.46[a]
Rainy (S2)	301	12.73±0.17[a]
Winter (S3)	496	13.22±0.25[a]
Genetic grade		
<50%HF (G1)	70	13.41±0.46[a]
=62.5%HF (G2)	965	12.73±0.17[a]
>75%HF (G3)	263	13.26±0.26[a]

Note: LS means bearing different superscripts within columns in sub groups differ significantly at p<0.05

significant. The present finding of non-significant influence of genetic groups on PY-1 agrees with the reports of Narayanaswamy (1981), Parmar *et al*. (1986) and Krishniah *et al.* (1987). However, Kaul *et al.* (1977), Rao and Sundaresan (1978), Arora and Desai (1979), Deshpande and Bonde (1981), Tomar and Tomar (1982), Tajane (1987) and Mukherjee (2005) reported significant effect of genetic groups on PY-1.

Table 2: Least squares analysis of variance (mean squares only) for PY-1 in Frieswal crossbred cattle

Source of variation	PY-1
Farm	69.54* (1)
Period	255.14* (3)
Season	10.30 (2)
Genetic grade	39.14 (2)
Error	13.27
R^2- value (%)	4.90

Note: Figures in parentheses indicate the degrees of freedom

* Significant at pá0.05

Farm had significant effect on PY.

Higher PY-1 mean of 13.83±0.23 kg was observed in cows at Secunderabad farm than that of 12.89±0.25 kg at MF, Belgaum. The variation in PY-1 in Frieswal cows maintained at two farms might be due to the differences in climatic conditions owing to different geographical location of the farms and other managemental factors as per the situation of the two farms. This is in concurrence with the reports of Rao and Sundaresan (1978), Deshpande and Bonde (1982), Mukherjee (2005), Gaur *et al.* (2007) and Anon. (2014-15) in Frieswal cattle.

Period of calving had significant influence on PY-1

The highest mean first peak yield estimates of 13.73±0.56, 13.50±0.21 and 13.44±0.27 kg were observed in cows calved during 1994- 1999, 2005-2009 and 2000-2004 periods respectively. Differences among these three periods were non-significant. PY-1 of cows calved during later period (2010-2013) was significantly lower (11.86±0.20 kg) compared to other three periods. Similar findings of significant influence of year/ period of calving on PY-1 were reported by Rao and Sundaresan (1978), Deshpande and Bonde (1982), Parmar *et al*. (1986), Tajane (1987), Sharma *et al.* (1996), Gaur (2001), Mukherjee (2005), Gaur *et al.* (2007) and Anon. (2014-15) in HF x Sahiwal crossbreds. This period effect in daughters may be due to genetic variations caused by different sires used during different periods in addition to the environmental changes over the years which led to severe stress and strain on the animals.

The mean PY-1 values of 13.24±0.46, 13.22±0.25 and 12.73±0.17 kg was observed in cows calved during summer (S-1), winter (S-3) and rainy seasons, respectively. However, the differences in the peak yields of cows calved during the three seasons were non-significant. Similar non-significant effect of season of calving on PY-1 was also reported in HF x Sahiwal crossbreds (Singh *et al.*, 1981; Tajane, 1987; Mukherjee, 2005). However, reports of Rao and Sundaresan (1978), Parmar *et al*. (1986), Gaur (2001), Mukherjee (2005) and Gaur *et al*. (2007) indicated significant differences in PY-1 due to season of calving.

CONCLUSION

The present study revealed the significant effect of farm and period of calving on the first lactation peak yield in Frieswal cows maintained at MFs Belgaum and Secunderabad. However, the effects of season of calving and genetic grades on first lactation peak yield were found be non-significant. The variations in the present and earlier reports might be attributed to differences in size of the data, type of analyses, and due to the prevalent management conditions at the different farms.

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Nutritional Evaluation of Common Weeds of Northern Dry Zone of Karnataka in Osmanbad Goats

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ABSTRACT

Four weeds *viz., Phyllanthus maderaspatensis*, *Corchorus trilocularis*, *Alycicarpus longifolius* and *Panicum repens* were evaluated for their nutritional quality in osmanbad goats. The CP level in all four weeds ranged from 9.33 to 15.05%. Higher intake of fresh leaves, DM and CP was noticed in *Alycicarpus longifolius* fed group. Digestibility of DM, OM, CP, EE and NFE were significantly higher (P<0.01) in *Phyllanthus maderaspatensis* fed group except CF. The DCP and TDN content of *Phyllanthus maderaspatensis*, *Corchorus trilocularis*, *Alycicarpus longifolius* and *Panicum repens* were 9.96 and 69.47, 9.38 and 68.80, 11.52 and 68.89, 6.83 and 54.94% respectively. It can be concluded that even though *Alycicarpus longifolius* and *Phyllanthus maderaspatensis* were found to be better in all respect, other weeds also serve as better feed resources for grazing ruminants.

Key words: Composition, digestibility, goats, nutrient density, weeds.

Small ruminants are the additional source of income for supporting most of the families of landless and small farmers who depend entirely on grazing to provide nutrients to them. The varied species of cereal and legume weeds available in the grazing land particularly after onset of monsoon are potentially good source of fodder which are rich in energy and protein (Kallah *et al.*, 2000). These weeds can meet the nutrient requirements of all purposes of small ruminants during entire grazing period as they are available plenty in the natural grazing land. But all the species of weeds may not be equal in palatability and their nutrient composition which invariably cause preferential grazing in small ruminants. Hence, an attempt was made to evaluate some most preferred weeds for their palatability, nutrient digestibility and density in Osmanabad goats.

MATERIALS AND METHODS

Most abundantly, commonly available and preferred weeds namely *Phyllanthus maderaspatensis*, *Corchorus trilocularis*, *Alycicarpus longifolius* and *Panicum repens* of Northern dry zone of Karnataka were collected from the grazing land of about 158 hectors belong to Regional Agricultural Research Station, Bijapur for the study.

Description of the weeds:

1. *Phyllanthus maderaspatensis*: Belongs to Euphorbiaceae family, perennial herb, thrives in tropical regions and used as fodder, dominant weed in sorghum (Kandasamy, 2000), sugar cane (Honyal, 1997) and cotton (Sreenivas, 2000).

2. *Corchorus trilocularis*: It belongs to Malvaceae family, wild jute branched annual herb, and the young tender leaves are cooked and eaten.

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3. *Alycicarpus longifolius*: It belongs to Leguminasiae family, grows in residual soil moisture during the dry season, provides green forage when most associated forages have dried up (Kallah, *et al.*, 2000). Its seeds are reported to be a famine food (Cook *et al.*, 2005). Suitable for semi-arid regions (Njarui, 2004).

4. *Panicum repens*: It comes under Graminae family, widespred in marshy areas, tolerates flooding and drought, perennial and palatable pasture grass resistant to grazing and trampling.

Phyllanthus maderaspatensis
Family: Euphorbiaceae (Kannad:Malamandi)

Twelve Osmanbad goats (14-19 kg B.wt., 6-8 month of age) were divided into four groups of three each. Each group was housed in a separate pen. W1, W2, W3 and W4 groups were fed *adlibitum* fresh weeds of *Phyllanthus maderaspatensis, Corchorus trilocularis, Alycicarpus longifolius* and *Panicum repens* respectively as sole feed without any supplementary feed. Daily fresh weeds intake were recorded. The digestion trial was conducted for five days at the end of the experiment. The feeding trial of 28 days comprised one week adjustment period, two weeks observation period and last one week collection period. The fresh weeds and fecal samples were analyzed for proximate principles (AOAC, 2000). The data was subjected to statistical analysis (Snedecor and Cochran, 1968).

Corchorus trilocularis
Family: Tiliaceae (Kannada: Chinchili)

Alycicarpus longifolius
Family: Leguminosae (Kannada: Alabu)

Panicum repens
Family: Graminae or Poaceae, Kannada**:** Simpiganullu)

RESULTS AND DISCUSSION

The chemical composition of four weeds (Table 1) revealed that CP was higher in *Alycicarpus longifolius* (15.05%) and lowest in *Panicum repens* (9.33%) whereas *Phyllanthus maderaspatensis* , *Corchorus trilocularis* had equal level of CP (13.0%). The variation in the CP level among the weeds was due to their species difference as *Alycicarpus longifolius* belonged to Leguminasiae family, and contain higher CP than *Panicum repens* which is perennial grass from Graminae family. The total ash content was higher in *Panicum repens* followed by *Phyllanthus maderaspatensis, Corchorus trilocularis* and *Alycicarpus longifolius*. Kallah *et al*. (2000) reported higher CP (15.05 v/s 16.4%) and TA (7.24 v/s 9.90) values for *Alycicarpus longifolius* weed and Kuo (1967) found similar trend for *Panicum repens* with regard to CP level (9.33 v/s 10.9) but not for TA (20.6 v/s 10.9) level. Assaeed *et al.* (1995) noticed lower CP and higher TA content in *Corchorus trilocularis* weed than the values reported in this study.

The fresh weeds intake was significantly higher (Pá0.01) in *Alycicarpus longifolius* fed group followed by *Phyllanthus maderaspatensis, Corchorus trilocularis* and *Panicum repens* fed groups. Similarly, significantly higher (Pá0.01) intake of DM, OM, CP, EE and CF were noticed in *Alycicarpus longifolius* and *Phyllanthus maderaspatensis* fed groups both on live weight and per cent body weight basis (Table 2) when compared to other weeds fed groups. Patel *et al*. (2000) reported similar findings with regard to *Alycicarpus longifolius* weed fed to cattle where green alfalfa was replaced to provide 50% of protein requirement without any adverse effect on live weight gain. Cook *et al*. (2005) also found similar results when 40% of CP requirement was met through *Alycicarpus longifolius* weed.

The digestibility of DM, OM, CP, EE and NFE except CF was significantly higher (Pá0.01) in *Phyllanthus maderaspatensis* fed group (Table 3) which were far higher than the values reported for *Alycicarpus longifolius* and *Panicum repens* weeds by Kuo, (1967) and Kallah *et al*. (2000) where they have reported 58.7 and 58.8% OM digestibility in ruminants respectively. Assaeed *et al*. (1995) noticed similar in vitro DM digestibility of 78.7% as against 78.4% in vivo digestibility reported in this study. The DCP and TDN contents of all the weeds ranged from 6.83 to 11.52% and 54.94 to 69.47%, respectively being significantly highest DCP in *Alycicarpus longifolius* and lowest in *Panicum repens* (Table 3) whereas TDN level was significantly higher (P<0.01) in *Phyllanthus maderaspatensis, Corchorus trilocularis* and *Alycicarpus longifolius* weeds than in *Panicum repens* weed. Kuo (1967) and Kallah *et al*. (2000) reported 53.64% TDN (8.1 MJ/Kg DM) for *Alycicarpus longifolius* and *Panicum repens* weeds and Aseeaed *et al.* (1995) reported lower TDN value (65.17%) for *Corchorus trilocularis* weed than the value reported in this study (75.71%). The DM, DCP and TDN received through weeds were more than the requirement (150g gain/day). The DM, DCP and TDN intake of goats of all four groups ranged

from 1.11 to 1.40, 1.3 to 2.7 and 1.1 to 1.6 times of the requirement respectively (Table 4).

Hence, it was concluded that among four weeds, *Phyllanthus maderaspatensis*, *Corchorus trilocularis* and *Alycicarpus longifolius* weeds were found to be better in palatability and nutritive value than *Panicum repens.* However, all fresh weeds alone were able to meet the requirement at least 1 to 1.6 times more than the usual requirement of goats without any supplementation.

Table 1: Chemical composition (% on DMB) of weeds fed to experimental goats

Particulars	W1	W2	W3	W4
OM	89.08	90.14	92.76	79.40
CP	13.03	13.10	15.05	9.33
CF	29.01	22.60	43.02	29.40
EE	1.95	2.69	2.65	1.24
NFE	45.09	51.75	32.04	39.43
TA	10.92	9.86	7.24	20.60

W1- *Phyllanthus maderaspatensis*, W2 - *Corchorus trilocularis*, W3 - *Alycicarpus longifolius* W4 - *Panicum repens*

Table 2: Average intake of nutrients (kg/d) of experimental goats.

Particular	W1	W2	W3	W4	SEM	P value
Fresh leaves (kg/d)**	4.25^{bc}	4.13^{b}	4.50^{d}	3.80^{a}	0.068	0.002
100 kg B.wt**	20.24^{a}	18.77^{b}	19.56^{ab}	18.09^{bc}	0.419	0.010
Nutrient intake						
DMI **	1.36^{b}	1.11^{a}	1.43^{b}	1.15^{a}	0.025	0.001
100kg B.wt. **	6.46^{a}	5.02^{c}	6.22^{a}	5.45^{b}	0.073	0.007
OMI **	1.21^{b}	1.0^{a}	1.33^{b}	0.91^{a}	0.042	0.012
100kg B.wt. **	5.75^{a}	4.53^{b}	5.77^{a}	$4.33b^{c}$	0.008	0.003
CPI **	0.18^{c}	0.14^{b}	0.22^{d}	0.11^{a}	0.010	0.001
100kg B.wt. **	0.84^{b}	0.66^{c}	0.95^{a}	0.52^{d}	0.008	0.010
EEI **	0.03^{b}	0.03^{b}	0.04^{b}	0.01^{a}	0.006	0.012
100kg B.wt. **	0.13^{bc}	0.14^{b}	0.17^{a}	0.07^{d}	0.007	0.011
CFI**	0.39^{c}	0.25^{a}	0.62^{c}	0.34^{b}	0.009	0.001
100kg B.wt.**	1.87^{b}	1.13^{d}	2.67^{a}	11.60^{c}	0.041	0.005
NFEI **	0.61^{b}	0.57^{b}	0.46^{a}	0.45^{a}	0.010	0.001
100kg B.wt. **	2.91^{a}	2.60^{b}	1.99^{d}	2.15^{c}	0.042	0.001

*** Pdá0.01, Means with different superscripts in a row differ significantly.*

W1- *Phyllanthus maderaspatensis*, W2 - *Corchorus trilocularis*, W3 - *Alycicarpus longifolius* W4 - *Panicum repens*

Table 3: Average nutrient digestibility (%) and nutrient density (%) of weeds in goats

Particular	W1	W2	W3	W4	SEM	P Value
Nutrient digestibility						
DM**	77.37^{a}	68.41^{d}	71.82^{bc}	73.86^{b}	0.764	0.002
OM**	79.76^{a}	73.60^{c}	76.35^{b}	74.18b^{c}	0.818	0.001
CP**	76.47^{a}	71.57^{bc}	76.56^{a}	73.19^{b}	0.925	0.012
CF**	68.75^{ab}	65.06^{b}	72.53^{a}	67.10^{b}	1.218	0.010
EE**	76.06^{a}	72.88^{b}	78.31^{a}	68.80^{c}	0.723	0.010
NFE**	80.34^{a}	77.89^{a}	67.11^{b}	67.13^{b}	1.092	0.005
Nutrient density						
DCP**	9.96^{b}	9.38b^{c}	11.52^{a}	6.83^{d}	0.328	0.001
TDN**	69.47^{a}	68.80^{a}	68.89^{a}	54.94^{b}	0.818	0.013

** pá*0.01*, Means with different superscripts in a row differ significantly.
W1- *Phyllanthus maderaspatensis*, W2 - *Corchorus trilocularis*, W3 - *Alycicarpus longifolius* W4 - *Panicum repens*

Table 4: Plane of nutrition of experimental goats fed with different weeds.

Group	Body wt., kg	Nutrients supplied, g/day			Nutrients required for 150g gain/day (g) *			Nutrient Intake (No. of times than the requirement)		
		DM	DCP	TDN	DM	DCP	TDN	DM	DCP	TDN
T *Phyllanthus*	21.0	1360	1356	945	974	59.7	583.6	1.40	2.3	1.6
T*Corchorus*	22.0	1110	104	764	1004	60.9	595.7	1.11	1.7	1.3
T*Alycicarpus*	23.0	1430	165	985	1034	62.0	607.8	1.38	2.7	1.6
T*Panicum*	21.0	1150	79	632	974	59.7	583.6	1.18	1.3	1.1

*Requirements for goats (Ranjhan, 1998)

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Nutritional Evaluation of Spineless Cactus (*Opuntia ficusindica*) Based Complete Diets in Sheep*

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ABSTRACT

An experiment was conducted to study the nutritional value of diets based on Spineless cactus (*Opuntia ficusindica*) for adult sheep. In a Switch over design, six rams of 23.5 kg average body weight were selected and divided into two groups of three each and offered two dietary treatments in two periods of six weeks each. Control Group (T1) received diet containing finger millet (*Eleusine coracana*) straw (40%) and a compounded feed mixture based on corn (60%). Group T2 was fed diet comprising FMS (20%) and CFM (30%) with spineless cactus (fed *ad libitum*) replacing 50 per cent of the diet. The spineless cactus contained 91.5 per cent moisture, 7.11 per cent CP, 39.8 per cent NDF and 26.2 per cent ADF. The ME content was 8.82 MJ/kg DM. The intake of dry matter and organic matter was lower in Group T2, fed spineless cactus ($p<0.05$), nevertheless, there was no significant difference in the body weight change (g) between the treatment groups. The digestibility of organic matter was higher in sheep of Group T2 ($p<0.05$), resulting in higher total digestible organic matter with inclusion of cactus in the diet. The voluntary intake of water was significantly lower in Group T2 ($p<0.05$) indicating that spineless cactus could be a potential source of water for sheep. It was concluded that the spineless cactus could be included in the diet of adult sheep replacing 20.5 per cent of the FMS and 22.3 per cent of the CFM, equivalent to 42.8 per cent of the total diet.

Keywords: Spineless cactus, dry matter intake, water intake, nutrient digestibility, average daily gain

The exploration of new feed resources which do not compete with the human food chain continues to be a key mandate of research in sustainable livestock production. Spineless cactus or *opuntia*, a drought resistant plant, provides an option as roughage source for ruminant feeding, with a potential to produce large quantities of green and succulent fodder throughout the year (De Kock, 1980). With an advantage of minimum agronomical input for its propagation, the productivity of spineless cactus has been comparable to most conventional crops and forages. The annual yield of spineless cactus (dry matter) per hectare has been reported to be 10 tonnes in arid zones, 10 to 20 tonnes in semi-arid zones and 20 to 30 tonnes in sub-humid areas under optimum management (Le Houerou, 1992). Costa *et al*. (2012) observed that the succulent pods or cladodes of spineless cactus were palatable to dairy cattle with the reported daily intake of chopped cactus, ranging from 2.5 to 11.0 kg in sheep (Ben Salem *et al.,* 1996; Sirohi *et al.,* 1997 and Costa *et al.,* 2012). The energy content of cladodes of *Opuntia* was reported to be 2600 kcal of digestible energy (DE) per kg dry matter (Shoop *et al.,* 1977). In earlier experiments, Gabremariam *et al*., (2006)

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substituted spineless cactus to the straw in the diet of sheep, while Abidi *et al*. (2009) and Costa *et al*. (2012) replaced cereals such as corn or barley in the diet with spineless cactus. The production responses of sheep in previous studies have been equivocal due to different feeding regimen and plane of nutrition, and hence, the optimum levels of inclusion of cactus in the diet have not been clearly defined. Furthermore, there is paucity of literature in India on the evaluation and utilization of spineless cactus for feeding small ruminants. Therefore, this experiment has been taken up on the nutritional evaluation of the diet based on spineless cactus for adult sheep.

MATERIALS AND METHODS

The study was carried out at the Department of Animal Nutrition, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bengaluru. The feeding trial was conducted at Instructional Livestock Farm Complex (ILFC), Veterinary College, Bengaluru. Permission for using the animals for the experiment was duly obtained from Institutional Animal Ethics committee (IAEC).

Six adult Bannur crossbred rams ranging from 24 to 60 months of age were divided into two groups consisting of three rams each of comparable body weight and age. The feeding trial was carried out in two periods of six weeks each, in a switch over design. Each period comprised of a base line (adjustment) period of two weeks followed by intake measurement period of four weeks. The diet of the experimental rams was made from finger millet straw (FMS), compounded feed mixture (CFM) and spineless cactus. The CFM contained maize (80%) wheat bran (15%), urea (2%) salt (1%) and mineral mixture (2%).The animals in control group (Group T1) were offered a diet comprising 40 per cent finger millet straw and 60 per cent compounded feed mixture while the diet of Group T2 comprised 20 per cent of the FMS and 30 per cent of the CFM along with *ad libitum* feeding of spineless cactus. The diets for the rams were formulated individually and the calculated quantities of ingredients offered to meet the energy and crude protein requirement as per ARC (1984). In order to replace the FMS and CFM by spineless cactus in Group T2, the quantities of FMS (20 per cent) and CFM (30 per cent) in the diet offered was restricted to 50 per cent of the calculated quantities. Fresh spineless cactus cladodes were chopped to an approximate size of three centimeter length and offered *ad libitum*. The Spineless cactus was offered at 08.00, 14.00, and 18.00 hours while FMS was offered at 08.00 and16.00 hours, and the CFM offered at 12.30 and 15.30 hours. Measured quantity of clean drinking water was provided in separate plastic troughs at 07.00 and 14.30 hours of the day. The duration of the trial was 12 weeks. Digestion trial was completed in all animals during the last week of each period, by total fecal collection method (5 days collection).

Standard procedures of AOAC (2005) and Van Soest *et al.* (1991) were used for chemical analyses of feed and the faecal samples. The metabolisable energy content in feed ingredients and diets was determined by *in vitro* incubation for gas production (RIVIGP) according to the procedures described in Menke and Steingass (1988).The experimental data on dry matter and nutrient intake, body weight change and digestibility were subjected to statistical analysis by t-test (unpaired) as per the procedure described in Snedecor and Cochran (1989).Graph Pad Prism (2007, Version 5.01) software was used for the data analysis.

RESULTS AND DISCUSSION

The DM content of spineless cactus was low (8.53 per cent, Table 1), with crude protein (CP) neutral detergent fibre (NDF) and acid detergent fibre (ADF) content of 7.11, 39.8 and 26.2 per cent respectively. The ME content of spineless cactus was 8.82 MJ/kg DM. Similar values of energy and protein were reported in spineless cactus previously by Misra *et al*. (2006) and Ajith *et al*. (2017). The chemical composition and ME values appear to be intermediate to the concentrate and roughage feeds, and therefore the spineless cactus could be considered to replace a part of complete diet in ruminants.

High water content of spineless cactus (91.47%, Table 1) could be expected to provide ample water to sheep to meet the water needs of the body. The mean voluntary intake of water was significantly lower ($p<0.05$) in Group T2 fed spineless cactus (0.618 kg per day) compared to control group (1.24 kg per day) (Table 2). Previously, Ben Salem *et al.* (1996) and Gebremaraim *et al.* (2006) also reported a significant decrease in the voluntary intake of water of sheep with increased level of cactus inclusion in the diet. Therefore, spineless cactus could be valuable as a passable source of water, especially in hot and humid seasons and in situations of draught, to meet the water requirement of sheep. The calculated NDF (about 40%) and ADF (about 24%) content of both the diets were estimated to be adequate to meet the recommended minimum levels for ruminal fermentation (Van Soest, 1994).

The intake of dry matter and organic matter decreased significantly with inclusion of cactus in the diet ($p<0.05$) (Table 2). Sirohi *et al*. (1997) also reported lowered intake of feed dry matter associated with feeding of spineless cactus. Contrary to this, Costa *et al*. (2012) observed an increase in the intake of dry matter and organic matter with the increasing levels of cactus supplementation in the diet of lambs. Although the CP and energy content of spineless cactus was considerably lower, the mean intake of energy and protein exceeded the requirements of ARC (1984) in both the experimental groups (Table 3). The intake of RDP was marginally lower for Group T2 (intake of 30.8g against the requirement of 35.8). Nevertheless, the higher intake of UDP in Group T2 (13.2 g) could be expected to recompense to meet the CP requirement.

Considering the change in body weight of the experimental rams, the average body weight gain (g) per day for T1 and T2 groups was 7.94 and 5.95 respectively (Table 4). Such a marginal gain in body weight indicated a positive energy balance of the diet to meet the maintenance requirement of adult sheep. The mean apparent digestibility (per cent) of nutrients for T1 and T2 groups with respect to DM, OM, CP, NDF and ADF were 68.9 and 76.2; 74.1 and 78.1; 70.8, and 75.2; 59.5 and 63.5; 53.9 and 58.2, respectively (Table 5). The digestibility of organic matter was significantly higher in Group T2, resulting in the higher content of DOMDM (73.3 per cent in group T2 vs. 67.1 per cent in group T1) ($p<0.05$), thus increasing the energy density of the diet.

The overall results of the present study indicated that Spineless cactus (containing 8.82 MJ, ME/kg) could be a potential source of energy to meet the requirement of adult sheep. The low CP content of the spineless cactus (7.11%) resulted in decreased CP content of the diets (9.42 per cent). The calculated rumen degraded protein content of diets (70.1 % of CP) was optimum to meet the requirement. Despite the lowered DMI of T2 group (484 g in T2 vs. 549 g in T1), the ME provided in diet of group T2 was adequate to support the maintenance requirement (ARC, 1984) of adult sheep. The total CP intake was also adequate to meet the requirement (44.1 g intake as against 35.8 g requirement, ARC 1984) yet, the RDP intake was marginally lower (intake of 30.8 g vs. 35.8 g of requirement) (Table 3). This warrants supplementation of NPN or other sources of protein to make up the requirement of RDP, when cactus is included in the diet. The UDP supplied in the diet however, far exceeded the requirement, and could be expected to counter the marginal deficiency of RDP, to meet the total CP requirement. In the present study, the mean intake of fresh spineless cactus by adult sheep (mean body weight of 24.0 kg) was 2.43 kg per day. The DMI as per cent of body weight was 1.05. The digestibility of organic matter was higher in diet T2 resulted in the higher content of DOMDM and thus meet the energy requirement at lowered intake of dry matter (Table 2).

CONCLUSION

Considering the overall performance of the animals in terms of feed intake, digestibility and body weight change, it was concluded that spineless cactus could replace 42.8 per cent of the total diet, substituting FMS (20.5 per cent) and CFM (22.3 per cent) in the diet of adult sheep.

Table 1: Chemical composition (per cent dry matter), energy and protein fractions of dietary ingredients used in the experiment

Parameter	Compounded feed mixture	Finger millet straw	Spineless cactus
Dry matter	89.7	95.2	8.53
Organic matter	95.9	91.4	86.1
Ether extract	3.28	1.26	3.01
Total ash	4.10	8.60	13.9
Neutral detergent fibre	22.0	75.3	39.8
Acid detergent fibre	8.50	44.5	26.2
Energy and protein fractions			
ME[1] (MJ per kg DM)	13.1	7.42	8.82
Crude protein (per cent)	15.8	4.95	7.11
RDP[2] (% of crude protein)	64.3	53.1	69.2
UDP[3] (% of crude protein)	35.7	46.9	30.8

[1]**ME**=Metabolisable energy: Determined by RIVIGP (Menke and Steingass, 1988)
[2]**RDP**=Rumen degraded protein, Determined by *in situ* procedure (Singh *et al*., 1995)
[3]**UDP**=Undegraded dietary protein: 100-RDP %

Table 2: Effect of feeding spineless cactus based diets on daily mean intake of dry matter and nutrients in experimental sheep

Parameter	Group T1	Group T2	SEM
Finger millet straw	252	132	44.8
Compounded feed mixture	296	144	49.7
Spineless cactus	—	207	—
Total	549[a]	484[b]	22.2
As per cent body weight	2.32[a]	2.02[b]	0.08
Nutrients (g/day)			
Organic matter	514[a]	437[b]	20.3
Crude protein	59.3[a]	44.0[b]	6.78
Neutral detergent fibre	255[a]	213[a]	9.34
Acid detergent fibre	137[a]	125[a]	5.76
Roughage: CFM[*]	46:54	27:73	
Voluntary intake of water			
kg/day	1.24[a]	0.618[b]	0.034
kg/kg feed dry matter intake	2.45[a]	0.22[b]	0.030

Group T1: Control diet; Group T2: Fifty percent of the diet replaced by spineless cactus (25 per cent offinger millet straw and 25 per cent of compounded feed mixture)
[*]Comprising compounded feed mixture and spineless cactus
[ab] Means within arrow not sharing a common superscript letter differ ($p<0.05$)
SEM: Standard error of means

Table 3: Daily mean intake of energy and protein by sheep during the experimental period

Parameter	Group T1	Group T2	SEM
ME (MJ/day)	5.74 (4.78)	4.91 (4.79)	0.248
Crude protein (g/day)	59.3 (35.4)	44.1 (35.8)	6.784
Rumen degraded protein (g/day)	39.0 (35.4)	30.8 (35.8)	4.951
Rumen undegraded protein) (g/day)	20.3 (0.0)	13.2 (0.00)	0.834

T1: Control diet; **T2:** Fifty per cent of the diet replaced by spineless cactus (25 per cent offinger millet straw and 25 per cent of compounded feed mixture)
Mean values do not differ significantly between the two experimental groups, for all parameters
Values in parenthesis indicate the requirement as stipulated by ARC (1984)
SEM: Standard error of means

Table 4: Effect of feeding spineless cactus based diets on body weight change of sheep during the experimental period

Parameter	Group T1	Group T2	SEM
Initial body weight (kg)	23.5	23.8	0.201
Compounded feed mixture	23.8	24.1	0.196
Body weight gain (kg)	0.333	0.250	0.059
Daily body weight change (gain, g/day)[1]	7.94	5.95	0.635

T1: Control diet; **T2**: Fifty per cent of the diet replaced by spineless cactus (25 per cent of finger millet straw and 25 per cent of compounded feed mixture)
Mean values do not differ significantly between the two experimental groups, for all parameters
SEM: Standard error of means

Table 5: Effect of feeding spineless cactus based diets on digestibility (per cent) of nutrients in sheep

Parameter	Group T1	Group T2	SEM
Dry matter	68.9[a]	76.2[b]	0.994
Organic matter	71.1[a]	78.1[b]	0.937
Crude protein	70.8[a]	75.2[a]	2.37
Neutral detergent fibre	59.5[a]	63.5[a]	2.31
Acid detergent fibre	53.9[a]	58.2[a]	2.85
DOMDM	67.1[a]	73.3[b]	0.484

T1: Control diet; **T2**: Fifty per cent of the diet replaced by spineless cactus (25 per cent of finger millet straw and 25 per cent of compounded feed mixture)
[ab] Means within arrow not sharing a common superscript letter differ ($p<0.05$)
DOMDM: Digestible organic matter in dry matter
SEM: Standard error of means

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Nutritional Evaluation of Urea Treated Sugarcane Bagasse (SCB) on Performance of Kenguri Sheep

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ABSTRACT

Nine Kenguri sheep were divided into three groups of three animals each in 3x3 LSD to evaluate urea treated sugar cane bagasse (2 and 4% level) on performance of Kenguri sheep. Three groups were allocated differently with *ad libitum* untreated sugar cane bagasse (SCB) as control (T-1), *ad libitum* of 2% urea treated SCB (T-2) and *ad libitum* of 4% urea treated SCB (T-3). All three groups were fed with 250g of concentrate feed mixture per day. No significant ($p<0.05$) difference was observed in DMI among treatment groups whereas digestibility of CP was significantly higher in T3 group. DCP content of the diets were 6.55, 7.91 and 11.0 in T1, T2 and T3 group respectively which was significantly different among the diets.There was a significant difference among the groups for pH, TVFA and serum urea nitrogen except total serum protein. The results of the study indicated that the palatability and daily weight gain were marginally improved in 4% urea treated SCB fed group.

Keywords: Sheep, sugarcane bagasse, urea

The feed supply for productivity of ruminant livestock is largely dependent on crop residues which are low in nutrients and often do not meet the maintenance requirement of animals (Blummel, 1994). Search for newer feed resources is mandatory to meet the shortage of dry fodder. One among such feed resources is sugarcane bagasse (SCB) from sugar industry. About 41.78 million SCB is available in India that could be used for livestock feeding which is presently used mostly either as fuel or for paper industry. Bagasse contains higher level of lignin which lowers the degradability by rumen microbes (Kewalramani *et al.*,1988). SCB have enough potential to be used as ruminants feed after palatability and digestibility is improved. Urea treatment is a better option to improve the nitrogen content there by improves the protein value of low quality roughages. Protecting the true protein from microbial degradation could force the microorganisms to use NPN more efficiently and maximize the availability of amino acids for absorption through the intestine (Chalupa, 1975). Hence, an attempt was made to improve the nutritional value of SCB by 2 and 4 % urea treatment and its nutritional evaluation in Kenguri sheep.

MATERIALSANDMETHODS

Nine Kenguri sheep (Age: 6 to 12 months, BW: 20-23 kg) were divided into three groups of three animals each in 3x3 Latin square design. Each period consisted of four weeks duration. The sugar cane bagasse (SCB) was treated with 2 and 4% urea (w/w) with 20 percent moisture level and stored for seven days.The control (T-1) group was fed with untreated SCB, treatment group (T-2) was fed with 2% urea treated SCB and T-3 was fed with 4% urea treated

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SCB. All animals of three groups were fed with 250 g concentrate feed mixture (maize grain-36%, wheat bran-36%, ground nutcake-12.5%, soya bean meal-12.5%, mineral mixture-1%, dicalcium phosphate-1% and salt-1%) with calculated nutritive value of 15% CP and 70% TDN. Daily feed intake and weekly body weights were recorded during the experiment. Rumen fluid was collected at two hours post feeding before and at end of the each period. pH was recorded immediately using electronic digital pH meter and total volatile fatty acids (TVFA) was analyzed according to method of Barnet and Reid (1956). Blood samples were collected at start of the experiment and at the end of each period from all experimental sheep using heparinized tube and blood serum was separated for analysis of total protein (Gornall,1981) and serum urea nitrogen (Fawcett and Scott,1960). Digestion trial for five days was conducted at last week of each period. The samples of feed, fodder and dung were analysed for proximate constituents (AOAC, 1995) and forage fibre fractions (Vansoest *et al*., 1991). The data analysed statistically using SAS (2012).

RESULTS AND DISCUSSION

The chemical composition of SCB, CFM and urea treated SCB are given in Table 1. The CFM formulated with locally available ingredients contained (%) 15.06 CP, 10.10 CF, 30.58 NDF and 12.12 ADF. The CP level in CFM was restricted to15% to meet the protein requirement for maintenance level. The untreated SCB used in these experiment contained (%) 1.95 CP, 0.58 EE, 45.50 CF and 2.67 TA. The CP, EE and TA observed in this study were lower than the values given by Shakweer (2003) except CF content, however, Preston (2003) reported lower CP (1%) and higher CF (49%) than the values for untreated SCB in the present study. The NDF, ADF and ADL of untreated SCB were 89.44, 62.65 and 1.93 percent respectively, which were almost comparable to the values reported by Preston, (2003) and Shakweer (2003). The level of proximate principles and fibre fractions in SCB were not comparable to other crop residues like ragi straw; CP 8.4%, NDF 7 6.6% and ADF 47% (Mruthunjaya *et al*., 2003), paddy straw; CP 4.28%, CF 30.28% (Suresh *et al*., 2009) and sorghum stover; CP 5.4%, NDF 66.96% and ADF 47.59% (Ramchandra *et al*.,2002) which were able to meet at least maintenance requirements of ruminants. The CP content of 2% urea treated SCB has increased to 4.89% from 1.95% and 10.47% from 1.95% in 4% urea treated SCB. The percent increase in CP level in 2 and 4% urea treated SCB were 150.8 and 436.9 respectively when compared to untreated SCB. Not much change was observed in the level of EE, CF, NDF and ADF values but there was a slight increase in ADL content and variation in NFE content in 2 and 4% urea treated SCB. Since NFE was calculated by difference, there was a variation in CP content and hence variation was observed in NFE also.

The average daily gain (g) observed in T-1, T-2 and T-3 groups were 19.56, 25.08 and 32.90 respectively (Table 2). T-3 group had significantly higher (Pá0.01) gain followed by T-2 and T-1 groups. The T-1 group showed negative body weight gain which was mainly due to significantly (pá0.01) lower digestible CP intake than requirement (ARC, 1984).

The mean DMI (g/d) from SCB and CFM, the total DMI, total DMI as percent body weight and as a proportion of metabolic body weigh (g/kg) are given in Table 2. The DMI of all the treatment groups found to be non significant (p>0.05), even though SCB was treated with 2 and 4% urea with 20 percent moisture level treated for seven days which indicated that moisture level and duration of treatment might not be sufficient to exert desired urea ammoniation of SCB. These results were corroborated with findings of Currier *et al*. (2004) where DMI and OMI of straw supplemented with CP or NPN source not affected. Similarly, Pin and Wanwisa, (2008) also reported significant improvement of DMI in goats fed with

urea treated straw. It was further evident in an experiment conducted by Salama *et al*. (2011) on lambs fed with plane SCB and 3% urea treated SCB that no significant difference was observed in TDMI among the groups.

The mean digestibility of nutrients of experimental sheep is given in Table 3. The mean apparent digestibility (%) of CP was significantly higher (pá0.05) in T-3 group compared to T-1, and T-2 group. This could be attributed to the higher CP level in T-2 and T-3 diets than T-1 diet which comprised untreated SCB. The results were similar to the observations made by Pin and Wanwisa (2008) with regard to digestibility of nutrients in goats fed with cassava chips. T-3 group (47.47 g) significantly (0.01) consumed higher DCP followed by T-2 (34.23 g) and T-1 (28.24 g) which was mainly due to the change in the level of CP in T- 2 and T-3 groups up on urea treatment. The DCP and TDN (g) and ME (MJ/d) intake of three experimental diets in three groups are presented in Table 4. There was no statistical significant difference in the ME (MJ/d) intake among the different treatment groups. The DCP of the T-3 diet was significantly (pá0.01) higher followed by T-2 and T-1 groups. The TDN requirements were met in T-1 group also but DCP intake in T-1 group was low as per the requirements of ARC (1984). This was reflected on the body weight gain which was on negative side in T-1group. The DCP and TDN of the diet (%) in T-1, T-2 and T-3 groups were 6.55 and 64.04; 7.91 and 66.42; 11 and 65.55 respectively. The DCP of the T-3 diet was significantly (pá0.01) higher followed by T-2 and T-1 groups. There was no significant difference in TDN content of different groups. However, the DCP and TDN content of all the diets were well within the level of meeting at least maintenance requirement of experimental sheep. Both DCP and TDN intake of experimental sheep met there quirements of adult sheep according to Ranjhan (1998) in T-2 and T-3 groups. The DCP and TDN content of the diet in this study were higher than the values reported by Gado (1999) for bagasse fed to goat (DCP-1.8% and TDN-10.1%).

The mean rumen pH, TVFA (mmol/dl), total serum protein (g%) and serum urea nitrogen (mg%) of experimental sheep are given in Table 4. The rumen pH recorded was well within the normal range (5.5 to 6.5). T-3 had significantly (pá0.01) higher in TVFA (8.59), followed by T-2 (8.07) and T-1 (7.11). This suggested that more fermentable carbohydrates availability in the rumen in urea treated SCB groups due to breakdown of lignocelluloses bonds.Total serum protein levels in all treatment groups were also within the normal range (William, 2005) but urea treatment did not influence the level of the total serum protein. Serum urea nitrogen was found to be significantly higher in T-2 group when compared to T-1 and T-3 groups and all the values were within the normal range (William, 2005). On contrary, Hassoun *et al*. (2002) reported significantly higher ($p<0.001$) plasma urea concentration in urea treated sugarcane residues than in untreated sugar cane residues fed dairy heifers.

CONCLUSION

It can be concluded that sugar cane bagasse (SCB) had moderate energy with very low protein which could not meet the maintenance requirement of sheep in spite of providing CFM. 4% urea treated SCB showed improvement in weight gain because of higher CP digestibility than untreated and 2 % urea treated SCB fed groups.

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Table 1: Chemical composition (%DMB) of CFM, SCB and urea treated SCB.

Parameters	CFM	T1 (SCB)	T2 (2% Ureatreated SCB)	T3 (4% Ureatreated SCB)
OM	85.58	97.33	97.18	96.85
CP	15.06	1.95	4.89	10.47
EE	1.94	0.58	0.56	0.59
CF	10.10	45.50	46.38	47.11
NFE	58.48	49.30	45.34	38.69
TA	14.42	2.67	2.82	3.15
AIA	1.45	1.88	1.86	1.97
NDF	30.58	89.44	88.66	88.54
ADF	12.12	62.65	60.86	61.35
ADL	0.91	1.93	2.38	2.22
Cellulose	9.39	49.61	47.28	48.34
Hemicellulose	18.46	26.79	27.80	27.19

Table 2: Average daily gain (g/d) and dry matter intake (g/d) of experimental sheep.

Parameters	T1	T2	T3	P
Initialweight(kg)	22.18	21.21	21.59	-
Finalweight(kg)	21.63	21.91	22.51	-
ADG	-19.56^{c}	25.08^{b}	32.90^{a}	**
DMI(g/d)				
CFM	231.15	231.15	231.15	-
SCB	211.68	212.92	207.96	NS
SCB,% B.Wt.	0.98	0.93	0.95	NS
SCB,$B.W^{0.75}$	21.01	21.32	20.46	NS
TotalDMI	442.83	444.07	439.11	NS
TDMI,%B.Wt.	2.04	2.07	1.99	NS
TDMI,$B.W^{0.75}$	44.02	44.45	43.21	NS

**pá0.01,Means bearing different superscript in a row differ significantly.

Table 3: Mean digestibility and nutritive value of diets fed to experimental sheep.

Parameters	T1	T2	T3	P
Digestibility (%)				
DM	57.42	56.25	60.06	NS
OM	61.14	60.12	63.68	NS
CP	72.21^{b}	74.09^{ab}	79.95^{a}	*
EE	73.37	72.24	75.35	NS
CF	49.52	51.84	50.15	NS
NFE	64.85	61.61	67.12	NS
NDF	49.39	51.84	52.63	NS
ADF	41.41	41.28	45.73	NS
Cellulose	52.11	50.00	54.77	NS
Hemicellulose	65.57	61.80	67.84	NS
Nutritive Value (%)				
DCP	6.55^{c}	7.91^{b}	11.00^{a}	**
TDN	64.04	66.42	65.55	NS
DOMD$^{\#}$	55.78	54.87	58.09	NS
DCP intake (g)	278.65	288.86	283.73	NS
TDN intake (g)	4.21	4.36	4.28	NS
ME intake (MJ/d)	4.21	4.36	4.28	NS

**pá0.01,*pá0.05, Means bearing different superscript in arrow differ significantly.
#Digestible organic matter in dry matter

Table 4: Mean rumen pH, TVFA (mmol/dl), total serum protein (g%) and serum urea nitrogen (mg%) of experimental sheep.

Parameter	T1	T2	T3	P
pH	6.63^{b}	6.76^{a}	6.58^{b}	**
TVFA	7.11^{c}	8.07^{b}	8.59^{a}	**
Total serum protein	6.40	6.43	6.68	NS
Serum urea nitrogen	11.01^{b}	14.10^{a}	12.22^{b}	**

**pá0.01, Means bearing different superscript in a row differ significantly.

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In vitro Evaluation of Complete Diets Based on Spineless Cactus (*Opuntia ficusindica*) and Moringa (*Moringa oleifera*) for Ruminant Feeding

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ABSTRACT

Spineless cactus and Moringa were analyzed for proximate principles by chemical analysis. The ME content was determined by *in vitro* incubation and gas production technique (RIVIGP) as per Menke *et al*. (1979). On dry matter basis with 8.41 per cent DM, the total ash, crude protein, ether extract, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) content of spineless cactus were 11.6, 5.14, 1.79, 28.3, 17.1 and 3.06 per cent respectively and the ME content being 8.11 MJ/kg DM. The DM content in moringa was 22.1 per cent with the crude protein and ME content of 23.3 per cent and 10.2 respectively. Based on the chemical composition and predicted ME content, five complete diets were formulated using varying proportions of spineless cactus and moringa. The proportions of spine less cactus and moringa were 65:35; 60:40; 55:45; 50:50 and 45:55 in diets D1, D2, D3, D4 and D5 respectively. The ME content (MJ/kg) and crude protein (per cent) in the diets ranged from 9.12 to 9.48 and 11.5 to 15.1. Diet D3 with a combination of fifty five per cent of spineless cactus and forty five per cent of moringa containing 9.32 MJ and 13.3 per cent protein per kg diet was considered to provide a balanced supply of ME and crude protein to meet the requirement for ruminants.

Key words: Spineless cactus, moringa, metabolizable energy, crude protein, balanced diet

Ruminant production system is mainly based on the availability of forage resources since the bulk component of the feeds is provided by the fibre rich roughage feeds. Varieties of non legume and legume forages, crop residues (straws) and top feeds have been used as a roughage component in the diet of ruminants. Nevertheless, the major constraints hampering the productivity have been the scarcity of fodder, in dry season and drought situations, coupled with highly lignified and low nutritive value of the available fodder.

Spineless cactus or *Opuntia* (*Opuntia-ficusindica*) is a xerophytic plant used for feeding animals, easy to grow and palatable (Shoop *et al*., 1977). As a drought resistant crop with an advantage of minimum agronomical input for its propagation, *Opuntia* provides a major source of water and energy in the feed of ruminants. The productivity of *Opuntia* is 10 to 30 tons of DM/ha/yr under conditions of optimum management (Le Houerou, 1992). The water content (per cent) and metabolisable energy (MJ/kg dry matter) in *Opuntia* was reported to be 91.60 and 8.41 respectively (Ajith *et al*., 2017). As a potential feed for ruminants, there is a paucity of literature in India to recommend it as a feed for ruminants.

Moringa (*Moringa oleifera*) is a popular legume tree, the foliage and pods of which are known vegetable foods for humans. Moringa belongs to family of Moringaceae, considered to have its origin in Agra and Oudh, however popular in all parts of Asian and African continents. It can be grown on marginal or limited land holding under high temperature and low water availability (Odee, 1998). With a high nutritional value and good biomass production of both protein and energy, moringa can

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provide a fodder security for livestock, as a nutritional supplement (Devendra, 1990). To produce green moringa fodder with maximum of protein and a minimum of lignin, cutting should be done every 33 to 40 days. During the dry season the yield of fresh matter per cutting is as low as 45 tons/ha while during the rainy season the yield per cutting is as high as 115 metric tons/ha (Sarwat *et al*., 2004). The protein content (per cent on dry matter basis) of moringa has been reported to be 25.1 (Foidl *et al*., 2001).

Under adequate fertilization and irrigation, with 9 cuttings per year, the average annual fresh biomass production of *Moringa oleifera* could be 230 tons per acre. With attributes of being drought resistance and low water requirement for cultivation, both spineless cactus and moringa offer a huge potential as feedstuffs to provide balanced nutrition for ruminant feeding. While the Spineless cactus could be a source for water and energy, moringa, as legume forage could supplement protein. Literature regarding nutritional evaluation of complete diets based on spineless cactus and moringa for feeding ruminants has not been reported. In this perspective, this experiment has been taken up with the following objective.

MATERIAL AND METHODS

Procurement and processing of samples

The experiment on *In vitro* evaluation of complete diets based on spineless cactus (*Opuntia ficusindica*) and moringa (*Moringa oleifera*) for ruminant feeding was conducted at the Department of Livestock Production Management (LPM), Veterinary College, Hebbal, Bengaluru. Sufficient quantities of fresh samples of Spineless cactus (*Opuntia ficusindica*) and Moringa (*Moringa oleifera*) required for the entire study was procured from the Fodder Museum of the Department of LPM, Veterinary College, Bengaluru. The cladodes of spineless cactus and the moringa forage (leaves and tender twigs) was processed for analysis as follows.

The fresh samples of spineless cactus and moringa were chopped to a length of about 2-3 cm using a sharp knife and allowed to dry in hot air oven at 65^{o}C for 72 hours. After determining the partial dry matter, the samples were ground in a Willey mill using 1mm sieve, and stored in air tight polyethylene containers until analyzed. Samples of the feeds were dried at 105 ^{o}C in Hot air oven for 10 hours for determination of dry matter. The total dry matter (DM) was determined as product of the partial dry matter and the DM content in the partially dried samples.

Analytical procedures

Chemical analyses

The ash content in the samples was estimated as residue obtained after incineration of samples at 600°C for 3 hours. Crude protein (N×6.25) was analyzed using Gerhardt digestion and distillation unit that agrees with macro Kjeldahl standards (AOAC, 1995). The ether extract (EE) content in the feed samples was analyzed after extraction with petroleum ether using the procedure of AOAC (1995). The neural detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin were determined according to the methods described by Van Soest *et al*. (1991).

Rumen *In vitro* incubation for gas production

Donor cow and collection of rumen fluid

A lactating dairy cow producing 3 kg of milk per day, fitted with a flexible rumen canula of large diameter (Bar Diamond, Inc. USA), receiving a basal diet consisting of finger millet straw (FMS) and a compounded feed mixture (CFM) (Maize 60% , WB 35% , mineral mixture 2%, urea 2%, salt 1%) was used as the donor cow for rumen fluid. The FMS and CFM were fed separately. Six kg FMS was offered in small portions four times in a day, starting at 9.00 am. The CFM was offered 3.0 kg per day in two equal portions at 5.00 a.m. and 1.30 p.m. Rumen fluid was collected in the morning between 4:45 a.m. and 5.00 a.m. before offering CFM.

Metabolisable energy (ME) determination

The ME content in spineless cactus and moringa was determined by *in vitro* incubation and gas production technique (RIVIGP) according to Menke *et al* (1979) using the following equations:

$ME = 2.2 + 0.1357\ GP + 0.0057\ CP + 0.0002859\ EE^2$

Where,

ME = Metabolisable energy, MJ/kg DM.

GP = Corrected Net Gas production, ml/200 mg. DM.

CP = Crude protein, g/kg. DM.

EE = Ether extract g/kg. DM.

Based on the chemical composition and predicted ME content of the spineless cactus and moringa, five complete diets were formulated using varying proportions of spineless cactus and moringa in the diet. The composition of experimental diet is as follows.

Table 1: Experimental diets

	Spineless cactus	Moringa
Diet 1	65	35
Diet 2	60	40
Diet 3	55	45
Diet 4	50	50
Diet 5	45	55

The experimental diets were analysed for crude protein (per cent) and ME (MJ/kg DM) content using rumen *in vitro* incubation for gas production.

RESULTS AND DISCUSSIONS

The results of the chemical analyses of spineless cactus and moringa are presented in Table 2. Spineless cactus contained a dry matter of 8.41 per cent. On dry matter basis, the total ash, crude protein, ether extract, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) content of spineless cactus were 11.6, 5.14, 1.79, 28.3, 17.1 and 3.06 per cent respectively. The ME content (MJ/kg DM) of Spineless Cactus was 8.11. With 8.41 per cent DM, cactus could be a good source the water (91.59 per cent) for ruminants. The low crude protein content (5.14 per cent) was comparable to non legume forages. The relatively low NDF and ADF content indicated the digestibility of cactus could be higher with possibly higher energy content. The ME content of 8.11 MJ/kg DM indicated that spineless cactus could provide a valuable source of energy in the diet of ruminants.

Moringa contained DM of 22.1 per cent. The crude protein content was higher (23.3 per cent) indicating that it can be a major protein source in the diet of ruminants. The ME content of 10.2 was higher than spineless cactus signifying that moringa could provide a good source of energy. The NDF and ADF content in moringa was higher than spineless cactus, however, lower than most of the roughage feeds used in feeding dairy cattle

The results of analysis of ME (MJ/kg DM) and Crude protein (per cent) composition of the experimental diets are presented in Table 3. The proportion of Cactus in the diet varied from 45 to 65 per cent, whereas moringa content in the diet ranged from 35 to 55 per cent. All the diets provided adequate energy (more than 9.00 ME, MJ/kg DM), whereas the protein content was low in diets 1 and 2. Crude protein content of more than 13.3 in the total diet 3 would ensure adequate amount of protein supply to meet the requirement of ruminants. Higher levels of

Table 2: Chemical composition*[1] (per cent dry matter basis) energy (ME, MJ/Kg DM) content of spineless cactus and moringa

Parameter	Spineless Cactus	Moringa
Dry Matter	8.41	22.1
Total Ash	11.6	14.3
Crude protein	5.14	23.3
Ether Extract	1.79	3.26
Neutral Detergent Fibre	28.3	41.7
Acid Detergent Fibre	17.1	20.2
Acid detergent Lignin	3.06	3.01
ME[2](MJ per kg DM)	8.11	10.2

* Except dry matter and ME

[1]Mean of two replicates. Variations in duplicate measurements were within ±3% of the mean

[2]Determined by RIVIGP (Menke*et al.*, 1979)

Table 3: Metabolisable Energy[1] (ME, MJ/KG DM) and crude protein[2] (per cent dry matter basis) composition of the experimental diets

Parameter	ME	Crude Protein
Diet 1	9.12 (57.1)	11.5
Diet 2	9.25 (58.3)	12.4
Diet 3	9.32 (59.2)	13.3
Diet 4	9.41 (59.1)	14.2
Diet 5	9.48 (63.1)	15.1

[1]Determined by RIVGP (Menke *et al.,* 1979)
[2]Mean of two replicates. Variations in duplicate measurements were within ±3% of the mean
Values in parenthesis; Gas volume for 200 mg sample/24h

moringa (more than 45 per cent) in diets 4 and 5 may not be obligatory since the protein from moringa forage could be saved. Spineless cactus served as a source of energy, whereas moringa provided adequate amount of protein and energy in the diet. The combination of spineless cactus and Moringa therefore would make up a balanced diet for feeding ruminants, not only to provide energy and protein to meet the requirement, but also the diet could be a good source of water, especially for feeding ruminants in dry season or drought prone areas.

CONCLUSIONS

The study concluded that nutritionally balanced and economical feeds could be formulated using spineless cactus and moringa forage for feeding ruminants. The proportion of spineless cactus and moringa at 55 and 45 per cent respectively in the diet was found to be optimum to provide adequate energy and protein to balance the requirement of ruminants. Diets based on spineless cactus could also provide adequate water to the ruminants and therefore mitigate the deficiency of water in extreme dry arid regions and in drought situations.

ACKNOWLEDGEMENT

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A Study on Efficacy of L-Carnitine and Fish Oil in the Management of Cardiac Diseases of Dogs*

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ABSTRACT

The present study was carried out to evaluate the utility of nutraceuticals L- carnitine and fish oil in the cardiac diseases of dogs. Dogs presented to Department of Veterinary Medicine, Veterinary College Hospital, Bangalore with cardiac insufficiency were selected and divided into 3 groups (regular treatment, regular treatment with L- carnitine and regular treatment with fish oil). The characteristic clinical signs observed were coughing, exercise intolerance, ascites, anorexia, dyspnea, edema of limbs, pale mucus membrane and cyanosis. The ECG findings were sagging of ST segment, tall QRS complex, Q dip, P- pulmonale, tall-T wave, deep S wave and ventricular premature complexes. The resolution of clinical signs was faster in the groups which received nutraceutical than the group which did not receive nutraceutical. Group which received L- carnitine showed better improvement than the group which received fish oil.

Key words: Nutraceutical, L- carnitine, fish oil

Nutraceuticals are becoming increasingly popular in the Veterinary field. A number of nutraceuticals are currently being used in the prevention and treatment of common diseases in animals such as cardiovascular diseases, osteoarthritis, periodontal disease, renal diseases and cancer.

Nutraceuticals have been described by the North American Veterinary Nutraceutical Council as a "non drug substance that is produced in purified or extracted form and administered orally to provide agents required for normal body structure and function with the intent of improving the health and well-being of animal" (Boothe, 2004).

Coenzyme Q10 (CoQ10), Vitamin E, L-carnitine, taurine and fish oil (Omega-3 fatty acids) have been evaluated in the prevention and treatment of many types of heart disease in dogs (Dove, 2001).

Both clinical observation and intervention trials with various breeds have provided clear evidence for the benefit of numerous supplements on canine heart disease. Appropriate levels of certain dietary nutrients have been shown to increase life span, improve life quality, reduce symptoms and physical evidence of disease and decrease mortality rates in these animals (Dove, 2001).

L-carnitine, a trimethylated aminoacid, transports long-chain fatty acids to beta-oxidation sites in the mitochondrial matrix and is essential for normal cellular energy metabolism. Deficiency of L-carnitine results in development of cardiomyopathy in several breeds of dogs (Hamlin and Buffington, 1989).

Omega-3 fatty acids, eicosapentanoic acid and docosahexanoic acid are found almost exclusively in seafood and especially fish oil from oily, cold water fish such as herring and mackerel. Fish oil possesses hypotriglyceridemic, anti-inflammatory, hypotensive, antiarrhythmic, antivasopressor and anti-intimal thickening activities (McCarty, 1996; Billman *et al*., 1999).

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Dietary supplementation with fish oil concentrate is an effective means of introducing these nutrients, and should be considered as supportive care for dogs diagnosed with heart disease (McCarty, 1996).

MATERIALS AND METHODS

Dogs presented to the Veterinary College Hospital, Hebbal, Bangalore with cardiac insufficiency were considered for the present study. Dogs were diagnosed as having cardiac insufficiency based on history, clinical signs and electrocardiography. After selection they were grouped and subjected to different therapeutic regimens.

The electrocardiography was recorded using the standard bipolar and augmented unipolar limb leads at 25 mm/s speed and interpreted as described by Tilley (1992).

The 18 cardiac insufficiency cases were randomly allotted to one of the three treatment groups, Group I, Group II and Group III each group having 6 dogs. Animals belonging to Group I was administered with regular treatment. Which includes digoxin (Lanoxin 0.25 mg tabs, Burroughs Wellcome) at the rate of 0.005 to 0.01 mg/kg b.i.d., p.o, and spiranolactone (Lasilactone® 100 mg tabs.) at the rate of 4 mg /kg b.i.d. orally.

Animals belonging to Group II were administered with regular treatment and the nutraceutical L- carnitine (Carni Paste ®) at the rate of 1000 mg b.i.d. orally for 42 days.

Animals belonging to Group III were administered with regular treatment and the nutraceutical fish oil (Mega 3 capsules, Reddy's laboratories) at the rate of 1200 mg/day orally for 42 days.

The 18 cases were monitored for a period of 42 days and the different treatment regimens were evaluated at weekly intervals (7^{th}, 14^{th}, 28^{th} and 42^{nd}) based on history, clinical signs and electro cardiography.

Observations recorded and results obtained in the study were subjected to two way ANOVA by using computer based graph pad prism statistical programme to arrive at a conclusion.

RESULTS AND DISCUSSION

The clinical signs in dogs with cardiac disease in the present study included coughing in 9 dogs (50%), exercise intolerance in 7 dogs (38.88%), ascites in 7 dogs (38.88%), anorexia in 7 dogs (38.88%), dyspnoea in 4 dogs (22.22%), edema of limbs, pale mucus membrane and cyanosis in 1 dog each (5.55%). Similar clinical signs were observed by Sisson and Thomas (1995) and Martin *et al*. (2009).

Exercise intolerance and pale mucus membrane could be due to poor tissue perfusion caused by decreased cardiac output as also indicated by Schlant and Sonnenblick (1990). Coughing may be due to pulmonary edema caused by left sided heart failure as indicated by Sisson and Thomas (1995).

Ascites in the present study was one of the clinical signs which has been reported as common clinical sign in cardiac disease which could be due to right sided heart failure as indicated by Sissons and Thomas (1995). Anorexia may be attributed to production of cytokines tumor necrosis factor–a (TNF) and interleukin I (IL- I). These cytokines reduce the energy intake in dogs with cardiac diseases. Dyspnea was seen in 22.22% of dogs.

Dyspnoea observed in the present study could be attributed to pulmonary edema caused by left sided heart failure as indicated by De Francesco (2002). Edema of limbs was obeserved in 5.55% of cases. Edema could be due to increased systemic venous and capillary pressures caused by congestive right sided heart failure as indicated by Mellins *et al*. (1970).

The electrocardiography findings in 18 dogs with cardiac disease in the present study indicated sagging of ST segment (72.22%), tall QRS complexes (66.66%), where amplitude of R wave was more than 3mV. Q dip (Fig. 3) (33.33%), with Q wave more than 0.5 mV. P- pulmonale (22.22%), where amplitude of P wave was more than 0.4 mV. Tall T wave (Fig. 2) (11.11%), where amplitude of T wave was more than one – fourth the height of the associated R wave. Deep

S wave (Fig. 1) (5.55%), where S wave was more than 0.35 mV and Ventricular premature complexes (Fig. 4) (5.55%).

S-T segment depression in leads II or those with dominant R waves indicated myocardial ischemia, hyperkalemia, hypokalemia and digitalis toxicity. Secondary S-T segment changes from abnormalities of the QRS complex is indicative of hypertrophy, bundle branch block, and VPCs. Tall R wave is a suggestive of left ventricular enlargement. In left ventricular enlargement amplitude of R wave will be greater than 3mV in lead II. Q dip is characterized by Q waves greater than 0.5 mV. Q dip indicates right ventricular enlargement (Tilley, 1992). This correlates with findings of Thomas (1987) who has reported that Q >0.5mV in leads II in right ventricular enlargement. P- pulmonale is characterized by taller P waves with amplitude of more than 0.4 mV. Tall P waves are suggestive of right atrial enlargement. T wave in dogs should not be more than one – fourth the height of the associated R wave. Large T wave can be seen with myocardial hypoxia, intraventricular conduction disturbance, ventricular enlargement and hypothermia and animals with heart disease and bradycardia (Tilley, 1992).

Deep S wave was seen in the 5.55% of dogs. This is commonly observed in right ventricular enlargement. Ventricular premature complexes were seen in 5.55% of dogs. VPCs are seen in dilated cardiomyopathy, CHF, myocardial infarction, bacterial endomyocarditis (Tilley, 1992).

In Group II, three animals showed improvement in ECG findings after supplementation with nutraceutical L- carnitine. There was reduction in the amplitude of R wave and Q dip after receiving L-canitine (Fig. 5 and 6).

In Group III, two animals showed improvement in ECG findings after supplementation with nutraceutical fish oil. Sagging of ST was present before treatment (Fig. 7). After supplementation with fish oil sagging of ST segment was disappeared (Fig. 8).

The mean number of days taken to resolve clinical signs in coughing resolved in 21, 7 and 7 days; exercise intolerance resolved in 21, 7 and 7 days; anorexia resolved in 33, 9 and 14 days; dyspnoea resolved in 28, 7 and 10 days in Group III, Group IV and Group IV respectively.

The resolution of clinical signs was faster in the groups which received nutraceutical L- carnitine and fish oil compared to control group. In the present study the group which received L- carnitine showed better improvement compared to the group which received fish oil.

The dogs supplemented with L- carnitine showed better improvement compared to control group because the heart obtained much of its energy from the breakdown fatty acids. L-Carnitine played very important role in the conversion of fatty acids into energy via both the citric acid cycle and beta-oxidation (Kendal, 1998).

Resolution of clinical signs was faster in Group II compared to Group I. This correlated with the findings of Keene *et al*. (1991) and Dove (2001) who reported that dogs diagnosed with dilated cardiomyopathy supplemented with L-carnitine improved greatly in their health and myocardial function. The L-carnitine supplementation in cardiac diseases of dogs improved the quality of life, reduced the symptoms and mortality rate in affected animals.

Resolution of clinical signs was faster in Group III compared to Group I. This may be attributed to the addition of nutraceutical fish oil. These findings correlate with findings of Freeman *et al*. (1998), Billman *et al*. (1999), Dove (2001) and Smith *et al.* (2007) have indicated that omega-3 fatty acids exhibit the ability to improve general health in animals with heart disease. Omega-3 fatty acids reduce the symptoms and mortality rates in animals affected with the cardiac disease.

In the present study groups which received netraceuticals L-Carnitine and fish oil showed better improvement compared to groups which received regular treatment alone. L-Carnitine and fish oil supplementation was superior to regular treatment in management of canine cardiac diseases. In the present study the group which received L-carnitine showed better improvement compared to the group which received fish oil.

Table 1: Clinical signs in dogs with cardiac disease (n=18)

Clinical signs	No. of animals exhibiting clinical signs	Per cent
Coughing	9	50
Exercise intolerance	7	38.88
Ascites	7	38.88
Anorexia	7	38.88
Dyspnoea	4	22.22
Edema of the limbs	1	5.55
Pale mucus membrane	1	5.55
Cyanosis	1	5.55

Table 2: Electrocardiographic findings in dogs with cardiac disease (n=18)

ECG finding	No. of animals	Per cent
Sagging of ST segment	13	72.22
Tall QRS complex	12	66.66
Q dip	6	33.33
P pulmonale	4	22.22
Tall T wave	2	11.11
Deep S wave	1	5.55
Ventricular Premature Complexes	1	5.55

Table 3: Mean number of days taken for resolution of clinical signs in dogs with cardiac disease (n=18)

Clinical signs	No. of days		
	Group I	Group II	Group III
Coughing	21	7	7
Exercise intolerance	21	7	7
Anorexia	33	9	14
Dyspnoea	28	7	10

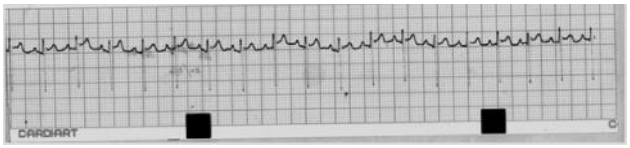

Fig. 1: Deep S wave - Deep S wave more than 0.35 mV

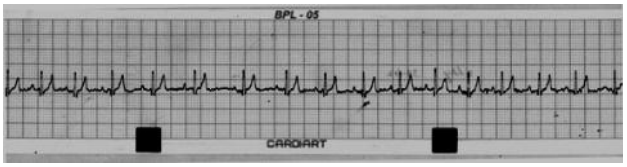

Fig. 2:Tall T waves

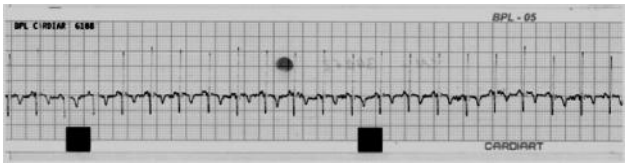

Fig. 3: Q dip - Deep Q waves more than 0.5 mV

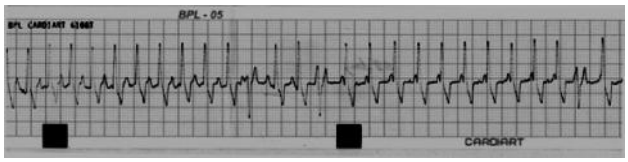

Fig. 4: Ventricular Premature Complexes

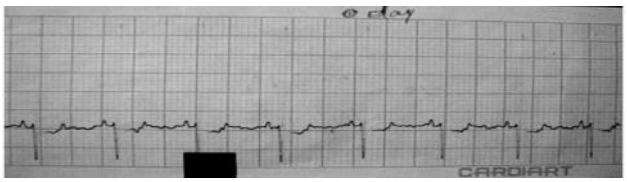

Fig. 5: ECG showing tall QRS complex and Q dip before treatment with L-carnitine (Group II)

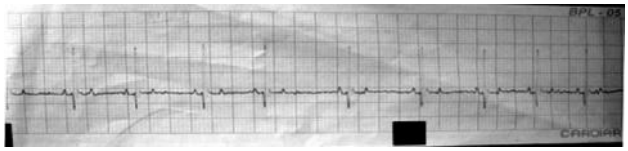

Fig. 6: ECG showing reduction in the amplitude of QRS complex and Q dip after treatment with L-carnitine(Group II)

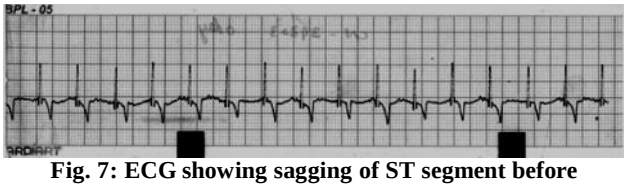

Fig. 7: ECG showing sagging of ST segment before treatment with fish oil (Group III)

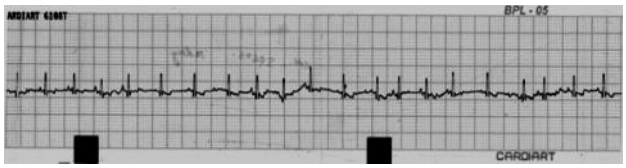

Fig. 8: ECG showing reduced sagging of ST segment after treatment with fish oil

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Hypertrophic Osteopathy in a Dog - A Case Report

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ABSTRACT

A 13 year old female dog was presented to Veterinary College Hospital, Bengaluru with a history of anorexia, dullness and inability to bear weight (recumbent). Clinical examination of the dog revealed dullness, depression with hard, swollen limbs and evidence of pain on palpation. Based on clinical examination, haematobiochemical analysis and radiography it was tentatively diagnosed as Hypertrophic Pulmonary Osteopathy.

Key words: Hypertrophic osteopathy, Marie's disease, metastatic masses

Hypertrophic osteopathy (HO) is a pathologic disease process, secondary in nature and most commonly occurs in humans and dogs (Carr, 1971; Nielson and Bishop, 1977; Grierson *et al.*, 2003).This disease in dogs is characterized by bilaterally symmetrical, non edematous soft tissue swellings affecting primarily the distal portions of all four limbs (Lenehan and Fetter, 1985).

The onset of Hypertrophic Pulmonary Osteoarthropathy in a dog is usually insidious. The initial soft tissue swellings are usually followed by a diffuse periosteal new-bone formation, which may ultimately affect all the bones of the limbs, resulting in severe disability. These skeletal changes are merely an outward manifestation of some underlying systemic disease, usually neoplastic in nature. Since most cases of hypertrophic osteopathy are associated with some form of pulmonary disease, the syndrome has most commonly been referred to as Hypertrophic Pulmonary Osteoarthropathy (Lonehan and Fetter, 1985).

A predominant number of dogs afflicted with hypertrophic osteopathy do, in fact, suffer from some kind of pulmonary disease, most commonly pulmonary neoplasia (Brodey, 1971). Further several authors have referred to the fact that metastatic pulmonary lesions remain the most common cause of hypertrophic osteopathy in the dog (Leighton and Stoyak, 1953; Jones and Schnelle, 1959).

HISTORY, CLINICAL SIGNS AND OBSERVATIONS

A 13 year old female non descriptive dog was presented to Veterinary College Hospital, Bengaluru with the history of anorexia, swollen limbs and inability to walk and bear weight. On examination it was found that animal was dull, depressed and recumbent. Swelling was present on all four limbs. All four limbs appeared hard (Fig. 1) and firm and the animal evinced severe pain on palpation.

Haematobiochemical studies revealed leucocytosis (21,000 cells/u L), anemia (Hb 8g% and RBCs 3.8×10^6 cells/u L) and elevated ALP levels (589 U/L) while other parameters were within the normal limits. Thoracic radiograph revealed presence of metastatic masses in the lungs (Fig. 2). Animal was treated with meloxicam (@ 0.2 mg/ Kg body weight) and enrofloxacin (@ 5 mg/Kg body weight) and the owner was advised about the prognosis. The owner reported a slight improvement in the condition following a few days of treatment.

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DISCUSSION

A majority of cases of HO are associated with underlying pulmonary disease, usually metastatic tumor. In the present case on examination it was found that distal portion of all the four limbs were swollen, firm and hard and thoracic radiograph revealed presence of intra thoracic masses in the lungs. Similar observations were made by Cetinkaya *et al*. (2011). The exact pathogenesis of the condition is not clearly understood, development of osteopathy is reported to be due to changes in the peripheral vascular supply induced indirectly by the underlying pulmonary disease (Gerbode *et al*., 1966).

The clinical signs and physical findings in the present case were similar to that reported byKshama and Kamran (2016). They further reported that the limbs are generally warm to touch, often pulsatile and sometimes painful upon deep digital palpation. Radiographically, hypertrophic osteopathy usually is seen as a bilaterally symmetric and generalized periosteal proliferative reaction that primarily the long bones of the appendicular skeleton with the occurrence of metastatic lesions in the thorax (Madewell *et al.*, 1978).

Resection of primary lung lesions with lobectomy might sometimes result in regression of clinical signs of hypertrophic osteopathy (Holman, 1963). However, dogs suffering from hypertrophic osteopathy due to secondary lung tumor have a very poor prognosis (Cetinkaya *et al.*, 2011).

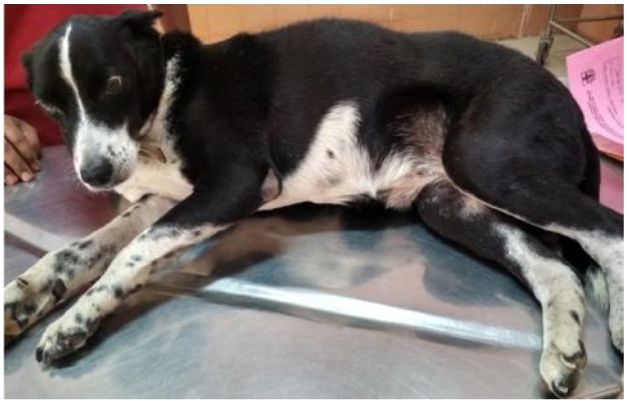

Figure 1: Affected dog with hard, swollen and stiff limbs

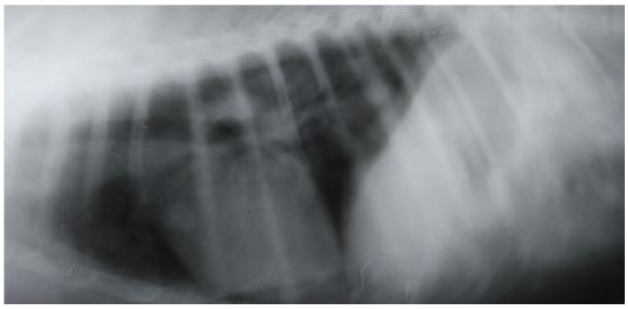

Fig. 2: Presence of metastatic masses in the lung

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Surgical Management of Urolithiasis in a Buck by Tube Cystostomy – A Case Report

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ABSTRACT

A 2 years old, non-descript buck presented with dysuria, stranguria, colic, anorexia and distended abdomen since 3 days, was diagnosed as urolithiasis based on clinical and physical examination. After proper stabilization of the patient and taking asceptic precautions, tube cystostomy was performed under xylazine sedation and local anaesthesia. Post operatively, buck was administered with ammonium chloride orally along with antibiotic and analgesic therapy. Normal urination was observed after 9 days post surgery and the catheter was removed. No recurrence was observed even after 3 months post surgery.

Key words: Buck, urolithiasis, stranguria, tube cystostomy, ammonium chloride.

Urolithiasis is lodging of urinary calculi or concretions of organic compounds anywhere in urinary system. In goats, most frequently calculi obstruct at the distal curvature of sigmoid flexure or at the end of urethral process (Radostits *et al*., 2000). Various nutritional, pathological, physiological, managemental and hereditary factors may contribute to calculi formation (Makhdoomi and Gazi, 2013). Amarpal *et al*. (2004) found that the incidence of urolithiasis was highest in caprine (49.83%), followed by cattle (32.87%), dogs (14.53%), horses (1.38%), sheep (1.04%) and cats (0.34%). Castrated males were reported to be affected more with urethroliths (Makhdoomi and Gazi, 2013). Urethral obstruction, if not attended on emergency basis, may lead to bladder distension and cystorrhexis leading to suroperitoneum which is detrimental to animal life. Various medical and surgical techniques have been reported to overcome the problem (Van Metre *et al*., 1996) of which, tube cystostomy followed by oral administration of ammonium chloride provided satisfactory results (Tamilmahan *et al*., 2014 and Sumiran *et al*., 2017). In this communication, we put forth a case of successful surgical management of urolithiasis in a buck by tube cystostomy.

CASE HISTORY AND OBSERVATIONS

A non-descript buck aged about 2 years and weighing about 20 kg was presented to the Dept. of Veterinary Surgery and Radiology, Veterinary College, Bengaluru with a history of dysuria, dribbling of urine and anorexia since 3 days. It was straining and making futile attempts to urinate. Anamnesis also revealed that it was fed with high concentrated diet and bore water for the past one month to hasten fattening. Clinically, the buck was dull and dehydrated. Signs like dysuria, stranguria, pollakiuria, colic (indicated by frequent getting up and lying down), bruxism, tachycardia and tachypnoea were observed. Distended urinary bladder was observed on abdominal palpation.

DIAGNOSIS AND TREATMENT

Based on history and clinical examination it was diagnosed as urolithiasis and surgical intervention was planned. The animal was stabilized with 500 ml Ringer's Lactate and dexamethasone 0.5 ml I/V.It was restrained in right lateral recumbency and body of the penis was carefully exteriorised. Urethral process was snipped off at its base and the condition could not be relieved completely. Hence, tube cystostomy surgery was performed.

Caudal left paramedian area cranial to rudimentary teats was prepared asceptically (Fig.1). Sedation and local anaesthesia were achieved by xylazine @ 0.1 mg/ kg BW and linear infiltration with 2% lignocaine hydrochloride parallel over the site of incision. A 6 cm linear incision was made over skin and muscles and distended urinary bladder was exteriorised (Fig. 2). A nick incision was made at cranio-ventral area of bladder and the urine was completely evacuated, which was dirty white in colour (Fig. 3). A sterile Foley's catheter (20 F) was tunnelled through skin and muscles about 10 cm cranial to surgical site. Tip of the catheter was inserted in to bladder, inflated and secured in position with purse string suture using polyglacin 910 no. 2-0 (Fig. 4) and then bladder was repositioned in to abdomen. Muscles were sutured in simple interrupted manner using polyglacin 910 no. 1and skin edges were opposed in horizontal mattress pattern using poly amide black no. 1 (Fig. 5). The other end of catheter was secured to ventral abdominal skin by stay sutures. The retrieved calculi were multiple, white in colour and very small in size (Fig. 6). Post operatively buck was administered with ceftriaxone @ 10 mg/ kg BW and meloxicam @ 0.2 mg/ kg BW along with daily dressing for 5 days. Ammonium chloride was advised @ 0.2 g/ kg BW and two Cystone tablets (Himalaya Drug Co. Bengaluru) BID orally till it urinated normally. After 5 days, the owner was advised to clamp catheter and check whether urine was voiding out of normal urethra or not. If not, advised to continue to feed ammonium chloride. Normal urine outflow was observed after 9 days and hence the tube was removed. Skin sutures were removed after 12 days of surgery and animal recovered well. No complications or recurrence were reported even after 3 months post surgery suggested the effectiveness of tube cystostomy in management of urolithiasis with oral medication of ammonium chloride.

DISCUSSION AND CONCLUSION

Various physiological, hereditary, pathological and nutritional factors result in urolithiasis in small ruminants. Etiology for the present case may be attributed to high concentrate diet for faster weight gain and providing mineralized artesian water which added more mineral content (Makhdoomi and Gazi, 2013). Calcium carbonate crystals are found to be formed in such cases and also influenced by pH of urine (6.5 to 7.5) which precipitate concretions (Ewoldt*et al*., 2008).Uroliths made of calcium carbonate are the most common type in goats (Videla and Amstel, 2016). Signs observed in this case like dysuria, stranguria, pollkiuria were also observed by Rakestraw *et al*. (2014) and Sumiran *et al*. (2017). The incidence was high in males compared to females because of gradually narrowing urethra and presence of sigmoid flexure .Initially, urethral process was resected at its base as indicated by Van Metre *et al.* (1996), but could not relieve the obstruction completely. Hence, tube cystostomy was performed as per the procedure followed by Rakestraw *et al*. (1995). Tube cystostomy is a type of urine diversion in emergency and re-establishes urethral patency. It also allowed removal of stones and normo grade flushing (Videla and Amstel, 2016). Ammonium chloride acted as a urinary acidifier that reduced urine pH and increased urine output thus aided in curing the disease (Ewoldt *et al*., 2008). For this buck, ammonium chloride was administered orally @ 0.2 g/kg BW as indicated by Mahesh *et al*. (2017).

Tube cystostomy followed by oral administration of ammonium chloride provided satisfactory recovery of the buck from urolithiasis.

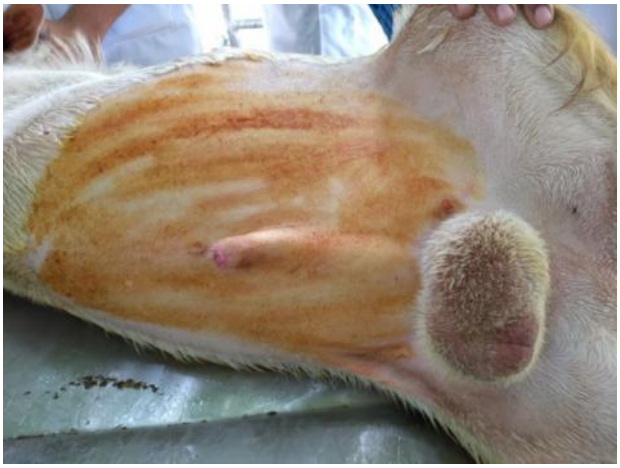

Fig. 1: Photograph showing asceptically prepared site anterior to rudimentary teats and left paramedian to prepuce.

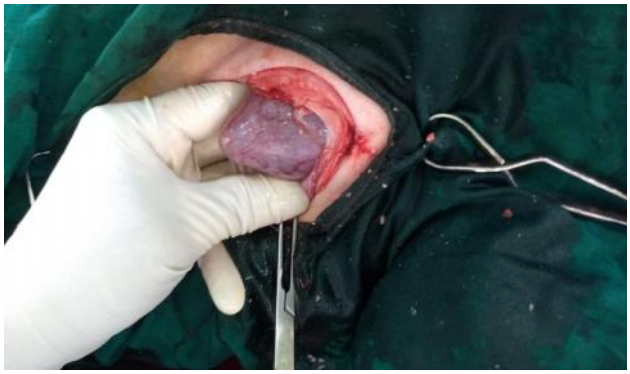

Figure 2: Photograph showing laparotomy incision and bladder exteriorization.

Fig. 3: Photograph showing cystotomy and urine evacuation.

Fig. 4: Photograph showing tunneling of foley's catheter. Note tip of the tube inflated and secured with purse string suture.

Fig. 5: Photograph showing skin sutures and retention sutures for Foley's catheter.

Fig. 6: Photograph showing calculi retrieved.

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Effect of Temperature on Growth of Psychrotrophic Molds in Malt Extract Broth*

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ABSTRACT

Malt extract broth was inoculated with psychrotrophic molds such as *Penicillium chrysogenum, Cladosporium cladosporioides, Alternaria alternate* and *Mucor mehei* at the rate of 6.0 $\log_{10}$ spores/ml individually and incubated at 30 ^{0}C and 7 ^{0}C to study the effect of temperature on biomass production. The spore count in inoculated broth was determined every day upto 5 days and once in 5 days up to 25 days at 30 ^{0}C and 7 ^{0}C respectively. The log spore count increased to 7.2, 7.3, 7.9, and 8.4/ml in case of *Alternaria alternata, Cladosporium cladosporioides, Mucor mehei,* and *Penicillium chrysogenum* respectively at 30 ^{0}C on 4th day. Rise in spore count was also observed at 7 ^{0}C and maximum count attained was 6.3, 6.4, 6.5 and 7.0 by *Alternaria alternata, Cladosporium cladosporioides, Mucor mehei* and *Penicillium chrysogenum* on 15th day respectively. The count reached peak on 4th day and 15th day at 30 ^{0}C and 7 ^{0}C respectively. The highest spore count was attained by *Penicillium chrysogenum* while lowest by *Cladosporium cladosporioides* both at 30 ^{0}C and 7 ^{0}C. According to the present study it clearly indicates that the lower temperature delays the growth of molds.

Key words: Spore count, temperature, psychrotrophic molds and haemocytometer

INTRODUCTION

Molds are eukaryotic multicellular micro organisms consisting of basal mycelia which bear hypha (structure bearing the spores), both may be septate or aseptate. Mycelium are the stringly growth structure of molds and spores were the reproductive or seed like structures. Reproduction in molds is either sexual (Sporangiospore or Ascospore formation) or asexual (budding or conidiospore formation). Spores of molds usually found dispersed in the air, due to their low density which serve as a major source of mold contamination. Growth of molds is evaluated by measurement of the average increase of the fungal spores at specific temperature against time interval. The psychrotrophic molds which grow at refrigeration conditions may be saprophytic causing defects in milk and milk products as well as toxigenic leading to adverse health conditions in consumers (Gervais *et al.*, 1988; Marth, 1998).

Airborne mold spores are ubiquitous, but, upon germination, they require oxygen to grow and sporulate. The molds are aerobic in nature and factors such as temperature, moisture, inoculum level, incubation period arefound to affect the growth (Jay, 2000). Molds are incredibly resilient and adaptable. The germination of spores may occur from 24 to 72 hours (Davis, 2001).

The mold spores germinate during growth and dichotomic branching in observed in hyphae resulting in spherical, thick agglomerates known as pellets or glomerules. Spore exhibits growth by increase in the size and density as incubation proceeds. At the end of biosynthetic cycle, fragments of pellet structure separate themselves as terminal hyphae (Leuca *et al*., 2008). However, little information is available on the effect of temperature on the germination of molds in broth medium. The objective of the present study was to evaluate the effect of two variable temperatures

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30 ^{0}C and 7 ^{0}C on the germination of psychrotrophic molds.

MATERIALS AND METHODS

The samples drawn were subjected to spore count using haemocytometer (Harris *et al.* 1963).

Broth culture of mold was placed on the marked area of Cleaned haemocytometer (Neubauer counting chamber) slide

↓

Spores present in all 16 square chambers were counted (40X objective)

↓

Total number of spores were calculated and expressed as spore count/ml

$$\text{Spore count/ml} = \frac{\text{Number of spores x 10000 (conversion factor for 0.1µl to ml.)}}{\text{Number of squares}}$$

Statistical Analysis

The data obtained in present study was analyzed using compound growth rate. The formula is given below

$Y = ab^t$

where Y = variable under study

t = time in days

a and b are the constants

b = 1+g where g is growth rate

The molds have their optimum growth temperature as 30 ^{0}C and always psychrotrophs are enumerated at 7 ^{0}C. Hence the samples were incubated and analyzed at these temperatures only.

RESULTS AND DISCUSSION

Production of spores at 30 ^{0}C in malt Extract broth

Psychrotrophic molds such as *Penicillium chrysogenum, Alternaria alternata, Mucor mehei* and *Cladosporium cladosporioides* were used to study the effect of 30 ^{0}C and 7 ^{0}C of temperature on spore count in malt extract medium. The mold spores were inoculated into malt extract broth (pH- 3.5) with known number of spores per ml and incubated at mesophilic temperature (30 ^{0}C). Every day samples were collected up to 5 days for spore count using haemocytometer (Fig. 1a and b).

Spore count of psychrotrophic *Penicillium chrysogenum* ranged from 6.3 to 7.2 but maximum of 8.4 $\log_{10}$ spores/ml was observed on Day 4 whereas spore count for *Alternaria alternate* ranged from 5.7 to 6.4 from Day 1 to Day 5 with peak of 7.2 $\log_{10}$ spores/ml on Day 4.

Psychrotrophic mold *Cladosporium cladosporioides* revealed log spore count of 5.5 to 6.0 from Day 1 to Day 5 with peak of 7.3 spores/ml on day 4 while it ranged from 5.7 to 6.2 for the psychrotrophic mold *Mucor mehei* from Day 1 to Day 5 with peak spore count of 7.9 spores/ml on Day 4. Growth of psychrotrophic molds such as *Penicillium chrysogenum, Alternaria alternata, Mucor mehei* and *Cladosporium cladosporioides* showed significant ($P>0.05$) increase of 5.37, 4.40, 4.53 and 4.12% per day respectively (Table 1 and Fig. 2a).

All the four psychrotrophic molds showed reduction in the spore count after 4th day of incubation in malt extract broth indicating the decline phase.

Production of spores at 7 ^{0}C in malt extract broth

Cultured *Penicillium chrysogenum, Alternaria alternata, Mucor mehei* and *Cladosporium cladosporioides* in malt extract broth at 7 ^{0}C were determined for spore count once in 5 days up to 25 days of incubation.

Counts of *Penicillium chrysogenum* ranged from 6.30 to 6.04 from Day 5 to Day 25 but the highest count of 7.0 $\log_{10}$ spores/ml was observed on 15th day. For the isolate *Alternaria alternate* spore count ranged from 5.7 to 5.9 $\log_{10}$ spores/ml (maximum on 15th day: 6.3) from Day 5 to Day 25. *Cladosporium cladosporioides* showed log spore count of 5.5 to 5.7 from 5th day to 25th day with high count on 15th day (6.4 spores/ml) while the spore count ranged from 5.7 to 6.2 for *Mucor mehei* from Day 5 to Day 25 with peak of 6.5 $\log_{10}$ spores/ml on Day 15.

Statistically significant ($p<0.005$) growth was observed by psychrotrophic molds at 7 ^{0}C from 0th to 25th day of incubation among *Penicillium chrysogenum, Alternaria alternata, Cladosporium*

cladosporioides and *Mucor mehei* as they exhibited increase in spore count of 0.11, 0.17, 0.18 and 0.27% per day (Table 2 and Fig. 2b).

Increase in spore count of *Penicillium chrysogenum* from initial 4.69 to final 0.08 mg/ml after 45 days while in the present study the spore count raised from initial 6.3 and reached peak of 8.4 later reduced to 7.2 from Day 1 to Day 5. The spore count decreased from 6.3 to 6.04 at 7 ^{0}C (Prabha Devi *et al*., 2009).

Germination rate of *Alternaria helianthi* ranged from 33% to less than 7% at 18-30 ^{0}C. Highest germination was observed at 18 ^{0}C with 33% followed by 28% (22 ^{0}C), 4% (26 ^{0}C) and 1% (30 ^{0}C) for 24h incubation (Abbas *et al*., 1995). *Alternaria alternata* in the present study exhibited maximum growth at 30 ^{0}C when compared to 7 ^{0}C. The spore count reached peak of 7.2 and 6.3 from initial 5.7 later reduced to 6.4 and 5.9 at 30 ^{0}C and 7 ^{0}C respectively.

Mucor mehei showed, more spore count on Day 4 with 7.9 spores/ml where as 6.5 $\log_{10}$ spores/ml was attained at 7 ^{0}C on Day 15 in the present study. *Mucorrace mosus* when inoculated in Potato Dextrose broth showed germination of 11% and 4.35% at 15 and 25 ^{0}C respectively (Dantigny *et al*., 2002). The present study also revealed that the low temperature reduced spore count.

Cladosporium spp growth temperature ranges from 18 to 28 ^{0}C, but growth till -6 ^{0}C was also possible (Gofron, 2007). *Cladosporium cladosporioides* revealed highest count of 7.3 spores/ml on 4th day of incubation at 30 ^{0}C than 6.4 spores/ml at 7 ^{0}C on 15th day of incubation.

On par with the present study Baert *et al*. (2008) observed that the growth temperature influenced the spore count with an effect on the growth phases. The rise in mold spore count of was delayed at 7 ^{0}C than at 30 ^{0}C which might be due to prolonged lag phase.

CONCLUSION

The two variable temperatures considered (30 ^{0}C and 7 ^{0}C) for study were optimum for mesophillic and psychrotrophic type of organisms. The psychrotrophic mold spore count was less at 7 ^{0}C when compared at 30 ^{0}C in malt extract broth. During study the highest spore count was observed in *P. chrysogenum* among the selected mold species. Even though the selected species of molds were psychrotrophic in nature they did not show much increase in spore count that might be due to adjustment of molds to the environmental conditions. The results reveal that 7 ^{0}C extended the lag phase of mold growth compared to 30 ^{0}C indicating that low temperature is bit safer for storage for food products.

Table 1: Production of spores by psychrotrophic molds at 30 ^{0}C in Malt Extract broth ($\log_{10}$ spores/ml)

Incubation period (Days)	Mold isolates			
	Penicillium chrysogenum	***Alternaria alternata***	***Cladosporium cladosporioides***	***Mucor mehei***
1	6.3	5.7	5.5	5.7
2	6.5	5.9	5.8	6.0
3	7.3	6.1	6.6	7.1
4	8.4	7.2	7.3	7.9
5	7.2	6.4	6.0	6.2
% Growth	5.37%	4.40%	4.12%	4.53%
T	37.32	47.64	30.87	25.12
	0.000002***	0.000001***	0.000004***	0.000008***

Note : 1) S- Haemocytometric spore count ($\log_{10}$/ml)
*** -Significant

Table 2: Production of spores by psychrotrophic molds at 7 ^{0}C in malt extract broth (log_{10} spores/ml)

Incubation period (Days)	Mold isolates			
	Penicillium chrysogenum	***Alternaria alternata***	***Cladosporium cladosporioides***	***Mucor mehei***
5	6.3	5.7	5.5	5.7
10	6.7	6.1	5.9	6.4
15	7.0	6.3	6.4	6.5
20	6.9	6.2	6.0	6.2
25	6.0	5.9	5.7	6.2
% Growth	-0.11%	0.17%	0.18%	0.27%
T	220.929689	362.22188	248.0725523	298.83041
Significance	0.0000000014***	0.0000000002***	0.0000000009***	0.0000000004***

Note : 1) S- Haemocytometric spore count (log_{10}/ml)
*** -Significant

Fig. 1a: Haemocytometer

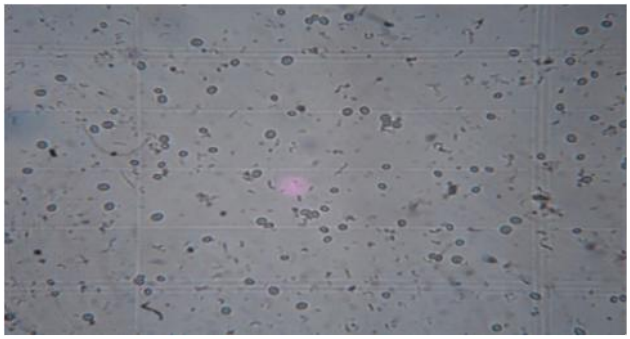
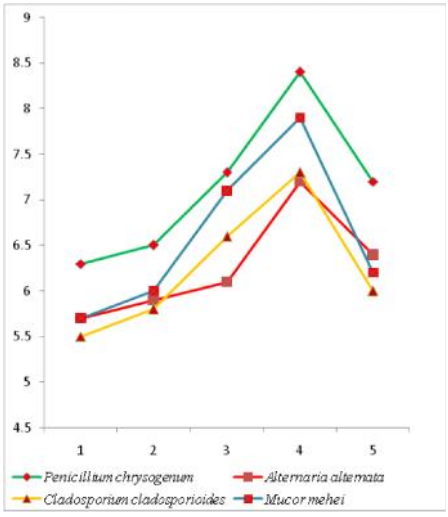
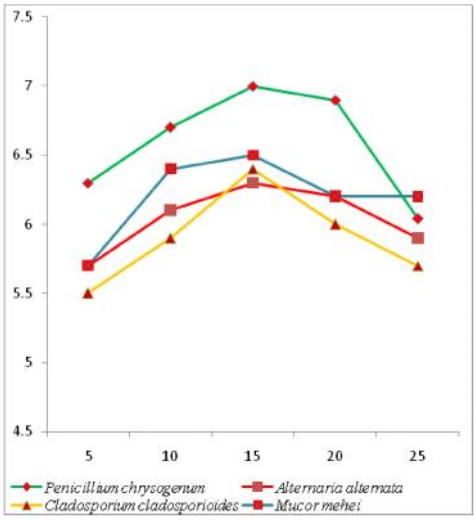


Fig. 1b: Microscopic spore count



a) Spore count at 30 ^{0}C



b) Spore count at 7 ^{0}C

Fig. 2: Effect of temperature on spore count of psychrotrophic molds in malt extract broth

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Occurrence of Fish Diseases in and around Bengaluru*

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ABSTRACT

A total of 101 fresh water edible and 210 ornamental fish were examined in the present study which were collected from eight lakes, three aquaculture farms, two whole sale and five local aquarium shops in and around Bengaluru. Occurrence of various bacterial, parasitic, fungal diseases and deaths due to poor water quality was observed. The infections/conditions in fish were identified by subjecting the fish for detailed post mortem examination, wet mount examination, bacterial and fungal isolation and analysis of water quality. Rohu and Gold fish were the most common fresh water edible fish and ornamental fish affected in the present study respectively. Among various causes of infections, parasitic causes were most common with high occurrence of protozoan infections. The various parasitic conditions encountered were Ich, Trichodinosis, Chilodonellosis, Tetrahymenosis, Ichthyobodosis, Epistylis infection, Myxosporidiosis, Lernaeaosis, Argulus infection, Dactylogyrosis and Gyrodactylosis. Among parasitic diseases, ich was the most common infection encountered in gold fish. The bacterial diseases encountered were infection with flavobacteria, aeromonas and streptococcus organisms. Flavobacterial infection was more frequent in ornamental fish and aeromonas in edible fish. Fungal infections included saprolegniosis, infection with achlya and epizootic ulcerative syndrome (EUS). Among fungal infections EUS was most common in edible fish followed by saprolegnia in ornamental fish. Analysis of water quality showed deaths due to ammonia toxicity, chloride toxicity and combined ammonia toxicity and hypoxia. The influence of water quality on occurrence of various diseases was analyzed and found that increased alkalinity, hardness, excess of ammonia and carbon dioxide precipitated stress in fish and predisposed them to infections.

Key words: Edible fish, ornamental fish, bacterial disease, parasitic disease, fungal disease

INTRODUCTION

In the recent years, the traditional aquaculture in India has turned into a super intensive fish culturing activity. Aquaculture and fisheries are plagued with disease problems as a result of tremendous growth, intensification and commercialization, affecting different culture facilities and different regions accounting for considerable economic loss. Diseases affect all kinds of fish which include fresh water, marine water and brackish water fish. Ornamental fish are also highly susceptible for infections in which the water quality is the main precipitating factor. The physiological conditions of fish and their entire environment play an important role in the course of diseases. The annual loss to the aquaculture industry due to diseases is estimated to be billion US dollars worldwide (Subasinghe, 2005).

Bengaluru, the capital city of Karnataka is growing at the rate of 23.4 per cent annually which has led to complications like poor waste management

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and pollution of air and water bodies. The city has a very poor drainage system in terms of segregation of biodegradable, household, toxic and non-toxic wastes which has led to dumping of the waste in to the lakes. These wastes entering into the water bodies have potential to alter physicochemical factors of the water and are expected to cause damage to the fish health directly and indirectly and predispose fish to numerous secondary infections and mass mortalities.

In addition to infectious causes, a number of environmental factors such as pH, hardness, water current, salinity of the water, dissolved oxygen, turbidity, temperature, light, pollutants etc. also play vital role in aquaculture.

MATERIALS AND METHODS

A total of 311 fish from eight lakes located at Hebbala, Madivala, Nagavara, Hulimavu, Hessaraghatta, Yelahanka, Rachenahalli and Sadaramangala, three aquaculture farms such as MRS farm, Fisheries Regional Investigation Centre (FRIC), Hebbala and FRIC, Hessaraghatta and two wholesale and five local aquarium shops in and around Bengaluru were collected. Ailing and freshly dead fish were collected and transported to laboratory immediately for further examination.

The occurrence of various diseases was confirmed by subjecting the fish for various laboratory tests like wet mount examination, post mortem examination, bacterial and fungal isolations. In addition the water quality was analyzed to determine its effect on occurrence of various diseases.

The methods used were according to standard protocols by Fernando *et al.,* (1972) and Lom and Dykova, (1992) for collection and processing of tissues, Bergey's Manual of Systemic Bacteriology, 2nd edn. (2009) for bacterial isolation and APHA, AWWA, and WEF Standard Methods Book, 20th edn. (1995), for the examination of water and waste water.

The obtained results were analysed through appropriate statistical methods wherever required.

RESULTS AND DISCUSSION

Out of 311 fish examined, 101 were fresh water edible fish and 210 were ornamental fish. Among edible fish, Rohu (37) was the most common fish type affected followed by Tilapia (33), Common carp (17), Silver carp (9), Murrel (4) and Mrigal (1) and among ornamental fish Gold fish, (67) was most affected followed by Guppy (50), combined (multiple types) (30), Gourami (20), Freshwater Shark (14), Molly (14) and others (Table -1). The most common types of fish reared in and around Bengaluru include mainly Carps, Rohu, Tilapia, Mrigal, etc. and this could be responsible for the over presentation of Rohu in the present study. Similarly, Gold fish is the most common ornamental fish preferred as aquarium fish because of its adaptability, colour and body shape.

Occurrence of diseases/conditions in freshwater and ornamental fish

Detailed postmortem and laboratory examinations of fish revealed occurrence of bacterial, parasitic, fungal infections and deaths due to poor water quality, in both freshwater edible and ornamental aquarium fish.

Since there was occurrence of multiple infections in fish with various combinations of ethiological agents, 503 numbers of various causes were diagnosed in 311 fish examined. Out of 503 conditions, 173 (33.39%) were in fresh water edible fish and 330 (65.61%) in ornamental fish. Parasitic causes of infections were more common (239), followed by bacterial as in 100 cases, fungal in 38 cases, problems with poor water quality in 125 cases and tumour in one case (Table 2).

Parasitic causes of infections in the present study included protozoa, copepods, monogeneans and tape worm. The protozoan causes of infections were most common in both edible and ornamental fish as also recorded by Pillay (1995); Scholz (1999) and Al-Rasheid *et al*. (2000).

Among 239 parasitic causes encountered, 142 (59.41%) were protozoa, 51(21.33%) were copepods, 45 (18.83%) were monogeneans and one cestode (0.42%) (Table 3). The protozoan causes included

Ichthyophthirius multifiliis (74), *Trichodina* (31), *Chilodonella* (2), *Tetrahymena* (12), *Ichthyobodo* (5), *Epistylis* (2) and *Myxospora* (16). Out of 51 copepod causes of infection, 38 were *Lernaea* and 13 were *Argulus*. Among monogenean parasitic causes, *Dactylogyrus* was observed in 23 cases and *Gyrodactylus* in 22 cases. *Bothriocephalus* cestode was observed only in one case (Table 3). Parasitic causes of infections were high in ornamental fish (180) with highest occurrence in Gold fish (121) and the most common parasitic infection encountered was with *Ichthyophthirius multifiliis* (72). Ich or white spot disease caused by *I. multifeliis* is very common and is characterized by white spots on the body due to the entrapment of trophonts of the parasite (Roberts 2012 and Noga 2010). Among edible fish, Tilapia (26 each) was most affected with a high occurrence of infection with Trichodina (13) (Table 4).

Bacterial causes of infections were observed in 100 out of 503 causes and included *Flavobacteria sp.* (45), *Aeromonas sp*. (40) and *Streptococcus sp.* (5). In fresh water edible fish bacterial infections (59) were more common compared to ornamental fish (41). Rohu was most commonly affected with bacterial causes of infection among edible fish (Table 6) and Gourami (20) in ornamental type (Table 7).

Among bacterial infections Aeromonas infection (35) was most common in fresh water edible fish and flavobacterial infection (36) in ornamental fish. Gopalakrishna (1961) and Austin and Adam (1996) also observed highest occurrence of aeromonas infection in fresh water edible fish and Clifford (2012) observed flavobacterial infection most common in ornamental fish (Table 7).

Columnaris infection is one of the most commonly occurring bacterial diseases of fish caused by *Flavobacterium columnare*. Flavobacteria are ubiquitous in nature and occurs as opportunistic bacteria (Roberts, 2012).They have a great potential to survive in water inspite of higher alkalinity, hardness and organic load (Fijan, 1968; Noga, 2010 and Roberts, 2012) and can readily infect stressed fish. In the present study also columnaris infection was associated with occurrence of several parasitic and fungal infections simultaneously wherein, the wounds created by other agents and the stress induced by them appeared to favor the occurrence of the infection. The common parasitic conditions associated with flavobacterial infection in the present study were lernaeaosis, trichodinosis, epistylis, chilodonellosis, ich and monogenean parasitic infestations.

Aeromonas organisms are ubiquitous in nature and occur in fresh water, coastal water and sewage water and cause acute haemorrhagic septicemia (Monfort and Baleusx, 1990 and Austin and Adam, 1996). They have a broad host range including humans (Ashdown and Koehler, 1993; Janda and Abbott, 1998). Aeromonas causes multiple organ damage with induction of multifocal haemorrhages and necrosis of parenchymatous cells.

The observation of the study also indicated that fungal infections are one of the common causes of death in fish in and around Bengaluru. Fungal infections have also been reported to contribute largely to significant economic losses in aquaculture (Neish and Highes, 1980; Noga, 1993; Bruno, 2011; Ramaiah, 2006 and Gonclaves and Gagnon, 2011). Frequently the fish are reported to be affected by oomycetes which include *Saprolegnia*, *Achlya* and *Aphanomyces* (Willoughby and Pickering, 1977, Blazer and Wolke, 1979, Noga, 1993) which was also the finding of the present study.

The only fungal infection observed in freshwater edible fish was epizootic ulcerative syndrome due to *Aphanomyces invadans* which occurred in 10 cases in the present study.

In the ornamental fish, saprolegniasis (25) was the most common fungal infection observed followed by infection with achyla (3) and Gourami fish (20) was found to be most commonly affected (Table-3). Fungi attack fishes of all age groups and can also prevent successful hatching by their invasion in to the fish eggs (West *et al*., 2006).

Roberts (1989), Bly (1993), Hibbett *et al*. (2007) and Quiniou (1998) stated that many of the fungi, especially those in the family Saprolegniaceae are

opportunists and attack the stressed and immunocompromised fish because of unfavorable environmental conditions, secondary to bacterial and viral infections or when they lose their mucus protection due to trauma or excessive handling. In the present study, the water quality was poor with an increase in mean alkalinity, hardness, ammonia content and the temperature of the water ranged from 24.5 ^{0}C to 28 ^{0}C which was conducive for the fungal growth (Dayal, 1963; Durbow, 2003; Mastan, 2015). Higher concentration of the ammonia in water can cause damage to the gills and alter osmoregulation process, stressing the fish and rendering them susceptible for the infections (Pickeringand Richards, 1982).

A single case of tumourous condition was observed which was diagnosed as squamous papilloma that occurred in fresh water shark.

Deaths due to poor water quality of water due to excess of ammonia and chloride and less dissolved oxygen was observed in 45 cases of freshwater fish and 80 of ornamental fish in the present study(Table-8). In fresh water edible fish ammonia toxicity was observed in 45 cases involving Rohu and Tilapia. Chloride toxicity (30) and ammonia toxicity with hypoxia (50) was observed in ornamental fish. Chloride toxicity was observed in 30 and Ammonia toxicity with low DO was observed in 50 cases of guppy fish (Table 8).

The occurrence of infections is closely related to the water quality and the general health of the fish. Several physicochemical factors such as water temperature, alkalinity, ammonia, free carbon dioxide, dissolved oxygen, pH and total hardness have strong influence on fish health and their resistance against the disease causing agents (Plumb *et al.*, 1988; Hossain, 1990; Hossain*et al.,* 2007). Poor quality of water is indicated by depletion of oxygen, excess of ammonia, carbon dioxide and temperature change which was an observation in the present study.

CONCLUSION

The study indicated that there is a prevalence of diseases due to various causes in edible and ornamental fish with the latter more frequently affected. Similar causes of affections occur in both the fish types however with variation in the frequency of occurrence of particular cause.

Table 1: Total number of edible and ornamental fish examined

Freshwater edible fish	Number of samples	Ornamental fish	Number of samples
Rohu	37	Gold fish	67
Silver Carp	9	Red kodango	1
Common Carp	17	Molly	14
Mrigal	1	Angel fish	3
Murrel	4	Eel	3
Tilapia	33	Flower horn	1
		Fresh water shark	14
		Pot belly	1
		Koi carp	2
		Red sword tail	1
		Black molly	1
		Guppy	50
		Silver dollar	2
		Gourami	20
		Combined fish	30
Total	**101**	**Total**	**210**

Table 2: Occurrence of various diseases in edible and ornamental fish

Type of fish / Etiology		Edible fish,	Ornamental fish,	Total	Percent
Parasitic disease	Number	59 (11.73%)	180 (35.78%	239	47.51
Bacterial disease	Number	59 (11.73%)	41 (8.15%)	100	19.88
Fungal diseases	Number	10 (1.99%)	28 (5.56%)	38	7.55
Poor water problem	Number	45 (8.95%)	80 (15.9%)	125	24.85
Tumour	Number		1 (0.19%)	1	0.19
Total number		**173**	**330**	**503**	
Per cent		**33.39**	**65.61**		**100**

Table 3: Occurrence of various diseases ofedible and ornamental fish

Diseases		Edible fish	Ornamental fish	Total	Per cent
Parasitic diseases					
Protozoan diseases	Ich (white spot disease)	2	72	74	14.72
	Trichodinosis	13	18	31	6.16
	Tetrahymenosis	0	12	12	2.39
	Icthyobodosis	5	0	5	0.99
	Chillodonellosis	2	0	2	0.39
	Epistylis infection	2	0	2	0.39
	Myxosporidiosis	6	10	16	3.18
	Total	30	112	142	
Copepods	Lernaeosis	11	27	38	7.55
	Argulus infestation	0	13	13	2.58
	Total	11	40	51	
Monogenean	Dactylogyrosis	9	14	23	4.58
	Gyrodactylosis	9	13	22	4.38
	Total	18	27	45	
Cestode	Bothriocephalus	0	1	1	0.19
	Total		1	1	
Bacteria	Flavobacterial infection/ Columnaris diseases	9	36	45	8.95
	Aeromonas bacterial infection	35	0	35	6.95
	Streptococcosis	15	5	20	2.99
	Total	59	41	100	

(Cont...)

Diseases		**Edible fish**	**Ornamental fish**	**Total**	**Per cent**
Fungus	Saprolegniosis	0	25	25	4.98
	Achlya infection	0	3	3	0.59
	EUS	10	0	10	1.99
	Total	10	28	38	
Deaths due to poor water quality	Ammonia toxicity	45		45	8.95
	Ammonia toxicity with low DO	0	50	50	9.94
	Chloride toxicity	0	30	30	5.97
	Total	45	80	125	
Tumour	Squamous cell pappiloma	0	1	1	0.19
		1	1		
Total		**173**	**330**	**503**	
Per cent		**30.81**	**69.19**		**100**

Table 4: Occurrence of various parasitic causes of diseases among edible fish

Etiology	**Cause of diseases/ Fish typ**	**Common carp**	**Tilapia**	**Silver carp**	**Rohu**	**Total**	**Per cent**
Protozoa	*Ichthyopthiris multifilis*	2				2	**3.38**
	Trichodina spp.	1	11		1	13	**22.03**
	Myxospora spp.	6				6	**10.16**
	Epistylis spp.	2				2	**3.38**
	Chillodonella spp.		1	1		2	**3.38**
	Icthyobodo spp.	2	1	1	1	5	**8.47**
Copepods	*Lernaea spp.*	11				11	**18.64**
Monogenean	*Gyrodactyla spp.*	1	7	1		9	**15.25**
	Dactylogyra spp.	1	6	2		9	**15.25**
Total		26	26	5	2	**59**	
Per cent		**44.07**	**44.07**	**8.47**	**3.39**		**100**

Table 5: Occurrence of various parasitic causes of diseases among ornamental fish

Causes of diseases/ fish type		Gold fish	Molly	Flower horn	Reds word tail	Black molly	Silver dollar	Combined fish	Total	Percent (%)
Protozoa	*Ichthyopthiri smultifilis*	60							72	**40**
	Trichodina spp.	16			1				18	**10**
	Tetrahymena spp.	10					2		12	**6.77**
	Myxospora spp.	10							10	**5.56**
Copopodes										
	Lernaea spp.	11	14			2			27	**15**
	Argulus spp.	13							13	**7.23**
Digenean parasites	*Dactylogyra spp.*	1					2	10	14	**7.78**
	Gyrodactyla spp.				1			12	13	**7.22**
Cestode	*Bothriocephalus spp.*			1					1	**0.56**
Total		121	14	1	3	2	4	22	180	
Per cent (%)		67.24	7.78	0.56	1.67	1.11	2.22	12.22		**100**

Table 6: Occurrence of various bacterial causes among edible fish

Fish name		Common carp	Mrigal	Tilapia	Silver carp	Murrel	Rohu	Total	Per cent (%)
Bacteria	Columnaris infection	3		2	3		1	9	15.3
	Aeromonas infection	10		13	1		11	35	59.32
	Streptococcosis		1	2		4	8	15	25.4
Total		13		17	3	4	20	59	
Per cent (%)		22.03	1.69	28.81	5.08	6.77	33.89		100

Table 7: Occurrence of various bacterial causes among ornamental fish

Fish Type	Bacteria		Total	Per cent (%)
	Columnaris infection	Streptococcosis		
Gold fish	12	2	14	34.14
Red kodango	1	1	2	4.87
Angel fish	2		2	4.87
Fresh water shark	1		1	2.43
Koi carp	2		1	2.43
Gourami	20		20	48.78
Total	36	5	41	
Per cent (%)	87.80	12.19		100

Table 8: Occurrence of deaths due to poor water quality among various types of edible and ornamental fish

Etiology/ Fish type	Ammonia toxicity	Ammonia toxicity with low DO	Chloride Toxicity	Total	Per cent (%)
Rohu	25			25	20
Tilapia	20			20	16
Guppy		50		50	40
Combined fish			30	30	24
Total	45	50	30		
Per cent (%)	**36**	**40**	**24**	**100**	**100**

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