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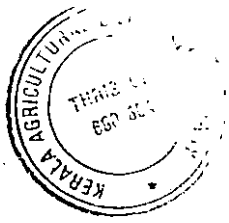
STRESS ASSESSMENT OF PIGLETS UNDERGOING ROUTINE SURGICAL PROCEDURES RELATED TO MANAGEMENTAL PRACTICES



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Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

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DECLARATION

I hereby declare that the thesis entitled **"STRESS ASSESSMENT OF PIGLETS UNDERGOING ROUTINE SURGICAL PROCEDURES RELATED TO MANAGEMENTAL PRACTICES"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that the thesis entitled **"STRESS ASSESSMENT OF PIGLETS UNDERGOING ROUTINE SURGICAL PROCEDURES RELATED TO MANAGEMENTAL PRACTICES"** is a record of research work done independently by **Dr. N. Geetha,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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We, the undersigned members of the Advisory Committee of **Dr. N. Geetha,** a candidate for the degree of Master of Veterinary Science in Livestock Production Management, agree that the thesis entitled **"STRESS ASSESSMENT OF PIGLETS UNDERGOING ROUTINE SURGICAL PROCEDURES RELATED TO MANAGEMENTAL PRACTICES"** may be submitted by Dr. N. Geetha, in partial fulfilment of the requirement for the degree.

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N.GEETHA

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Introduction

1. INTRODUCTION

Stress is defined as an environmental effect on an individual, which overtaxes its control systems and thus reduces its fitness or appears likely to do so. It also includes situations in which behavioral, physiological or immunological measures indicate that coping is difficult even if biological fitness is not reduced (Broom, 1996a).

Stress has profound impact on comfort, health and performance of all living beings. It may potentially be the most costly of negative influences considered by those who manage enterprises or care for animals, (Ewing *et al.*, 1999) as it has significant biological and emotional importance.

Pig is one of the most efficient feed converting domestic animal species. Among the meat producing livestock, pigs are litter-bearing animals with the shortest generation interval and faster growth rate. Due to various research and development efforts; pig husbandry and pork production has gained impetus during the recent past and the concept of pig farming is changing from that of a zero input to that of a semi-commercial one (Bajarbaruah and Taneja, 2002). Welfare evaluations entail significant impact on success and profitability of pork production operation to impel pig performance in the scientific community. Any slight change in management and husbandry may erode into the economics of production system. The routine handling and surgical procedures in pig husbandry, such as castration and ear notching can be one among the major causes of stress. Effective stress management can reduce the impact in production. In order to ensure this, it is essential to assess the stress generated by routing surgical procedures and the handling associated with them, which in turn are influenced by the age of the animal (Anil *et al.*, 2002)

The concern in modern management related to livestock husbandry and welfare is whether the routine husbandry procedures cause undue stress in farm animals. Currently such information is lacking and the findings of the present

study are expected to provide this knowledge, which will help to re-orient the management in swine farms and to make it suitably sustainable.

Castration provides a useful model system to validate behavioural indicators of stress. It is generally agreed that pigs express their discomfort by three mechanisms, such as behavioural alterations, vocalization and physiological response. Thomas *et al.* (2002) opined that for sustainable livestock production there should be economic viability, technical feasibility, social acceptability and resource conservation ability. Present trend of management is to give greater emphasis on the study of behaviour and apply control through intelligent manipulation of their behaviour than through inflicting painful anti-welfare procedures.

Vocalization is an outward response of the animal and could be used to estimate the animals' current behavioural modality and its welfare. In stress studies and evaluation, changes in concentration of circulating hormone corticosteroids, released from the adrenals are often used as major physiological indicator. Salivary cortisol measurement is an effective technique with reliable end point of stress (Greenwood and Shutt, 1992). Assessment of salivary cortisol has acquired momentum with the advent of radio immuno assay techniques. Stress assessment has become precise and perfect utilizing modern techniques.

The present study is envisaged with the following objectives:

1. To assess stress due to handling and performing the routine surgical procedures such as ear notching and castration.
2. To compare the effect of individual and combined performance of the routine surgical procedures.
3. To assess effect of age on stress due to handling and castration.

Review of Literature

2. REVIEW OF LITERATURE

2.1 STRESS AND WELFARE

Stress refers to the behaviour and physiology of animals in purposive terms, implying that an animal adjusts its functioning in order to maintain itself and its environment within certain limits. An animal is said to be in a state of stress if it is required to make abnormal or extreme adjustments in its physiology or behaviour in order to cope with adverse aspects of its environment and management. A husbandry system can be said to be stressful if it makes abnormal or extreme demands on the animals (Fraser *et al.*, 1975).

Mercy *et al.* (1985) opined that a technique of non-surgical method of sterilizing rams using injections of a sclerosing agent is simple, safe, quick and effective method, causing minimal discomfort to the animal.

Blackshaw (1986) inferred that welfare indicators are health, productivity, physiological and biochemical changes, and behavioural patterns; they can often be correlated from a husbandry system to give a good indication of the welfare status of the animals in the system. The most obvious to monitor is behaviour, as even a slight change in it can indicate a change in welfare status.

Brooks and Pearson (1986) studied the steroid hormone pathways in the pig, with special emphasis on boar odor. They suggested that auto immunization techniques appeared to inhibit the production of compounds (C_{19} -Δ^{16}-steriods) responsible for boar odor in male pigs. It was reported that improved efficiency and greater leanness was maintained in pigs raised for meat production.

Welfare is a wide term that embraces both the physical and mental well being of the animal. Any attempt to evaluate welfare, therefore must take into account the scientific evidence available concerning the feelings of animals that can be derived from their structure and functions and also from their behaviour (Duncan, 1993).

Stress adversely affects well-being only when the stress is of such a magnitude that it results in a significant alteration in biological function. The greater the period of stress, the greater the change in biological function. The greater the change in biological function, the greater is the biological cost and in turn the greater is the risk to the animals' welfare. By using biological risk assessment it is possible to make decisions about welfare by weighing the biological risk versus the need of such activities and also to alter the level of acceptable biological cost (Moberg, 1993).

The challenge for the livestock industry has been to integrate the emerging body of knowledge about clinical ethology in food animals, which attempts to document and quantify animal distress and suffering, into efficient production practices. Primary concern is to determine scientific measures of well being in food producing animals. Development of long-term management options and short-term production practices about animal well-being was to be accomplished (Crook and Heider, 1994).

According to Broom (1996a) the word 'stress' should be used for that part of poor welfare, which involves failure to cope. If the control systems regulating body state and responding to dangers are not able to prevent displacement of state outside the tolerable range, a situation of different biological importance is reached. Stress concerns situations where there is failure to cope.

Welfare is solely dependent on animal's sentience (capable of feeling), (Duncan, 1996). He further suggested that methods to assess welfare should be based on the animal's feelings or emotions and not on non-sentient states such as surgical procedures.

Pederson (1996) suggested that methods used as welfare indicators should not necessarily be generalized to all species, but within species the search for the most reliable method should be intensified.

In order to evaluate welfare of animals, the measurement of habituation to specific challenges could provide valuable information. It is important to know which aspects of the environment and which behavioural demands or needs are significant to the animal and also how the animal ranks them (Sande *et al.*, 1996).

Assessment of animal welfare must be based on scientific knowledge and practical experience related to behaviour and physiology (Simonsen, 1996). It was believed that physiological and behavioural requirements differ regarding the mental experience of the animal.

The ability to quantify the degree of pain experienced by animals is an important component in the assessment of animal welfare. This is especially where husbandry procedures such as tail docking, mulesing, dehorning, disbudding, beak trimming, branding and castration are routinely performed on fully conscious animals without the use of any anesthesia or analgesia. The most important tool for assessing pain and suffering, especially in field conditions, is observing the behavioral variables and physiological measurements. The continuing efforts to measure pain will make required contributions to assess and improve animal welfare (Barnett, 1997).

The animal's response could be determined by a complex interaction of genetics and previous experience. Studies to assess animal welfare during handling should contain both behavioural and physiological measurements. Cattle and pig producers need to select animals with a calm temperament because excessive excitability creates serious animal welfare problems during handling (Gradin, 1997).

Odendaal (1998) suggested that enhancing animal welfare and well being in practice requires evaluation of the animals' interaction with their daily surroundings. Animal health in this sense would be to create equilibrium between the animal and its environment.

Schutte *et al.* (1998) documented that animal welfare is rightly considered by consumers to be an essential link in the production chain of providing safe, high-quality food. Therefore, welfare should always be addressed integrally with environmental and agronomic concerns. It was reported that hands-on system of defining animal welfare was useful to evaluate various production systems.

Ewing *et al.* (1999) opined that presence of acute stress does not compromise an animals' well being. The effects of stressors were coped with biological mechanisms. The issue of well being comes into play when stress was prolonged to create a pathological condition or aberrant behaviour.

Fraser (1999) believed that scientists and philosophers concerned with animal ethics work to provide a framework that will help us understand and articulate our proper relationship to animals of other species, and to translate this understanding into appropriate action.

It is necessary to monitor current findings about the physiological basis of the distress caused by painful husbandry practices, so that efforts can be made to minimize distress. In husbandry procedures more emphasis was placed to evaluate and minimize the pain and distress (Mellor and Stafford, 1999).

Rowan *et al.* (1999) reported that controversial elective surgeries include castration, tail docking, beak trimming, dehorning, ear notching and branding. These procedures are often performed with the idea that short-term suffering may be induced, but the welfare benefits are lasting.

Curtis (2000) described four kinds of stress, such as understress (as in a barren environment), eustress (exciting but pleasurable), overstress (unpleasant stimulation with which the animal cannot cope) and distress (severe overstress).

Kruip and Van (2000) opined that introduction of new biotechnologies into farm animal husbandry should be accompanied by scientifically valid and

systematic studies into the effects on animal welfare, with the help of a comprehensive protocol.

As per (Mellor *et al.*, 2000) stress response refers to the full range of physiological reactions. This resulted from the small deviations to the maximum physiological changes of body systems, under extreme challenge.

2.2 BEHAVIOUR MEASURES

Sanford *et al.* (1986) ascertained that some species communicate their experiences by means of bodily attitude and other postural mechanisms. Also, particular changes, in posture are often elicited by painful foci in particular parts of the body.

Kitchell (1987) studied the problems in defining pain and peripheral mechanisms of pain. He opined that pain in animals is an aversive sensory and emotional experience, which led to learned avoidance by protective motor actions.

Marks *et al.* (1988) conducted a study to compare the influence of early castration at day one, seven or 14 on performance and carcass traits in swine. Result suggested that day one castrates had significantly longer carcass and more carcass back fat than their 14-day counterparts.

Fraser and Broom (1990) inferred that in describing, recording and measuring behaviour, the animal's attitude, disposition and temperament should be assessed before any handling procedures. Its alertness and apparent awareness of its general environment should be noted. Appraisal of the animal's willingness to move and the nature of its gait are important considerations. Behavioral examinations are best performed in a quiet space or enclosure with limited light where distractions will be minimal. They have also evaluated the initial response of a pig to a disturbing situation, such as the close approach of a person or a sudden noise.

Behavioral responses caused by castration with rubber rings in day old lambs and kids; and in hand reared calves aged one to seven days, were estimated by Mellor (1991). According to him, the distress was greatest in lambs, intermediate in kids and least but not necessarily absent in hand reared calves.

In behavioral studies, the recording of an animal's response to a particular set of environmental circumstances can provide much information on the mental, as well as the physical, state of the animal under observation. Also, the response of an animal to a choice of environment provides an insight into an animal's mental and physical state (Moss, 1992) in connection with livestock health and welfare.

McGlone *et al.* (1993) studied the development of pain in young pigs associated with castration. They have observed that castration caused measurable changes (reduced suckling, reduced standing and increased lying times, $P<0.5$) in the behaviour of young pigs compared to that of intact pigs. Effects of age and interactions between age and castration treatment were not significant ($P<0.10$) for any behaviour evaluated. Pigs showed similar behavioral changes when castrated from one to 20 days of age. Pig performance data favoured castration at 14^{th} day rather than at first day of life. .

Broom (1996b) opined that it is important to distinguish between normal and abnormal behaviour in the welfare assessment. In pigs' stereotypy such as sham chewing and bar biting were abnormal behaviours.

Jensen *et al.* (1996) reported the effect of long-term stress on the hypothalamic-pituitary-adrenocortical axis and the role of the stressor. He suggested that in addition to the strength of the stressor, other factors such as the animal's perception of controllability and predictability of the stressor are believed to influence the stress response.

Pederson (1996) suggested that when assessing welfare of farm animals both behavioural and physiological measurements were needed to make reliable conclusions.

Mench and Mason (1997) recognized that behaviour observation is a measure of welfare, providing excellent cues about the preferences, needs and internal states of animals. Animal associated with states of pain, disturbance, distress and fear indicated reduction in welfare. Since behaviour varied with situations, knowledge of species, strain, behaviour of individuals and their social groups was a pre-requisite to assess welfare.

Assessment of acute pain in farm animals using behavioural measurements was done by (Molony and Kent, 1997). They have concluded that pain response modify animal's behaviour that minimize pain and assist healing (eg.. lying or standing still). Local anaesthetic treatment could reduce or eliminate behavioural changes produced by castration.

Houpt (1998) studied the domestic animal behaviour and opined that, the tail in piglets was a good index of general well being in most breeds. A straight one indicated fright or isolated distress. Irritation of skin caused tail twitching and increased defecation rate. It was indicated that normal pigs does not wander. Castrated piglets stood, suckled less and laid more, at any age from day one of life, indicating pain perception. In confined environment, piglets explored by rooting, mouthing, biting and chewing. In 3-4 weeks of age, typical porcine startle reaction (a woof and freezing behaviour) could be elicited.

Enlightened design criteria increased the ease with which the animals were handled. Similarly understanding the animals' movement and confinement could enhance safety for both animals and handlers (Ewing *et al.*, 1999)

Mellor *et al.* (2000) opined that pain as a stressor elicited a range of physiological and behavioural responses. This aided to assess the impact of pain-causing stimuli on animals and devise strategies to alleviate pain.

Aversion learning techniques was used by Pajor *et al.* (2000) to determine the handling practices in cattle. Results proved that these techniques were effective to find the aversive or rewarding practices.

Wemelsfelder *et al.* (2000) believed that spontaneous qualitative assessment of behaviour provides empirical access to behavioral expressions and thus potentially to associated states of welfare in pigs.

Bataille *et al.* (2002) suggested that grinding down or clipping of the temporary incisors and docking should be carried out as routine practices in pigs, only when problems such as vice of tail biting and damage to the teats occur.

Effect of age at castration on certain growth characteristics of Hampshire barrows, was studied by Mili *et al.* (2002). Results showed that pigs castrated at 7^{th} week had highest weight gain (0.296 kg) while it was lowest (0.272 kg) in, 5^{th} week castrated group. They opined that castration greatly influenced growth rate in meat animals and was considered an important tool to enhance meat production.

Thornton and Pearson (2002) evaluated the behavioural responses to castration in lambs. It was found that castration resulted in significant reductions in the time spent performing play behaviour in one-week old lambs and lying behaviour in 4 to 6 week old lambs. There was also a significant increase in abnormal postures following castration, in 4-6 weeks old lambs.

Individual differences in animal behaviour could elucidate the differences in stress coping style (Van *et al.*, 2002). Other factors such as time and test situation has important role in determining an individual's behavioural reaction.

Hay *et al.* (2003) assessed the behavioral signs indicative of pain over five days following castration of piglets. Results showed significantly more pain or castration related behaviour (trembling) during the first hour following the treatment. Tail wagging existed for two days after castration. It was confirmed

that castration induced pain and behaviour responses were exacerbated during the first few hours following the procedure.

2.3 VOCAL RESPONSE

Houpt and Wolski (1982) opined that vocal signals are probably the most important means of communication in pigs. They have inferred that isolation in a strange place caused vocalizations. The common 'grunt' is given in response to familiar sounds or while a pig is rooting, which lasts 0.25 to 0.4 seconds. The staccato grunt or short grunt is shorter, for 0.1 to 0.2 seconds and is given by an excited pig. A startled pig gives bark. Long grunt persisting for 0.4 to 1.2 seconds, appears to be a response to pleasurable stimuli and squeal is the more intense vocalization. Common sequence is to proceed from common grunts to staccato grunts to repeated grunts without interruption and to grunt squeals to screams as the animal is approached, chased, picked up and injected.

Oldham (1985) stated that pigs vocalize at the slightest provocation (squealing or long grunting), and this may indicate anything from severe pain to very minor discomfort as in attempted handling.

Pigs normally squeal and attempt to escape when handled but these reactions may be accentuated in pain. Squealing is characteristic, when painful areas are palpated. The lack of positive signs of normal social behaviour and vocalization are indicators of a pig in pain (Sanford *et al.*, 1986).

Braithwaite *et al.* (1994) used vocalization produced by piglets to distinguish between the distress caused by the handling necessary to perform a castration and sham castration. Results clarified that the rate of vocalization (calls/s) was significantly greater (1.1+0.1vs.0.7+0.2), with higher frequency (4483+209 vs.3483+412 HZ), longer duration (650+53 vs.446+65 ms) and larger amplitude (8 dB greater) in castrated than in sham castrated ones. They have suggested that while the handling during castration is stressful, the actual castration procedure would be significantly more distressing.

Weary and Fraser (1995) determined that, 'non-thriving' and 'unfed' piglets called more and used more high-frequency calls, longer calls than their 'thriving' and 'fed' littermates.

White *et al.* (1995) studied the vocalization response of pigs during castration. Result showed that pigs castrated without 'lidocaine' had higher frequency of highest energy measurements of vocalization ($P<0.05$). They have suggested that castration without anesthetic is of greater stress for pigs eight days of age or older.

Duncan and Fraser (1997) postulated that suckling piglets elicited characteristic vocalizations and experienced a particular kind of distress when separated from the mother.

Vocal response to pain in piglets subjected to castration without anaesthetic was studied by Weary *et al.* (1998). Their result showed that castrated piglets produced significantly more high frequency calls (>1000 Hz) than sham castrates. Castrated piglets produced calls with a longer mean duration than sham piglets. It was also suggested that increased rate of high calls was a reliable indicator of pain. But the way in which the piglets were restrained does not affect the pain caused by castration.

Vocal responses of piglets, as indicators of well-being were monitored by Cloutier *et al.* (2000). They have observed that playing music or other sounds provided no improvement in conditions for piglets during handling and weaning.

Research on automatization of livestock management using animal voice, as the expression of animal demands was undertaken by Ishii and Ikeda (2000). They have reported that the average rate of exact recognition of the condition was 92.40 percent.

Taylor and Weary (2000) identified the vocal responses of piglets to castration without anaesthesia. Compared to sham operated animals, castrated

piglets produced much more high-frequency calling. It was suggested that use of local anaesthetic might potentially increase the stress of handling and restraint.

Watts *et al.* (2000) evaluated the variability in vocal and behavioural responses to visual isolation between full-sibling families of beef calves. It was assessed that endogenous characteristics such as sex, age and weight also influenced vocal response

Marchant *et al.* (2001) identified the vocalization of adult female domestic pig during a standard human approach. It was observed that the animal performed a number of distinct vocalizations during isolation. Short single grunts appeared to be associated with investigatory behaviour. Long single grunts might be a form of contact call, the rate of which was related to physiological and behavioral activity. Squeals had similar function but resulted from a higher level of arousal. Short, rapidly repeated grunts appeared to have either a greeting or threat function.

Taylor *et al.* (2001) tested the vocal and behavioural responses of piglets during castration. It was assessed that during the castration, piglets produced higher frequency calls (>1000 Hz) at more than three times the rate of sham-castrated groups. The results concluded that, while the factors affecting both the shams and castrates varied with age, the pain of castration was not affected within the range of ages tested at three, ten and seventeen days.

2.4 HORMONAL ASSESSMENT

Dantzer (1979) noted that the stress of social isolation and exposure to a new environment, induced hyperactivity, vocalizations and ACTH release in recently weaned piglets. It was further identified that these reactions were increased by pretreatment with Morphine (0.5 and 1 mg/kg) and decreased by Naloxone (1 mg/kg).

Moberg *et al.* (1980) reported that in one-week-old lambs, the plasma corticosteroid (ng/ml) concentration were, 14.5, 48.9 and 48.9 at rest, after restraint and after administration of exogenous ACTH respectively. He suggested that exposure to open field arena was stressful to lambs regardless of the age. Also, previous exposure to the testing procedure has not decreased the response of adrenal axis to the stimulus.

Fell *et al.* (1985) compared the cortisol concentration (mean ± SE) in samples of saliva and blood serum from eight ewes under two managemental procedures. Results indicated that, salivary steroid measurement was a powerful diagnostic tool, as it measured the 'free', biologically active fraction rather than 'total' steroid.

Fell *et al.* (1986) studied the effects of castration in 4-11 weeks aged calves. The short-term salivary cortisol response was significantly higher after surgical castration than after elastrator castration and it was significantly higher than in the control group. Maximum concentrations (mean ± SE) were reached one hour after treatment in these groups. The recorded concentrations were 1.1 ± 0.1 (nmol/l) in control group, 10.2 ± 2.6 (nmol/l) in surgical castrated group, and 3.2 ± 0.6 (nmol/l) in elastrator group and1.1 ± 0.1 (nmol/l) in control group. Salivary cortisol level was elevated over a period from 15 minutes to three hours.

The rise in plasma corticosteroids that occurred in response to exposure to a particular husbandry procedure determined the degree of suffering or distress experienced by an animal (Rushen, 1986).

Sanhouri *et al.* (1989) measured the effect of different types of transportation on plasma cortisol in male goats. According to them, cortisol concentrations remained within the basal range in male goats 1-5 (ng/ml) during the control periods. Maximum values of cortisol 13-24 (ng/ml) were achieved by fifteen minutes after transport in the noisy trolley. Concentrations of greater than

27 (ng/ml) were recorded immediately after motorized van transport. This concluded that adrenal cortex activity was stimulated more by noise than motion.

Barnett and Hemsworth (1990) commented on the validity of physiological and behavioural measures of animal welfare. They agreed that stress was accompanied by a number of physiological changes including the elevation of corticosteroid concentration.

Cohen *et al.* (1990) determined the efficacy and stress of chemical versus surgical castration of cattle. Result showed that plasma cortisol concentrations were greatest at six hour following surgical castration 23.2 ± 0.64 (µg/l) and at three hours following chemical castration 16.8 ± 0.49 (µg/l). Mean cortisol concentration in calves not castrated, was 7.1 ± 0.26 (µg/l). It was concluded that acute stress occurred for six to twelve hour following castration and that surgical castration caused greater stress than chemical castration.

Sanhouri *et al.* (1991) ascertained that Pentobarbitone (20 mg/kg iv) could block plasma cortisol release and inhibit the stress response to transport in male goats. Cortisol (ng/ml) concentration ranged from 0.2 to 6 (ng/ml) for control groups and increased to 26.6 ± 0.5 (ng/ml) within 15 minutes of van journey, reaching baseline values by 1.5 to 2 hours. Pentobarbitone kept cortisol concentration between 7.7 and 20.3 (ng/ml) during the recovery period.

Greenwood and Shutt (1992) assessed cortisol level in saliva and plasma as an index of stress in one to three weeks old goats. Transport of adult goats caused significant increases ($p<0.001$) in free cortisol in saliva and in free and total cortisol in plasma. It was demonstrated that salivary cortisol was a useful measure of stress in adult goats.

Andrews (1992) conducted a study on corticosteroid levels, in livestock health and welfare. Results suggested that, corticosteroid levels rose abruptly when pigs were subjected to acute stress. But, decrease in level to chronic stress was due to decrease in receptors to corticotrophin release factor.

Fisher *et al.* (1996) determined the effect of castration methods in bull calves on their plasma cortisol level. Values reported were 5.0 (ng/ml) in control group, 30.9 (ng/ml) in surgical castrates without local anaesthetics and 19.7 (ng/ml) in surgical castrates following local anaesthetics administration. It was concluded that local anaesthetic reduced the pain associated with castration.

Jensen *et al.* (1996) stated that increased plasma concentration of glucocorticosteroids reflected the strength of stressor applied.

Ruis *et al.* (1997) examined the circardian rhythmicity of salivary cortisol in growing pigs. The cortisol values were 1.19 ± 0.05 (ng/ml) in 12 weeks, 1.03 ± 0.06 (ng/ml) in 16 weeks, 0.74 ± 0.05 (ng/ml) in 20 weeks, and 0.71± 0.06 (ng/ml) in 24 weeks of age. The concentrations were, 1.01 ± 0.05 (ng/ml) in barrows and 0.86 ± 0.04 (ng/ml) in gilts. Stress applied in morning was 1.38 ± 0.46(ng/ml) higher in 12 weeks and 0.31 ± 0.14(ng/ml) higher in 20 weeks than in the evening.

The most frequently monitored physiological responses to acute stress were increased secretion of glucocorticoids from the hypothalamo-pituitary adrenal axis into the blood. An increase in body temperature, immunosuppression, increase in plasma ion levels and reduction in weight gain may accompany stress. Animals tend to differ consistently in stress reactivity (Terlouw, 1997).

Kent *et al.* (1998) recorded plasma cortisol changes in week old lambs subjected to castration or tail docking. Results suggested that injection of anaesthetics into the testes was less effective than injection into the neck of the scrotum.

Ewing *et al.* (1999) reported that levels of one or more corticosteroid hormones in blood, saliva or urine along with response duration and variability were often determined to assess stress status of the animal.

Changes in plasma cortisol concentrations do not measure pain as such. But they provided an indication of the overall noxiousness of the experience. Both physical and emotional components were included in case of pain induced distress (Mellor *et al.*, 2000)

Rosochacki *et al.* (2000) determined the influence of restraint immobilization stress on the concentration of plasma cortisol of Pietrain and Duroc pigs. The concentration of plasma cortisol increased with the duration of stress and there was a significant interaction between the breed of animals and time of stress.

Schonreiter and Zanella (2000) assessed salivary cortisol in swine by a new methodology. They have found that Oral Diffusion Sink (ODS) method aided continuous measuring, without any manipulation of the animal.

Gallagher *et al.* (2002) collected saliva from neonatal piglets in order to assess the secretory dynamics of cortisol in newborn piglet. Values showed that salivary cortisol concentration was high perinatally and declined with age. A circardian pattern was evident in female pigs from day six, whereas males displayed a similar pattern from day ten. Results suggested that collection of saliva in the piglet provided a viable alternative to blood sampling.

In assessment of pain induced by castration in piglets, there were no clear effect of castration on urinary corticosteriods and catecholamines (Hay *et al.*, 2003)

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL LOCATION

The study was conducted at the Center for Pig Production and Research (CPPR) of Kerala Agricultural University, Mannuthy; located seven km East of Thrissur, at an altitude of 50m to 928m above msl (mean sea level) with latitude 10° 20` to 10° 56`N (north) and longitude 76° 05` to 70° 45` E (east). The state is endowed with humid tropical climate with maximum rainfall by South West monsoon from June to August and North East monsoon from September to October.

The experiment was scheduled for two months, from February to March 2003. The mean maximum and minimum ambient temperature was 34.7°C and 23.6°C in February and 34.6°C and 24.1°C in March respectively. The mean diurnal atmospheric humidity percentage was 83 in February and 86 in March. Rainfall recorded was 162 mm in February and 95 mm in March 2003.

3.2 ANIMALS

Purebred Large White Yorkshire; Landrace; Duroc crossbred and Indigenous pigs were scientifically maintained in the farm. The piglets were reared till weaning in natural system, with their respective sows.

3.3 MANAGEMENT SYSTEM

The animals; housed in intensive system, were provided with 3 x 4 meters of floor space per sow with litters. Animals were identified based on their ear notches. Ear notching was done when they were one-week old. They had a body weight of one to one and a half kg. Castration was performed at eight weeks when they weighed 9 to 10 kg.

3.4 EXPERIMENTAL DESIGN

60 male piglets formed the sample for the study. Ten treatments with six replicates were evolved from these piglets. The same handler under the same standard conditions did the handling and routine surgical procedures, throughout the experiment to avoid variation. The experiment was performed in isolated pens of 3 x 4 meters floor space per animal. Behaviour and vocalization were observed and recorded. Salivary cortisol level was also determined. The ten treatment groups were:

T_{1A} One-week-old control group with no treatment,

T_{1B} 8 weeks old control group with no treatment,

T_2 Handling for ear notching at 1st week of birth without performing ear notching

T_3 Handling for ear notching at 1st week of birth with performing ear notching

T_4 Handling for castration at 1st week of birth without performing castration (sham castration)

T_5 Handling for castration at 1st week of birth with performing castration

T_6 Handling for castration at 8th week of age without performing castration (sham castration)

T_7 Handling for castration at 8th week of age with performing castration

T_8 Handling for ear notching and castration simultaneously at 1st week of age without performing the procedures

T_9 Handling for ear notching and castration simultaneously at 1st week of age with performing the procedures

Sham castration: Restrained identically but not castrated

3.4.1 Method of castration

Subjects selected for castration, were restrained by local anaesthesia. 'Open covered' method of castration was followed. Piglets allotted to 'Sham castration' were handled similarly except that no operation was performed.

3.4.2 Method of ear notching

Experienced personnel in the farm performed ear notching using ear notcher. All male piglets were notched first followed by females or vice versa.

3.5 RECORDING OF BEHAVIOUR

Behaviour patterns were recorded using 'Hitachi' color video camera cum Videocassette recorder with provision of horizontal resolution of 625 lines and 25 frames of auto speed and digital signal processing and it was transcribed into Compact Discs. Still pictures were taken using f 50 Nikon camera with Fuji crystal 200 films. 'Focal animal sampling' method, described by Martin and Bateson (1988) was used in the present study to observe each individual for a specified amount of time.

Behaviour scorecard used for the experiment:

Sl. No	Behaviour	Description	Scores*
1	Nosing, Mouthing	Snout is close to or in contact with a substrate	10
2.	Biting, Chewing	Biting and/or nibbling the bar or substrates	20
3.	Rooting	Digging the floor or substrate with the snout	30
4.	Attempt to escape	Attempting to escape when approached or handled by personnel	40
5.	Trembling	Shivering, in sitting, standing or recumbent posture	50
6.	Startle reaction	Typical porcine reaction, with a 'woof and freezing' posture	60
7.	Tail twitching	Twitching of tail or Straightened tail	70
8.	Elimination	Defecation and/or Urination	80
9.	Ventral recumbency	Motionless; body weight supported by belly. Sternum in contact with floor	90

* Scoring relates to intensity of stress

Observations were made in four different time frames *viz*: 30 min before the treatment, during the treatment, 30 minutes after the treatment and 24 hours after the treatment, respectively. Behaviour pattern scored in four different time frames were tested using Non-parametric, 'Mann-Whitney U test' (Siegel, 1988).

3.6 RECORDING OF VOCALIZATION

Vocalizations of piglets were recorded using VHS video camera with sensitive Audio port. Care was taken to avoid background noises in most cases. Observations were made in four different time frames. The intensity, duration and frequency of vocalization were determined. The intensity was measured from the graphs made using 'Sound Forge' and 'SoX' Software.

'Sound Forge' is the digital audio editor that includes a powerful set of audio processes. It could view, select, edit and encode audio files. It has got non-destructive extreme speed and accuracy. Spectrum analysis tool analyze waveform by frequency to identify noise problems.

'SoX' (Sound Exchange) is a sound-processing software. This command line utility could convert various audio files into other formats.'SoX' was created by *Lance Norskog* and has been around since 1992.

Duration referred to the total length of time for which all occurrences of behaviour lasted over some specified time.

Frequency was the total number of occurrences of behaviour over specified period. "Analysis of variance" (ANOVA) was found using the model ($y_{ij} = \mu + \alpha_i + e_{ij}$)

Where,

y_{ij} = Value of the variate of the j th replicate of the ith treatment, where i varies from 1 to 9 and j varies from 1 to 6

μ = General mean effect

α_i = Effect due to ith treatment

e_{ij} = Random error which is assumed to be independently and normally distributed within mean value = 0 and variance $\sigma^2 e$.

Effect of age interactions was tested by t-test (Snedecor and Cochran, 1985).

3.7 SALIVA COLLECTION AND CORTISOL ANALYSIS

The non-invasive technique for cortisol analysis was using the biomaterial saliva. The sample was collected by insertion of cotton buds into the mouth of piglets. The animals were allowed to chew for 1-2 minutes until the buds were thoroughly moistened. The buds were packed in cotton gauze and placed in centrifuge tubes as inner cases. They were centrifuged for five minutes

at 3500 rpm at room temperature to remove the saliva. Usually 1-2 ml of saliva was retrieved, which was then stored at –20°C till cortisol analysis.

Concentrations of cortisol were measured in saliva samples, using Clinical Assays™ Gamma Coat™ Cortisol ^{125}I radioimmunoassay kit ("Diasorin", Stillwater, Minnesota, USA). The procedure was based on the competitive binding principles of radioimmunoassay. Standards (10μL) and unknown samples (10μL) of saliva were incubated with cortisol tracer (1.0 ml tracer-buffer reagent) in antibody (rabbit anti-cortisol serum) coated tubes. The antibody was immobilized onto the lower inner wall of the Gamma Coat tube. After incubation for 45 minutes in a 37±2°C waterbath, the contents of the tube were decanted. The counts per minute (cpm) bound for each tube were counted in 1480 WIZARD™ Automatic Gamma Counter for one minute with the window suitably adjusted for iodine-125. A standard curve was plotted with cpm values and cortisol concentration standards 1, 3, 10, 25 and 60 (μg/dL) on semi-logarithmic graph paper. Unknown values were interpolated form the standard curve. Results were tested by "analysis of variance"(ANOVA), using the model ($y_{ij} = \mu + \alpha_i + e_{ij}$).

Results

4. RESULTS

4.1 MEASURING BEHAVIOUR

Mean behaviour scores to assess stress, due to handling and performing the routine surgical procedures such as ear notching and castration, are presented in table 1. During the handling for ear notching, piglets attempted to escape, twitched their tail and defecated. During ear notching, the animals trembled, twitched their tail, urinated and attempted to escape more appreciably than handled for the same. In piglets handled for castration, trembling, startle reaction (typical porcine reaction with a 'woof and freezing' posture) are noticed during handling. During castration, piglets showed reduced attempts to escape and decreased tail twitching. These behaviour patterns are found after 30 minutes and 24 hours of castration. When the subjects handled for ear notching, was immediately handled for castration, they attempted to escape. Piglets with combined performance of ear notching and castration showed pain related behaviours such as startle reaction, tail twitching, straightened tail and they wandered less. These behaviours are observed around 30 minutes after the procedures and are not appreciable after 24 hours. Control group piglets with no treatment are observed to be nosing, mouthing, biting, chewing or rooting the substrates. Different behaviour pattern in piglets subjected to various stressors are picturised in Plate 1. Mean behaviour scores ranged from 36.66 ± 5.58 to 148.33 ± 7.03 at one week of age and from 43.33 ± 5.58 to 126.67 ± 13.33 at eight weeks of age.

4.1.1 Scores in one Week Old Piglets

4.1.1.1 Observation during Second Time Segment (During the Treatment)

Treatment group (T_3) that was ear notched had higher behaviour scores and differed significantly ($p<0.05$) from the control group with no treatment. Castrated group (90.00±12.90) with higher behaviour scores differed significantly

A

B

C

Plate.1. Behaviour Pattern in Piglets

A. Nosing

B. Attempt to escape

C. Trembling

D

E

F

Plate.1. (Contd.)
D. Startle reaction
E. Tail twitching
F. Ventral recumbency

Table 1. Stressors and mean behaviour scores in piglets of different ages during four-time segments

Treatments	Time segment			
	First	Second	Third	Fourth
One Week of Age				
T_{1A}	43.33 ± 5.58	43.33 ± 5.58[a]	43.33 ± 5.58[a]	43.33 ± 5.58[a]
T_2	40.00 ± 5.16	90.00 ± 10.95[b]	50.00 ± 6.83[a]	50.00 ± 5.77[a]
T_3	40.00 ± 2.58	123.33 ± 6.14[b]	101.67 ± 16.00[b]	50.00 ± 7.30[a]
T_4	40.00 ± 3.65	103.33 ± 7.15[b]	51.67 ± 5.42[a]	55.00 ± 5.63[a]
T_5	40.00 ± 2.58	90.00 ± 12.90[b]	133.33 ± 9.89[b]	145.00 ± 10.24[b]
T_8	40.00 ± 3.65	102.27 ± 9.50[b]	53.00 ± 9.18[a]	55.00 ± 6.19[a]
T_9	36.66 ± 5.58	106.67 ± 14.29[b]	123.33 ± 6.66[b]	148.33 ± 7.03[b]
Eight Weeks of Age				
T_{1B}	46.67 ± 6.66	46.67 ± 6.66[a]	46.67 ± 6.66[a]	46.67 ± 6.66[a]
T_6	48.00 ± 1.67	91.66 ± 17.59[a]	80.00 ± 3.65[b]	58.33 ± 6.00[a]
T_7	43.33 ± 5.58	85.00 ± 7.19[b]	126.67 ± 13.33[b]	120.00 ± 5.16[b]

Note: Means bearing different superscripts within the same column differ significantly ($p<0.05$)

First time segment : 30 minutes before the treatment
Second time segment : During the treatment
Third time segment : 30 minutes after the treatment
Fourth time segment : 24 hours after the treatment

($p<0.05$) from the control group (43.33 ± 5.58). Treatment group that has been exposed to ear notching and castration scored high in behaviour and differed significantly ($p<0.05$) from the control group.

4.1.1.2 Observation during Third Time Segment (30 Minutes after the Treatment)

Behavioural scores were higher in ear notched group, which differed, significantly ($p<0.05$) from the treatment group handled for ear notching. Castrated group (133.33 ± 9.89) with higher behaviour scores significantly differed ($p<0.05$) from sham-castrated (restrained identically but not castrated) group (51.67 ± 5.42). Treatment groups with combined performance of ear notching and castration scored high in behaviour and differed significantly ($p<0.05$) from the group exposed to only handling for ear notching and castration.

4.1.2 Scores in Eight Weeks Old Piglets

Castrated group (T_7) had shown lower behaviour scores and differed significantly ($p<0.05$) with the sham-castrated group (T_6) during the treatment. In fourth time segment, castrated group scored high in behaviour and differed significantly ($p<0.05$) from the sham-castrated group.

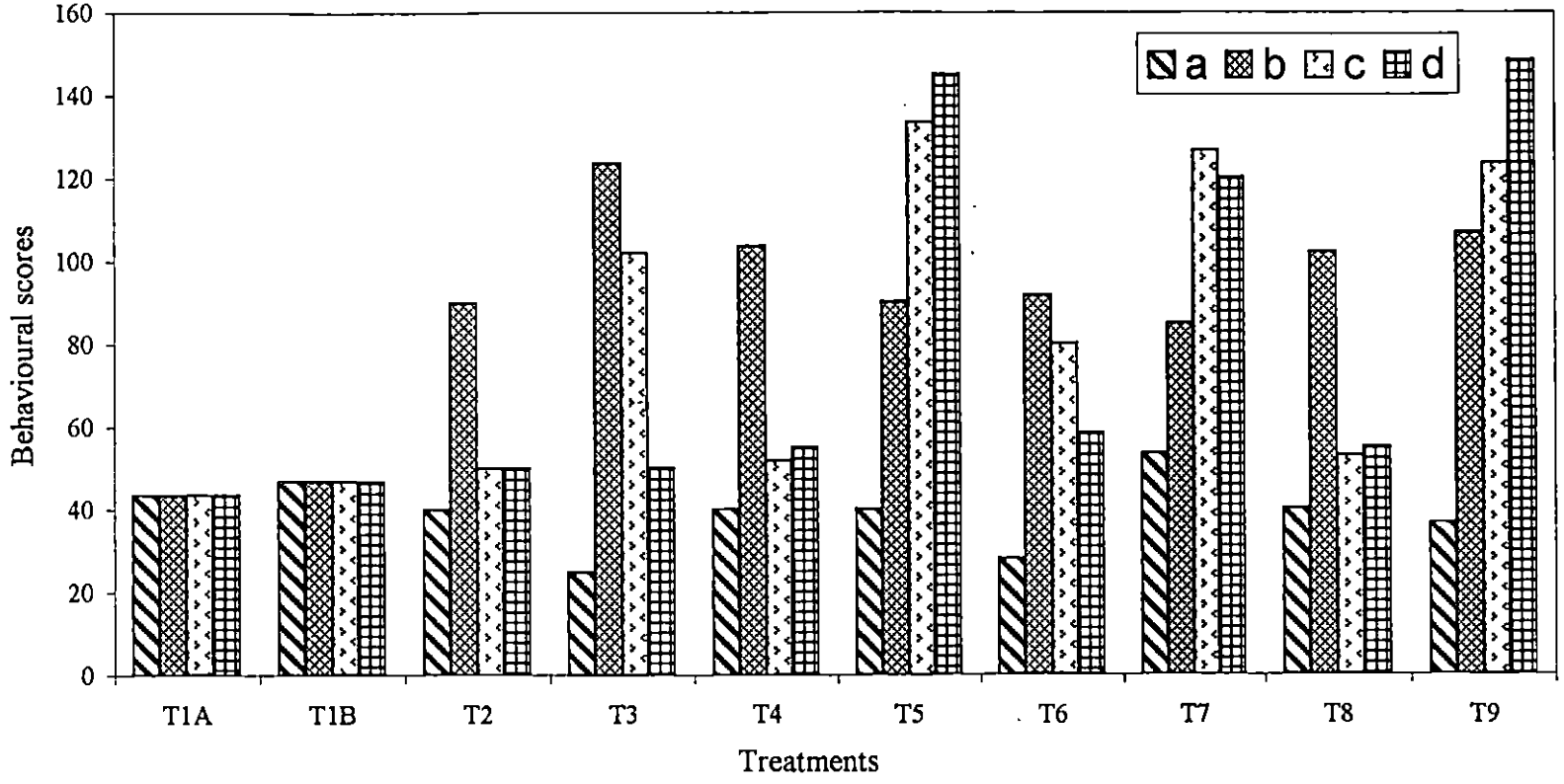
4.1.3 Age Interaction

Assessment of effect of age on stress is given in table 2. In the treatment groups of sham-castration, groups of eight week olds; attained higher behaviour scores and differed significantly ($p<0.05$) from one-week-old groups. This was observed during third time segment. Fig. 1. depicts the mean behaviour scores in piglets exposed to nine different stressors.

Table 2. Effect of age with the mean behaviour scores under two different stressors

Treatments	Time segment			
	First	Second	Third	Fourth
	Sham castration			
T_4	40.00 ± 3.65	103.33 ± 7.15	51.67 ± 5.42[a]	55.00 ± 5.63
T_6	48.00 ± 1.67	91.66 ± 17.59	80.00 ± 3.65[b]	58.33 ± 6.00
	Castration			
T_5	40.00 ± 2.58	90.00 ± 12.90	133.33 ± 9.89	145.00 ± 10.24
T_7	43.33 ± 5.58	85.00 ± 7.19	126.67 ± 13.33	120.00 ± 5.16

Note: Means bearing different superscripts within the same column differ significantly ($p<0.05$)



a. First time segment b. Second time segment c. Third time segment d. Fourth time segment

Fig.1. Stressors and mean bahaviour scores in piglets

4.2 MEASURING VOCALIZATION

4.2.1 Intensity of Vocalization (Decibels)

Mean intensity of vocalization to assess stress due to handling and performing the routine surgical procedures are depicted in table 3. Influence of various stressors on mean intensity of vocalization in piglets is given in Fig. 2. Mean vocalization intensity varied from 38.50 ± 1.56 to 53.50 ± 2.15 at one week olds and from 47.17± 1.51 to 53.33 ± 1.91 at eight weeks old. Fig. 5. has explained the intensity of vocalization (normalized between 0.4 and –0.4 decibels) in piglets of control group with no stressors [Sound Exchange (SoX) Software].

In one week olds, the intensity of vocalization was higher during simultaneous handling for ear notching and castration that differed significantly ($p<0.01$) from the group in which ear notching and castration was performed. Effect of handling for ear notching and castration on intensity of vocalization (normalized between 1.2 and –0.8 decibels) in piglets [Sound Exchange (SoX) Software] is given in Fig. 6. In Fig. 7. the effect of performance of ear notching and castration on intensity of vocalization (normalized between 1.0 and –0.6 decibels) in piglets [Sound Exchange (SoX) Software] is picturised.

4.2.1.1 Age Interaction

Assessments of effect of age on stress with the criterion, 'mean intensity of vocalization' are given in table 4.This shows that, in third and fourth time period, vocalization was recorded with higher intensity in one week old castrates (T_5) that differed significantly ($p<0.05$) from eight weeks old castrates (T_7).

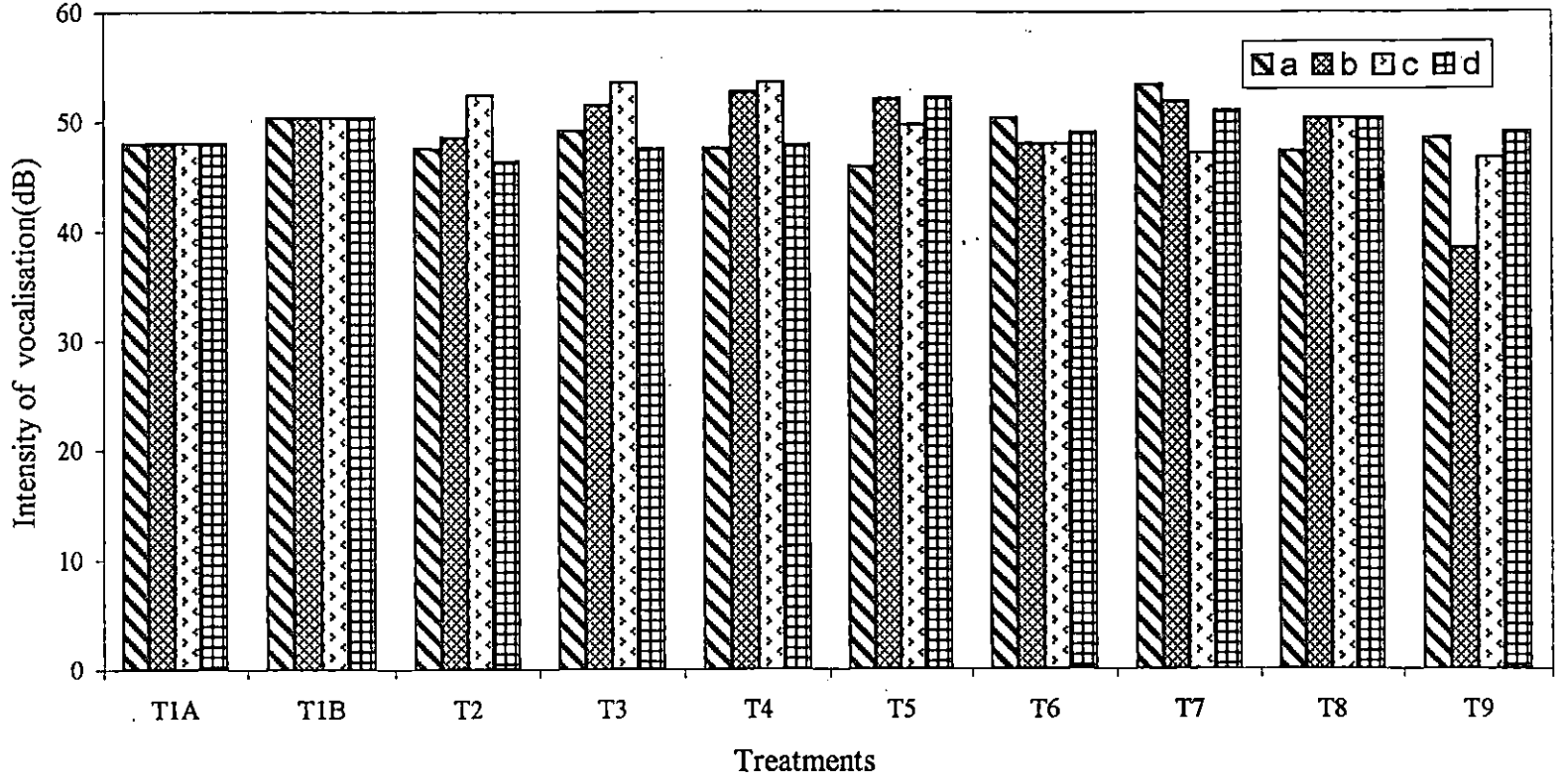
4.2.2 Duration of Vocalization (Minutes)

Mean duration of vocalization to assess stress due to handling and performing the routine surgical procedures are depicted in table 5. Mean duration

Table 3. Influence of stressors on mean intensity of vocalization, decibels

Treatments	Time segment			
	First	Second	Third	Fourth
One Week of Age				
T_{1A}	48.00 ± 1.81	48.00 ± 1.81[a]	48.00 ± 1.81	48.00 ± 1.81
T_2	47.50 ± 1.59	48.50 ± 2.79[a]	52.33 ± 1.73	46.33 ± 2.03
T_3	49.17 ± 0.54	51.50 ± 2.14[a]	53.50 ± 2.15	47.50 ± 2.50
T_4	47.50 ± 1.58	52.67 ± 2.57[a]	53.50 ± 2.06	47.83 ± 1.99
T_5	45.83 ± 1.90	52.00 ± 3.15[a]	49.67 ± 0.33	52.17 ± 1.30
T_8	47.33 ± 1.58	50.33 ± 2.35[a]	50.33 ± 2.46	50.33 ± 2.35
T_9	48.50 ± 1.82	38.50 ± 1.56[b]	46.67 ± 4.22	49.00 ± 2.68
Eight Weeks of Age				
T_{1B}	50.33 ± 2.35	50.33 ± 2.35[b]	50.33 ± 2.35	50.33 ± 2.35
T_6	50.33 ± 2.35	48.00 ± 1.81[a]	48.00 ± 1.81	49.00 ± 2.68
T_7	53.33 ± 1.91	51.83 ± 1.76[a]	47.17 ± 1.51	51.00 ± 2.53

Note: Means bearing different superscripts within the same column differ significantly ($p<0.01$)



a. First time segment b. Second time segment c. Third time segment d. Fourth time segment

Fig.2. Influence of stressors on mean intensity of vocalization, decibels

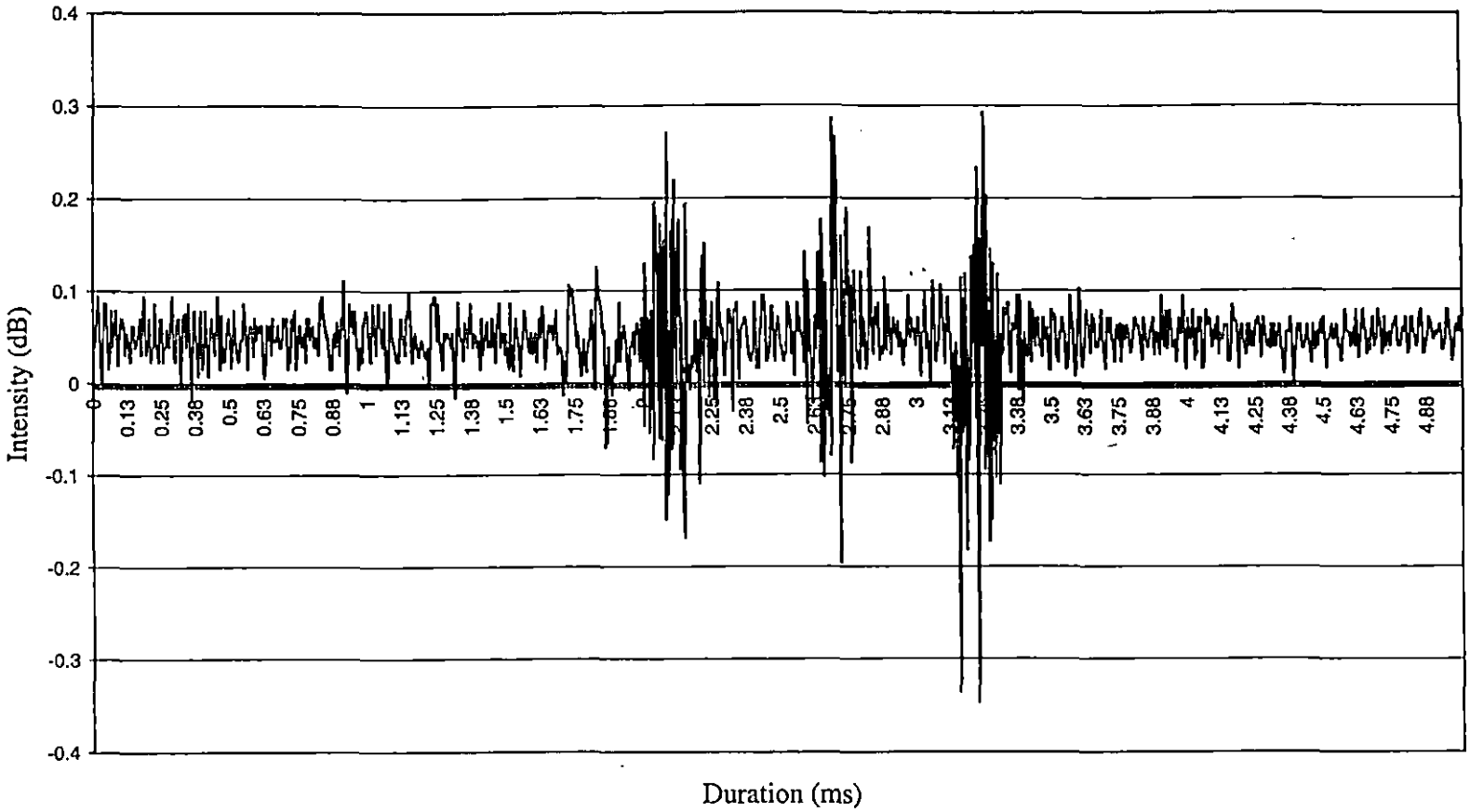


Fig. 5. Intensity of vocalization in control group no stressors
[Sound Exchange (SoX) Software]

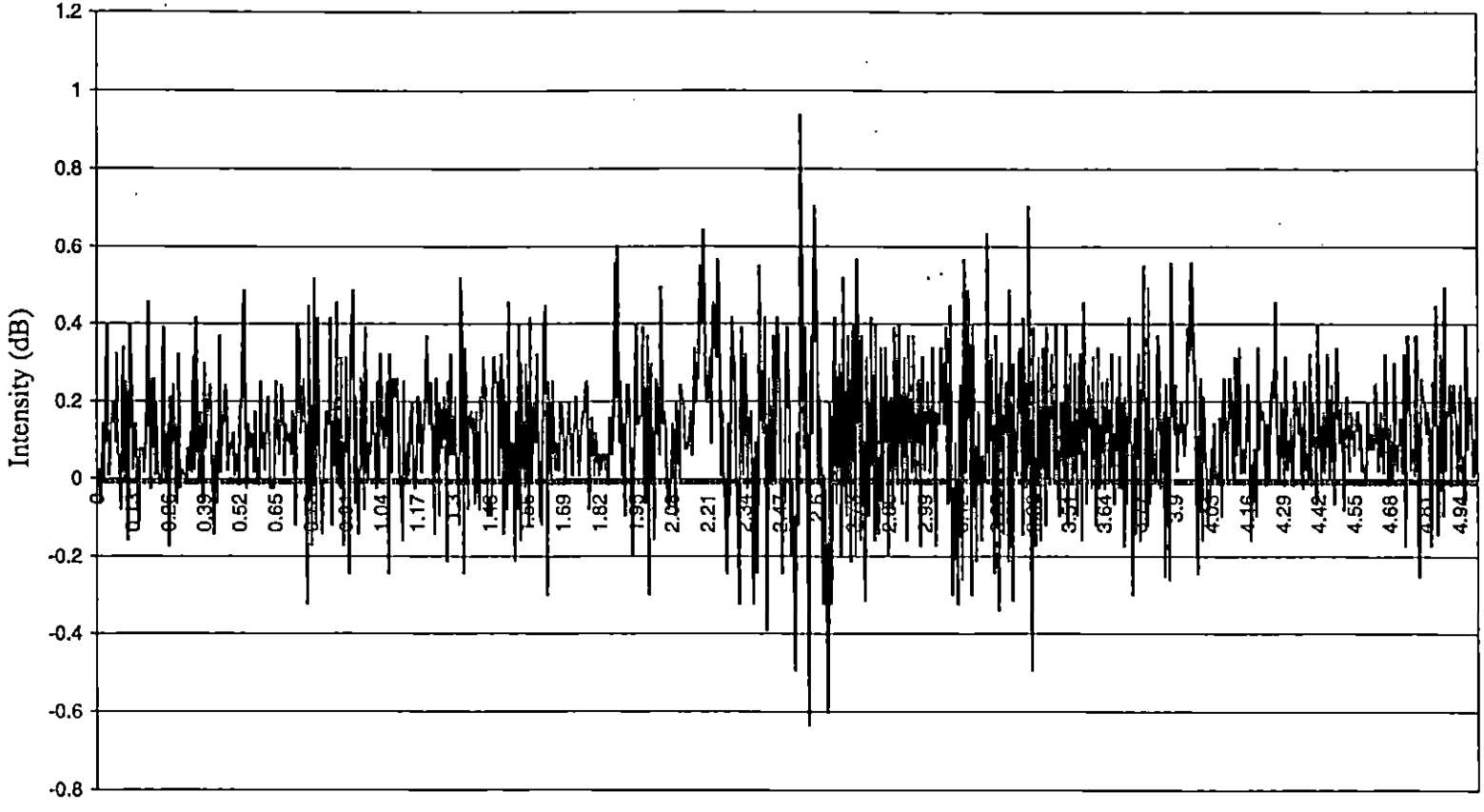


Fig. 6. Effect of handling for ear notching and castration on intensity of vocalization
[Sound Exchange (SoX) Software]

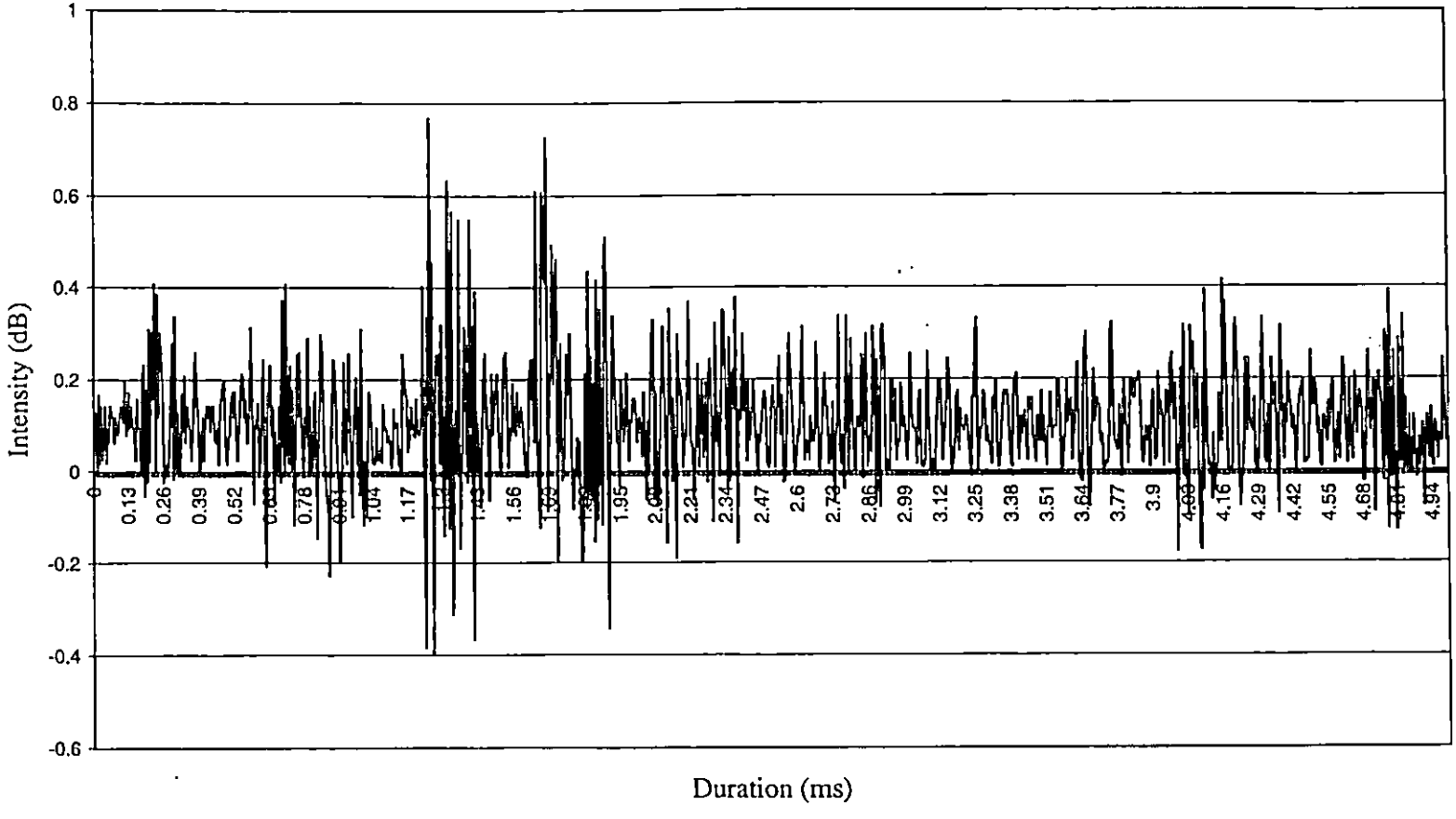


Fig. 7. Effect of performance of ear notching and castration on intensity of vocalization
[Sound Exchange (SoX) Software]

Table 4. Effect of age and stressors with the mean intensity of vocalization, decibels

Treatments	Time segment			
	First	Second	Third	Fourth
	Sham castration.			
T_4	47.50 ± 1.58	52.67 ± 2.57	53.50 ± 2.06	47.83 ± 1.99
T_6	50.33 ± 2.35	48.00 ± 1.81	48.00 ± 1.81	49.00 ± 2.68
	Castration			
T_5	45.83 ± 1.90	52.00 ± 3.15	49.67 ± 0.33[a]	52.17 ± 1.30[a]
T_7	53.33 ± 1.91	51.83 ± 1.76	47.17 ± 1.51[b]	51.00 ± 2.53[b]

Note: Means bearing different superscripts within the same column differ significantly ($p<0.05$)

Table 5. Influence of stressors on mean duration of vocalization, minutes

Treatments	Time segment			
	First	Second	Third	Fourth
One Week of Age				
T_{1A}	12.16 ± 0.70^{a}	12.16 ± 0.70^{a}	12.16 ± 0.70^{ab}	12.16 ± 0.70^{a}
T_2	17.15 ± 0.76^{b}	12.66 ± 0.71^{ab}	12.50 ± 0.76^{ab}	7.66 ± 0.71^{bc}
T_3	17.50 ± 0.76^{b}	14.16 ± 0.70^{b}	13.33 ± 0.49^{b}	7.00 ± 0.57^{c}
T_4	22.83 ± 0.60^{c}	22.33 ± 0.56^{c}	18.66 ± 0.99^{c}	12.50 ± 0.56^{a}
T_5	21.00 ± 0.53^{cd}	7.33 ± 0.56^{d}	9.00 ± 0.36^{d}	9.83 ± 0.70^{d}
T_8	19.66 ± 1.26^{cd}	12.66 ± 0.66^{ab}	12.50 ± 0.76^{ab}	7.50 ± 0.76^{bc}
T_9	17.83 ± 0.70^{b}	11.83 ± 0.60^{a}	11.33 ± 0.49^{a}	9.16 ± 0.60^{bd}
Eight Weeks of Age				
T_{1B}	7.00 ± 0.86^{d}	7.00 ± 0.86^{b}	7.00 ± 0.86^{b}	7.00 ± 0.86^{d}
T_6	12.66 ± 0.71^{c}	14.33 ± 0.88^{c}	8.50 ± 0.43^{b}	7.33 ± 0.42^{d}
T_7	12.50 ± 0.76^{c}	5.33 ± 0.42^{b}	7.16 ± 0.06^{c}	7.66 ± 0.66^{a}

Note: Means bearing different superscripts within the same column differ significantly ($p<0.01$)

of vocalization varied from 7.00 ± 0.57 to 22.83 ± 0.60 minutes, at one week olds and from 5.33 ± 0.42 to 14.33 ± 0.88 in piglets aged eight weeks.

In first time frame, the duration of vocalization was longer in one-week-old control group, which differed significantly ($p<0.01$) from eight weeks old control group. Influence of stressors on mean duration of vocalization is shown in Fig. 3.

4.2.2.1 Duration of Vocalization in One-Week-Old Piglets

4.2.2.1.1 Second Timeframe

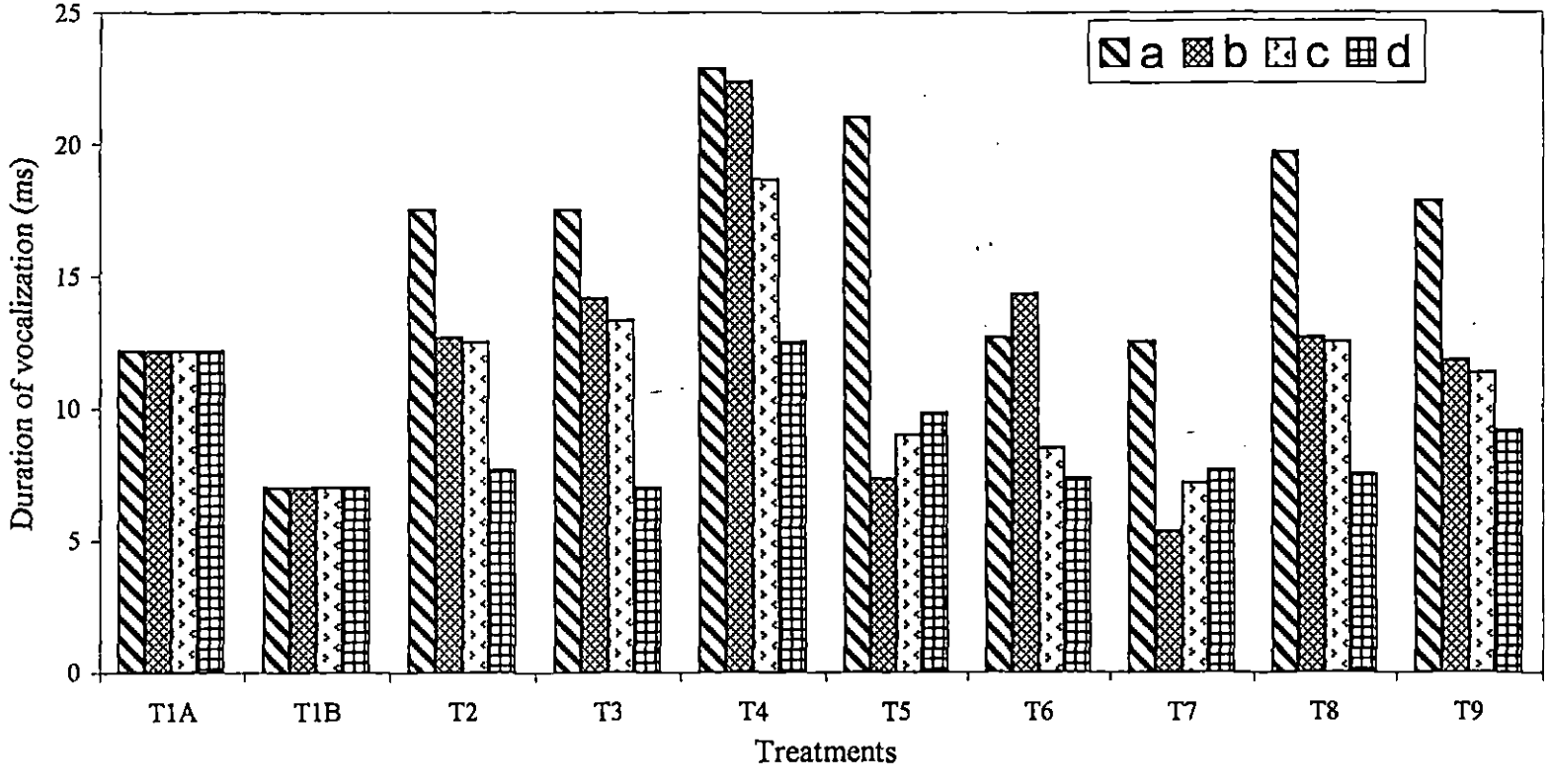
Ear notched group (T_3) had longer duration of vocalization which differed significantly ($p<0.01$) from the group handled for ear notching (T_2). Castrated group vocalized for shorter duration (7.33 ± 0.56) than the sham-castrated group (22.33 ± 0.56) that differed significantly ($p<0.01$). Significant difference ($p<0.01$) was noticed in ear notched group that had longer duration of vocalization than the treatment group exposed to ear notching and castration. The above results are given in table 5.

4.2.2.1.2 Third Timeframe

The vocalization was of longer duration in ear notched group, which differed, significantly ($p<0.01$) from the group handled for ear notching. Sham-castrated group vocalized for longer duration (18.66 ± 0.99) and differed significantly ($p<0.01$) from the castrated group (9.00 ± 0.36). These results are shown in table 5.

4.2.2.1.3 Fourth Timeframe (24 hours after the treatment)

From table 5. the duration of vocalization was shorter in castrated group, which significantly differed ($p<0.01$) from sham-castrated group. The treatment group that has been exposed to ear notching and castration had longer duration of



a. First time segment b. Second time segment c. Third time segment d. Fourth time segment

Fig.3. Influence of stressors on mean duration of vocalization, minutes

vocalization than the treatment group exposed to only ear notching which differed significantly ($p<0.01$).

4.2.2.2 Duration of Vocalization in Eight Weeks Old Piglets

In second time frame, the duration of vocalization was longer in sham-castrated group, which differed significantly ($p<0.01$) from the control group with no treatment. The vocalization duration was shorter in castrated group (7.16 ± 0.06) than sham-castrated group (8.50 ± 0.43) which significantly differed ($p<0.01$) in third time frame. These results are evident from table 5.

4.2.2.3 Interaction of Age

Assessment of effect of age on stress is elaborated in table 6. Sham-castrated group with longer duration of vocalization at one week of age differed significantly ($p<0.01$) from the sham-castrated group at eight weeks of age in second, third and fourth time frame. One week old castrates produced vocalization for longer duration (T_5) which significantly differed ($p<0.05$) from eight weeks old castrates (T_7) in second, third and fourth time frame.

4.2.3 Frequency of Vocalization (Number of Calls)

Mean frequency of vocalization to assess stress due to handling and performing the routine surgical procedures such as ear notching and castration are given in table 7. Mean frequency ranged from 13.16 ± 0.70 to 63.83 ± 1.08 at one week of age and from 7.50 ± 0.76 to 53.00 ± 0.73 at eight weeks of age. In Fig. 4. the mean frequency of vocalization (number of calls) influenced by different stressors in piglets is depicted. Following figures relate the frequency of vocalization (20 to 20,000 hertz) and intensity (0 to –100 decibels). Fig. 8. shows the vocalization of week old piglets in control group with no stressors [Sound Forge Software]. The vocalization of eight weeks old piglets in control group with no stressors (Sound Forge Software) is given in Fig. 9.

Table 6. Effect of age and stressors with the mean duration of vocalization, minutes

Treatments	Time segment			
	First	Second	Third	Fourth
	Sham castration			
T_4	22.83 ± 0.60**	22.33 ± 0.56**	18.66 ± 0.99**	12.50 ± 0.56**
T_6	12.66 ± 0.71**	14.33 ± 0.88**	8.50 ± 0.43**	7.33 ± 0.42**
	Castration			
T_5	21.00 ± 0.53**	7.33 ± 0.56*	9.00 ± 0.36*	9.83 ± 0.70*
T_7	12.50 ± 0.76**	5.33 ± 0.42*	7.16 ± 0.06*	7.66 ± 0.66*

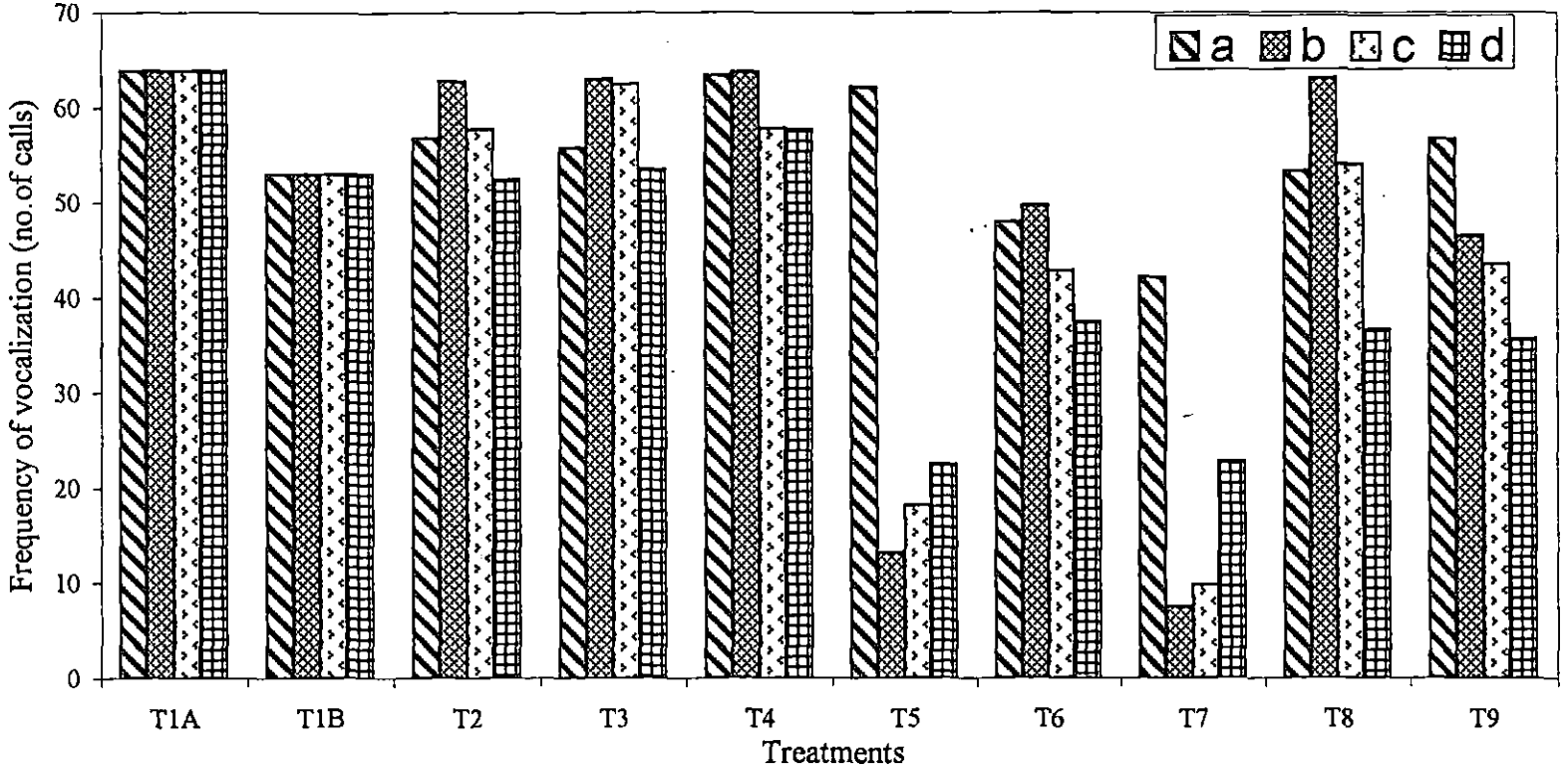
Note: ** within the same column is highly significant ($p<0.01$)

* within the same column is significant ($p<0.05$)

Table 7. Influence of stressors on mean frequency of vocalization (number of calls)

Treatments	Time segment			
	First	Second	Third	Fourth
One Week of Age				
T_{1A}	63.83 ± 1.08^{a}	63.83 ± 1.08^{a}	63.83 ± 1.08^{a}	63.83 ± 1.08^{a}
T_2	56.83 ± 0.79^{b}	62.83 ± 0.70^{a}	57.83 ± 0.70^{b}	52.50 ± 0.76^{b}
T_3	55.33 ± 1.02^{b}	63.00 ± 0.73^{a}	62.50 ± 0.76^{a}	53.50 ± 0.76^{b}
T_4	63.33 ± 0.71^{a}	63.67 ± 0.84^{a}	57.83 ± 0.60^{b}	57.66 ± 0.71^{c}
T_5	62.16 ± 0.60^{a}	13.16 ± 0.70^{b}	18.16 ± 0.79^{c}	22.50 ± 0.76^{d}
T_8	53.33 ± 1.26^{b}	63.00 ± 0.73^{a}	54.00 ± 0.36^{d}	36.66 ± 2.47^{e}
T_9	56.66 ± 1.54^{b}	46.50 ± 1.99^{c}	43.50 ± 0.84^{e}	35.66 ± 1.64^{e}
Eight Weeks of Age				
T_{1B}	53.00 ± 0.73^{a}	53.00 ± 0.73^{a}	53.00 ± 0.73^{a}	53.00 ± 0.73^{a}
T_6	48.00 ± 0.57^{b}	49.66 ± 0.66^{c}	42.83 ± 0.70^{b}	37.50 ± 0.99^{c}
T_7	42.16 ± 0.79^{c}	7.50 ± 0.76^{b}	9.83 ± 1.49^{d}	22.83 ± 0.70^{b}

Note: Means bearing different superscripts within the same column differ significantly ($p<0.01$)



a. First time segment b. Second time segment c. Third time segment d. Fourth time segment

Fig.4. Influence of stressors on mean frequency of vocalization (number of calls)

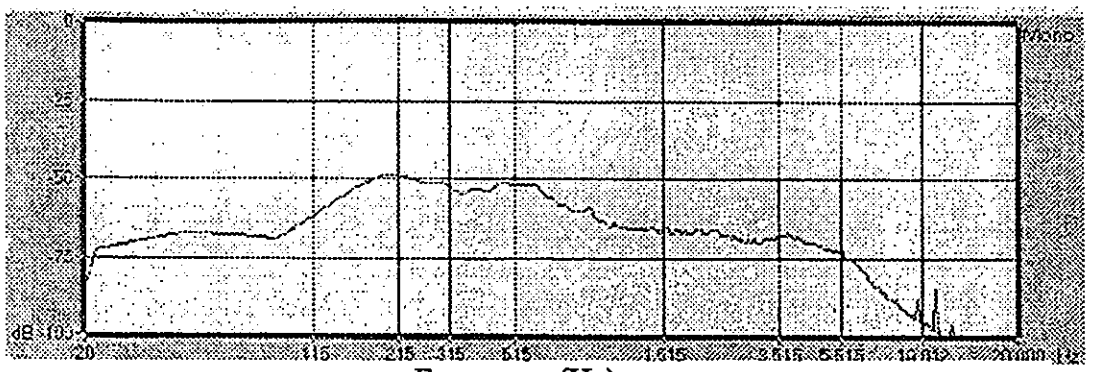


Fig.8. Vocalization of week old piglets in control group with no stressors
(Sound Forge Software)

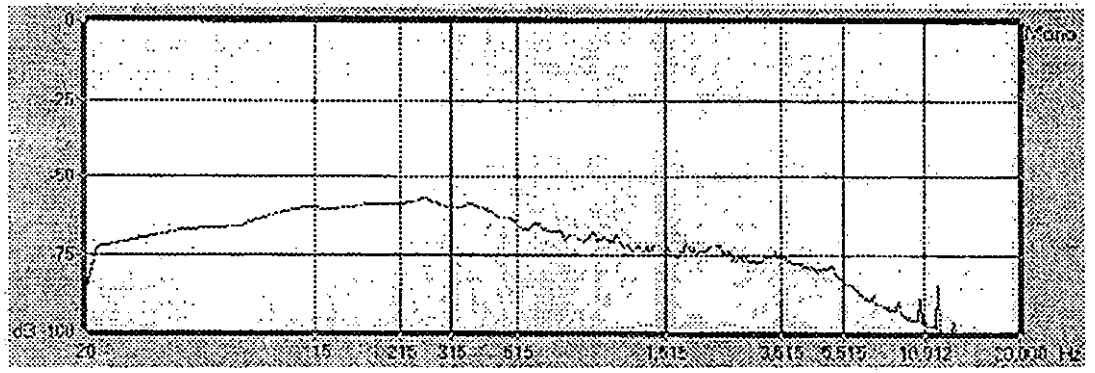


Fig.9. Vocalization of eight weeks old piglets in control group with no stressors
(Sound Forge Software)

4.2.3.1 Frequency of Vocalization in One Week Aged Groups

4.2.3.1.1 Second Time Segment

Mean frequency of vocalization was higher in sham-castrated group (T_4), which differed significantly ($p<0.01$) from the castrated group (T_5). Fig. 10 has given the influence of handling for castration on vocalization of week old piglets (Sound Forge Software). The influence of performing castration on vocalization of week old piglets (Sound Forge Software) is picturised in Fig. 11. In both the above figures, frequency of vocalization (20 to 20,000 hertz) and intensity (0 to −100 decibels) are denoted. The treatment group handled for ear notching and castration showed higher frequency of vocalization that significantly differed ($p<0.01$) from the ear notched and castrated group.

4.2.3.1.2 Third Time Segment

Ear notched group produced higher frequency of vocalization and differed significantly ($p<0.01$) from the group handled for ear notching. In the following figures, frequency of vocalization (20 to 20,000 hertz) and intensity (0 to −100 decibels) were measured. In Fig. 12. the influence of handling for ear notching on vocalization of week-aged piglets (Sound Forge Software) is depicted. Fig. 13. shows the influence of performing ear notching on vocalization of week-aged piglets (Sound Forge Software). Frequency of vocalization was higher in sham-castrated group, which differed significantly ($p<0.01$) from the castrated group. The treatment group exposed to handling for ear notching and castration showed higher frequency of vocalization and differed significantly ($p<0.01$) from the treatment group in which ear notching and castration was performed. In Fig. 14. the influence of handling for ear notching and castration on vocalization of one week aged piglets (Sound Forge Software) is drawn. Influence of performing ear notching and castration on vocalization of one week aged piglets (Sound Forge Software) is clear from Fig. 15. From the above figures, frequency of vocalization (20 to 20,000 hertz) and intensity (0 to −100 decibels) are observed.

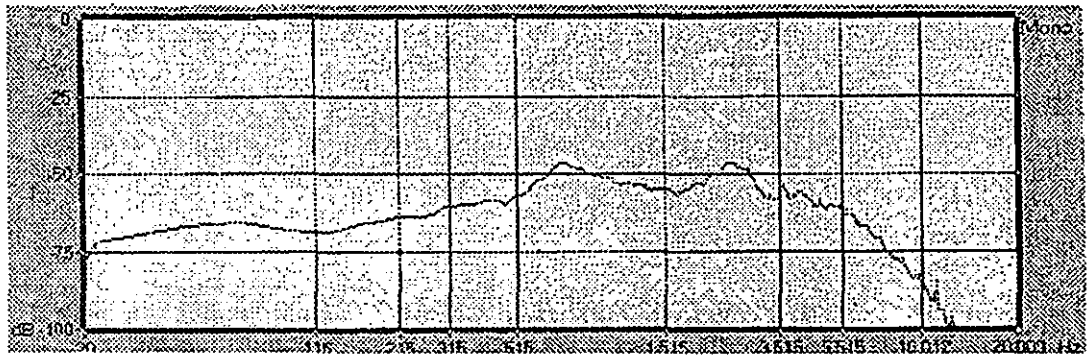


Fig.10. Influence of handling for castration on vocalization of week old piglets (Sound Forge Software)

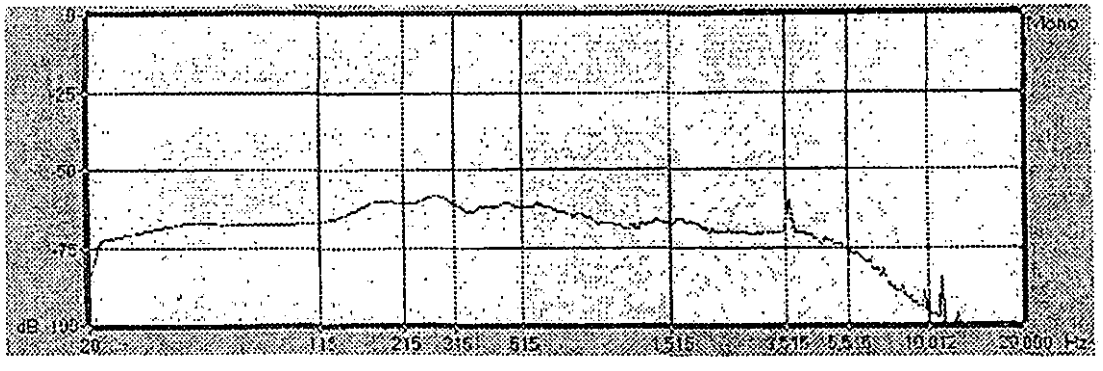
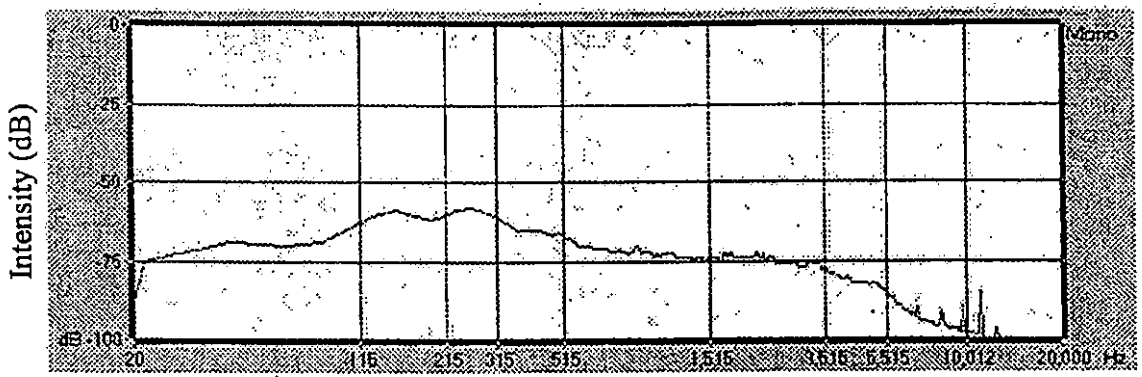
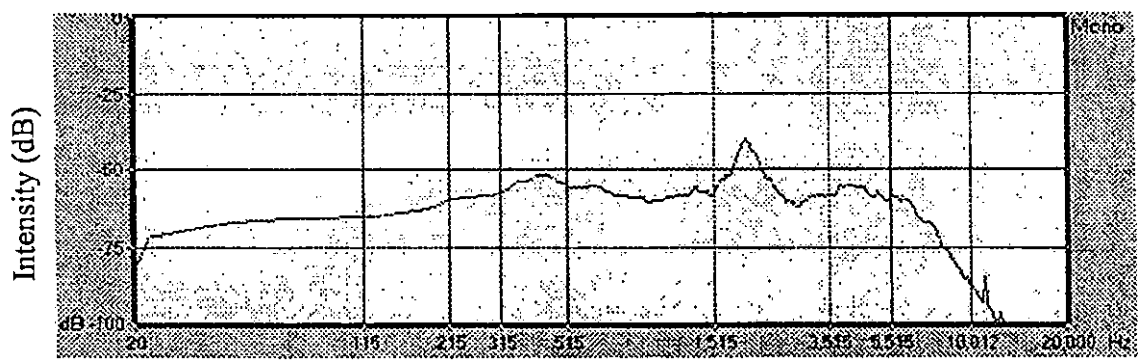


Fig.11. Influence of performing castration on vocalization of week old piglets (Sound Forge Software)



Frequency (Hz)

Fig.12. Influence of handling for ear notching on vocalization of week aged piglets (Sound Forge Software)



Frequency (Hz)

Fig.13. Influence of performing ear notching on vocalization of week aged piglets (Sound Forge Software)

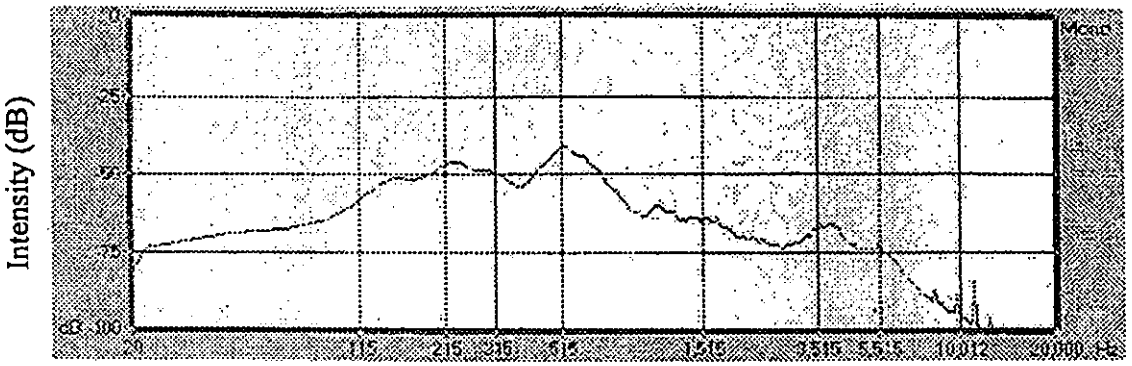


Fig.14. Influence of handling for ear notching and castration on vocalization of one week aged piglets (Sound Forge Software)

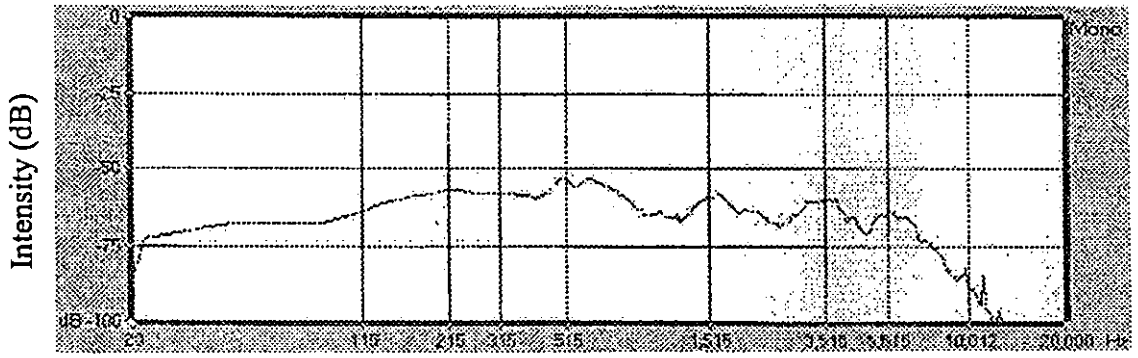


Fig.15. Influence of performing ear notching and castration on vocalization of one week aged piglets (Sound Forge Software)

4.2.3.1.3 Fourth Time Segment

Frequency of vocalization was lower in castrated group, which differed significantly ($p<0.01$) from the sham-castrated group. Following figures measured frequency of vocalization (20 to 20,000 hertz) and intensity (0 to −100 decibels). From Fig. 16 the influence of handling for castration on vocalization of eight weeks aged piglets (Sound Forge Software) is drawn. Fig. 17 shows the influence of performing castration on vocalization of eight weeks aged piglets (Sound Forge Software).

4.2.3.2 Frequency of Vocalization in Eight Weeks Aged Groups

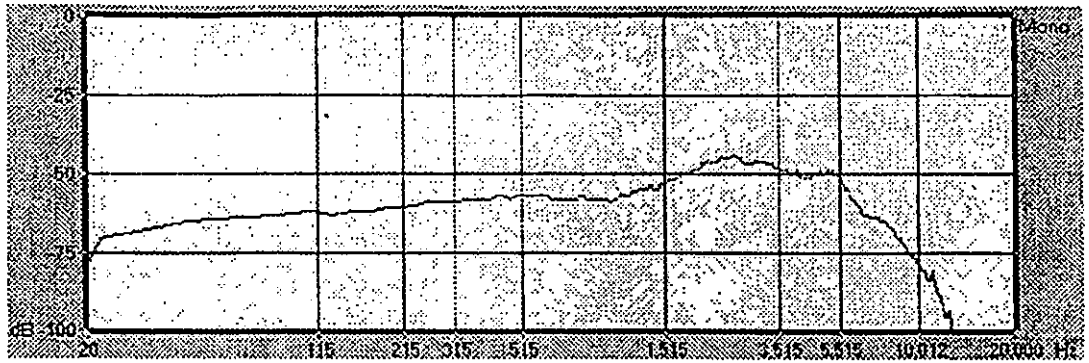
Sham-castrated groups produced higher frequency of vocalization and differed significantly ($p<0.01$) from the castrated group in second, third and fourth time segment.

4.2.3.3 Age Interaction

Mean frequency of vocalization to assess effect of age on stress is given in table 8. One-week-old sham-castrates had higher frequency of vocalization, which differed significantly ($p<0.01$) from eight weeks old sham-castrates in second, third and fourth time segment. Frequency of vocalization was higher in one-week-old castrated group that significantly differed ($p<0.01$) from eight weeks old castrated group in second and third time segment.

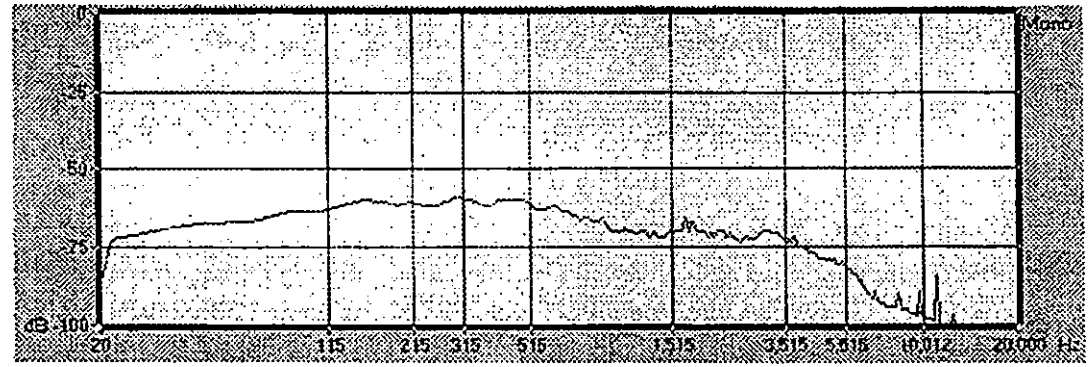
4.3 MEASURING CORTISOL LEVEL (µg/dL)

Mean cortisol levels to assess stress due to handling and performing the routine surgical procedures such as ear notching and castration are depicted in table 9. Minimum and maximum cortisol values are from 0.52 ± 0.19 to 3.50 ± 0.63 at one week and from 0.42 ± 0.18 to 2.07 ± 0.13 at eight weeks respectively. Fig. 18 depicts the cortisol level (µg/dL) in piglets under different stress conditions.



Frequency (Hz)

Fig.16. Influence of handling for castration on vocalization of eight weeks aged piglets (Sound Forge Software)



Frequency (Hz)

Fig.17. Influence of performing castration on vocalization of eight weeks aged piglets (Sound Forge Software)

Table 8. Effect of age and stressors with the mean frequency of vocalization (number of calls)

Treatments	Time segment			
	First	Second	Third	Fourth
	Sham castration			
T_4	63.33 ± 0.71^a	63.67 ± 0.84^a	57.83 ± 0.60^a	57.66 ± 0.71^a
T_6	48.00 ± 0.57^b	49.66 ± 0.66^b	42.83 ± 0.70^b	37.50 ± 0.99^b
	Castration			
T_5	62.16 ± 0.60^a	13.16 ± 0.70^a	18.16 ± 0.79^a	22.50 ± 0.76^a
T_7	42.16 ± 0.79^b	7.50 ± 0.76^b	9.83 ± 1.49^b	22.83 ± 0.70^a

Note: Means bearing different superscripts within the same column differ significantly ($p<0.01$)

Table 9. Influence of stressors on mean cortisol level (µg/dL)

Cortisol level (µg/dL)	Treatments									
	One Week of Age							Eight Weeks of Age		
	T_{1A}	T_2	T_3	T_4	T_5	T_8	T_9	T_{1B}	T_6	T_7
Mean ± SE	0.52± 0.19 [a]	1.65± 0.44 [b]	1.37± 0.25 [ab]	1.60± 0.29 [b]	3.50± 0.63 [c]	1.33± 0.29 [ab]	2.45± 0.89 [bc]	0.42± 0.18 [b]	1.62± 0.15 [c]	2.07± 0.13 [c]

Note: Means bearing different superscripts within the same row differ significantly ($p<0.01$)

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The cortisol value was higher in one-week-old control groups with no treatment, which differed significantly ($p<0.01$) from eight weeks old control groups. One week olds; castrates had higher cortisol concentration which differed significantly ($p<0.01$) from sham-castrates. Ear notched (T_3) group had lower cortisol level which differed significantly ($p<0.01$) from castrated group (T_5) at one week of age.

Assessment of effect of age and stressors on mean cortisol level are presented in table 10.

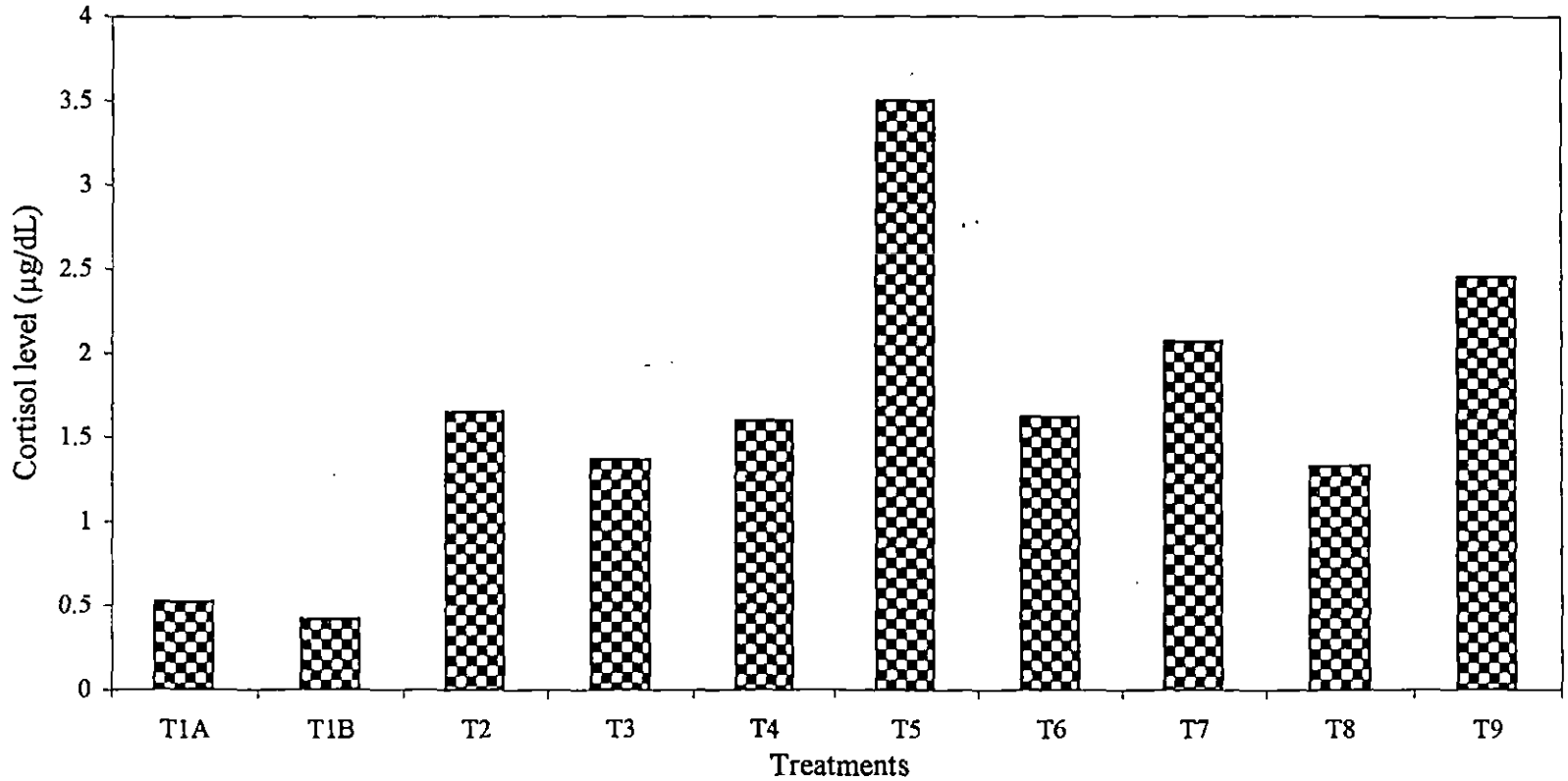

Fig. 18. Cortisol level under different stress conditions (µg/dL)

Table 10. Effect of age and stressors with the mean cortisol levels (µg/dL)

Cortisol level (µg/dL)	Treatments			
	Sham castration		Castration	
Mean ± SE	T_4	T_6	T_5	T_7
	1.60 ± 0.29	1.62 ± 0.15	3.50 ± 0.63	2.07 ± 0.13

Discussion

5. DISCUSSION

Influence of different stress factors on behaviour, vocalization and hormonal level of piglets during routine surgical procedures related to managemental practices are discussed.

5.1 MEASURING BEHAVIOUR

5.1.1 Behavioural Observation in One-Week-Old Piglets

The pig behaviours described by Houpt (1998) in the study on domestic animal behaviour and Hay *et al.* (2003) indicative of behavioural signs of castration in piglets, were akin to the methodology used in the present study.

During the handling for ear notching, piglets attempted to escape, twitched their tail and defecated. This is in agreement with the reports of Fraser and Broom (1990) who inferred that the animal's willingness to move and other behavioural examinations are important considerations in behavioural studies. They have also suggested that behavioural examinations are best performed in a quiet space or enclosure with limited light, where distractions were minimal. Sanford *et al.* (1986) had equivocal indication that pigs attempt to escape when handled.

During ear notching, the animals trembled, twitched their tail, urinated and attempted to escape more appreciably than handled for the same. Present findings are akin to Houpt (1998) who reported that skin irritation caused tail twitching and increased rate of elimination. Mench and Mason (1997) stated that, disturbance, distress and fear indicated stress in animals, which is in agreement with the present study. He also suggested that knowledge of the species, strain, behaviour of individual and their social group is a prerequisite to assess welfare of animals.

In piglets handled for castration, trembling, startle reaction (typical porcine reaction, with a 'woof and freezing' posture) were noticed, during handling. Present findings are in tune with Fraser and Broom (1990). They inferred that attitude; disposition and temperament of the animal, should be assessed before any handling procedures.

During castration, piglets showed reduced attempts to escape and decreased tail twitching. These behaviours were found after 30 minutes and 24 hours of castration. This may be due to pretreatment with local anesthetic. Findings of Houpt (1998) are in disagreement with the present findings. He has reported that, pigs castrated without anaesthesia stood and suckled less and laid more. It was concluded that this response occurred in piglets castrated at any age from day one of life, indicating that they are able to perceive the pain. Observations of McGlone *et al.* (1993) also contradict the present results. He observed that castration caused measurable changes such as reduced suckling, reduced standing and increased lying times in the behaviour of young pigs. The longer time segment for observation in the present study may be the reason for this difference.

When the subjects, handled for ear notching, were immediately handled for castration, they attempted to escape. This behaviour in pain-inflicted piglets may be due to aversive sensing as part of protective action. This is similar to the observations of Sanford *et al.* (1986).They have reported that pigs attempted to escape when handled and there reactions may be accentuated in pain. Kitchell (1987) opined that pain experienced by the animals led to learned avoidance by protective motor action. Present findings are in agreement with that of Fraser and Broom (1990). Their results showed that pigs responded to a disturbing situation such as close approach of a person by suppression of normal activities and preparation for flight, defence or hiding. Pederson (1996) suggested that behavioural measurements are needed to make reliable conclusions.

Piglets with combined performance of ear notching and castration showed pain related behaviours such as startle reaction, tail twitching, straightened tail and they wandered less. These behaviours were observed around 30 minutes after the procedures and were not appreciable after 24 hours. Present results are in accordance with Houpt (1998). He has reported that normal pigs do not wander and their tail positioned straight indicated fright or distress. Mellor *et al.* (2000) opined that pain-causing stressor elicited a range of behavioural responses. Findings in the present study are in line with that of Jensen *et al.* (1996). They suggested that in addition to strength of the stressor, animals' perception of controllability and predictability influenced the stress response.

Control group piglets with no treatment were observed to be nosing, mouthing, biting, chewing or rooting the substrates. This is in tune with reports of Houpt (1998) in piglets. It was reported that piglets in confined environment explored by rooting, mouthing, biting or chewing. Broom (1996b) recognized that stereotypy such as sham chewing, bar-biting were abnormal behaviours in pigs.

Similar finding was also reported by Sanford *et al.* (1986), that some species communicate their experience by means of bodily attitude and other postural mechanisms. Moss (1992) inferred that an animal's response to a particular set of environmental circumstances provided an insight into animals' mental and physical state.

5.1.2 Behaviour Observations in Eight Weeks Old Piglets

Piglets handled for castration, showed increased attempts to escape. They trembled and twitched their tail frequently during handling. Equivocal responses were reported by Ewing *et al.* (1999). They have suggested that enlightened design criteria increased the ease of animal handling and understanding the animal behaviour in confinement could enhance safety of animals and its handlers.

Castrated piglets depicted reduced attempts to escape, reduced tail twitching but increased time in ventral recumbancy, after 30 minutes of castration. The intensity of these behaviours declined after 24 hours. This may be due to the performance of castration by experienced personnel. Findings of Hay *et al.* (2003) confirmed that castration of piglets without anaesthetics induced pain. It was also reported that tail wagging existed for two days after castration and behaviour responses were exacerbated during the first few hours following castration. The results of Wemelsfelder *et al.* (2000) are in agreement with the present findings. They believed that spontaneous qualitative assessment of behaviour provided empirical access to behavioural expressions associated with welfare of animals.

5.1.3 Age Interaction

Piglets castrated at one week of age were observed to be in ventral recumbency and with startle reaction, after 30 minutes of the treatment. This indicated perception of pain due to castration at younger age. The observations of McGlone *et al.* (1993) contradict from the present findings and it may be due to the breed difference. Subjects castrated at eight weeks of age showed rooting, nosing and mouthing the substrates after 30 minutes and 24 hours of treatment. The difference in age and the ability to withstand stress may be the possible reasons for the difference observed in the present study. Present findings are in agreement with the behavioural measurements of Molony and Kent (1997). They have reported that local anesthetics reduced or eliminated behavioural changes produced by castration. This report justifies the present finding. Present results are in accordance with the findings of Van *et al.* (2002). According to them, time; test situation and individual difference, determined the animal's behavioural reaction. Mili *et al.* (2002) assessed that pigs castrated at 7^{th} week had higher weight gain than at 5^{th} week. This confirmed that castration greatly influenced growth rate in meat animals. Observations of Thronton and Pearson (2002) are not in agreement with the present findings. Their results concluded that castration reduced the time spent performing play behaviour in one-week-old

lambs and lying behaviour in 4 to 6 week old lambs. There was also a significant increase in abnormal postures following castration in 4-6 weeks old lambs. The difference in the species and strain may be a possible reason for this difference. Bataille *et al.* (2002) suggested that clipping of the temporary incisors and docking should be carried out as routine practices in pigs, only when vice of tail biting and damage occurred.

5.2 MEASURING VOCALIZATION

The common 'grunt' was given in pigs in response to familiar sounds usually prior to the application of treatments. An excited pig handled and restrained for manipulation gave the staccato grunt or short grunt. Pig Intense vocalizations were confirmed by squeals observed during castration. The above observation is akin to the reports that vocal signals are the most important means of communication (Houpt and Wolski, 1982). They have reported that common sequence of vocalization is to proceed from common grunts to staccato grunts to repeated grunts without interruption and to grunts to squeals to screams as the animal is approached, chased, picked up and injected.

5.2.1 Vocalization Intensity (Decibels)

In one week old piglets, the intensity of vocalization was higher in ear notched and castrated group than the groups handled for the same. The present results are in agreement with that of Braithwaite *et al.* (1994). He has suggested that since the handling for castration is stressful and the actual castration procedure would be significantly more distressing. Oldham (1985) stated that pigs vocalized at the slightest provocation indicating minor discomfort to severe pain, which was evident in the present observation too.

5.2.1.1 Interaction of Age

One-week castrates had higher intensity of vocalization than eight weeks castrates. Studies by Weary and Fraser (1995) were in accordance with the

current study. They determined that, 'non-thriving' and 'unfed' piglets called more, than 'thriving' and 'fed' littermates. Duncan and Fraser (1997) recorded a similar observation. They have reported that suckling piglets elicited characteristic vocalizations when separated from the mother. Assessment of Watts *et al.* (2000) is in line with the present study. According to them, endogenous characteristics such as age and weight influenced vocal response.

5.2.2 Vocalization Duration (minutes)

In the present study one-week-old control groups, with no treatment produced longer duration vocalization than eight weeks old control group. This is in agreement with the findings of Oldham (1985) and Weary and Fraser (1995). They have indicated that the condition of the piglets influenced vocalizations.

5.2.2.1 Duration of Vocalization in One-Week-Old Piglets

Ear notched groups vocalized for longer duration than the groups handled for ear notching. This was noticed during the procedure and after 30 minutes, of time period. This may be due to the fact that skin irritation due to ear notching has influenced vocalization as reported by Houpt (1998).

Duration of vocalization was longer in 'sham-castrated' group than castrated group. This condition was observed during the procedure, 30 minutes after and 24 hours after the treatments. Present results are not in agreement with that of Braithwaite *et al.* (1994). They have reported that vocalization was of longer duration in castrated than in sham-castrated piglets. The difference in local anaesthetic pretreatment may be a possible reason for this. Findings of Weary *et al.* (1998) are not in agreement with the present study. Their results confirmed that castrated piglets produced calls with a longer duration. It was suggested that the way in which piglets were restrained did not affect the pain caused by castration.

The duration of vocalization was longer in piglets, which underwent combined performance of ear notching and castration, than the treatment group with only ear notching. This was observed after 24 hours of the procedure. Present findings are in agreement with that of Sanford *et al.* (1986). They have recognized that vocalization indicated pain in piglets and their attempt to escape and squeal might be accentuated in pain.

5.2.2.2 Duration of Vocalization in Eight Weeks Old Piglets

The duration of vocalization was longer in 'sham castrates' than the control group. After 30 minutes of castration, castrates vocalized for lesser duration than the sham-castrates. The results obtained are not in agreement with Braithwaite *et al.* (1994) who have reported that castration was more stressful than sham-castration. The difference in restraint procedure, husbandry system adopted and the care system followed may be a reason for this difference.

5.2.2.3 Interaction of Age

One-week-old 'sham-castrates' had longer duration of vocalization than eight weeks old 'sham-castrates'. Also piglets castrated at one week of age-produced vocalization for longer duration than those castrated at eight weeks of age. There observations were made during the procedure, 30 minutes after and 24 hours after the application of treatments. The difference in age of exposure to the stressors may be a possible reason for the difference in duration of vocalization. Taylor *et al.* (2001) opined that while the factors affecting both the shams and castrates varied with age, the pain of castration was not affected within the range of ages tested at three, ten and seventeen days.

5.2.3 Vocalization Frequency (number of calls)

5.2.3.1 Frequency of Vocalization in One-Week-Old Piglets

The frequency of vocalization was higher in ear notched group than the group handled for ear notching. This observation persisted for 30 minutes after

the treatments. Piglets handled for ear notching and castration simultaneously produced vocalization with higher frequency, during the procedure. This may be due to the application of combined stressors. This finding is in line with the observations of Cloutier *et al.* (2000). They have opined that vocal response of piglets indicated their well-being. It was also reported that no improvement in condition of piglets was observed during handling, either by playing music or other sounds.

The frequency of vocalization was higher in 'sham-castrated' groups during the procedure compared to the castrated groups. Above findings are not akin to that of Braithwaite *et al.* (1994) in pigs. Their results showed that higher frequency of vocalization was observed in castrated than in 'sham-castrated' piglets. Weary *et al.* (1998) showed that castrated piglets produced significantly more high frequency calls than 'sham castrate'. It was suggested that increased rate of high calls was a reliable indicator of pain. The difference in husbandry system adopted and the care system followed may be a reason for the difference in the observation in the present study.

5.2.3.2 Frequency of Vocalization in Eight Weeks Old Piglets

'Sham-castrated' piglets produced high frequency vocalization than the castrates during the treatments. Contrary to the present study, Braithwaite *et al.* (1994) suggested that actual castration procedure was more distressing than the handling during castration in frequency of vocalization. The reports of Taylor and Weary (2000) are also in contradiction with the present finding. Their result stated that compared to sham-operated animals, castrated piglets produced much more high-frequency calls. It was suggested that use of local anaesthetics potentially increased the stress of handling and restraints. Present results are in disagreement with the findings of Taylor *et al.* (2001). Their test affirmed that castrated piglets produced high frequency calls at more than three times the rate of 'sham-castrate' group.

5.2.3.3 Interaction of Age

Piglets castrated at one-week of age, produced higher frequency of vocalization than those castrated at eight weeks of age. This was observed during the procedure and after 30 minutes of castration. The decreased stress response in eight weeks old castrates could be due to proper restraint and appropriate drug administration, in addition with experienced personnel doing the surgery. The present experiment is in agreement with the findings of Weary and Fraser (1995). They have determined that weaker piglets of younger age produced more high-frequency calls. Similar experiment was performed by White *et al.* (1995). Their results showed that pigs castrated without 'lidocaine' made higher frequency ($P<0.05$). They have suggested that castration without anaesthetic was of greater stress for pigs of eight days of age or older.

The frequency of vocalization was higher in one-week-old 'sham-castrates' than eight weeks old 'sham-castrates' during the procedure. This may be due to the influence of age on vocalization as assured by Watts *et al.* (2000).

Short single grunts in isolated piglets appeared to be associated with investigatory behaviour. After testing, when piglets were left along with their littermates and mother, long single grunts in the form of contact calls were noticed. Short, rapidly repeated grunts were observed when attempted to handle the piglets. This was akin to the identification of distinct vocalizations (Marchant *et al.*, 2001) in domestic pig during isolation.

5.3 MEASURING CORTISOL LEVEL (µg/dL)

In the present study cotton buds were used to collect the biomaterial, saliva from the piglets. Similarly (Fell *et al.*, 1985), developed a salivary cortisol method for detecting changes in plasma 'free' cortisol arising from acute stress in sheep. Apparatus to aspirate saliva from parotid gland was devised and 'synacthen' (Synthetic corticotrophin) was used for adrenal stimulation. Their result reported that values for salivary cortisol were intermediate between the

'free' levels in the two serum samples. Schonreiter and Zanella (2000) compared the common method of saliva sampling with cotton buds to a new one called 'Oral Diffusion Sink' (ODS) which aids continuous measuring without any manipulation of the animals. For this, defined stressors of isolation and transport were assessed in 10 German Landrace pigs. Their result suggested that ODS samples correlated significantly with cotton bud samples ($P<0.04$) and thus ODS may be a good method for continuous measuring of acute stress.

The cortisol value was higher in one-week-old control groups with no treatment, than eight weeks old control groups. The results of present study are akin to report of Dantzer (1979). According to him the stress of social isolation and exposure to a new environment induced ACTH release in piglets. Similar report was made by Moberg *et al.* (1980). They have suggested, that exposure to open field arena was stressful to lambs regardless of age. It was also indicated that exposure to the testing procedures has not decreased the response of the adrenal axis to the stimuli.

In the present experiment, cortisol level varied between one week olds and eight weeks old piglets. This result also indicated that younger piglets were more susceptible to stress. The above findings are in agreement with those of Ruis *et al.* (1997). Their results revealed that cortisol values declined as age increased from 12 weeks to 24 weeks of age in piglets. The present finding that different cortisol concentrations existed based on stressors in piglets are supported by findings of Terlouw (1997) who ascertained that animals differed consistently in stress reactivity. Gallagher *et al.* (2002) reported that salivary cortisol concentration was high perinatally and declined with age, in piglets. Their results suggested that collection of saliva in the piglet provided viable alternative to blood sampling. Greenwood and Shutt (1992) reported that transport of three weeks old goats had increased free cortisol in saliva, compared to one-week-old goats.

Ewing *et al.* (1999) opined that levels of one or more corticosteroid hormones in saliva, along with response duration were often determined to assess stress status of the animal.

Variations in cortisol levels in the present study are differing from reports of Hay *et al.* (2003). They have reported that, there were no clear effect of pain induced by castration on urinary corticosteroids and catecholamines.

Castrated piglets had higher cortisol concentration than 'sham-castrated' ones during one week and eight weeks of age. The present findings are in agreement with those of Fell *et al.* (1986). They have reported that short-term salivary cortisol response was significantly higher after surgical castration in calves. Equivocal response was given by Cohen *et al.* (1990) in calves. Their results of plasma cortisol concluded that the acute stress occurred for six to twelve hours following surgical castration. Mellor *et al.* (2000) opined that changes in plasma cortisol concentration provided an indication of the overall noxiousness of the experience.

Ear notched groups had higher cortisol level than the groups handled for ear notching. Combined performance of ear notching and castration showed elevated cortisol value compared to the group handled for the same. Present findings are in agreement with those of Rushen (1986). He has inferred that the rise in plasma corticosteroids occurred in response to exposure to a particular husbandry procedure, which would determine the degree of distress experienced by the animal. Present results are in congruence with reports of Andrews (1992). He stated that corticosteroid level rose abruptly when pigs were subjected to acute stress and the decrease in level on exposure to chronic stress was due to decrease in receptors to corticotrophin release factor. The increased cortisol value in combined performance of ear notching and castration observed in the present study was in agreement with reports of Jensen *et al.* (1996). He has suggested that increased plasma concentration of glucocorticosteroids reflected the strength of stressor applied.

5.3.1 Age Interaction

In the present study, cortisol concentration was higher in one-week-old castrates than eight-week-old castrates. The difference in age in hormonal secretion may be one of the reasons. This is in agreement with the findings of Ruis *et al.* (1997). Fisher *et al.* (1996) suggested that local anaesthetic reduced the pain associated with castration in calves. Kent *et al.* (1998) evaluated that in week old lambs subjected to castration, the injection of anaesthetics into the testis was less effective than injection into the neck of the scrotum. This holds true for piglets also under certain conditions. Rosochacki *et al.* (2000) determined that the concentration of plasma cortisol increased with the duration of stress. It was reported that these was a significant interaction between the breed of animals and time of stress.

Summary

6. SUMMARY

The experiment was conducted at the Center for Pig Production and Research (CPPR) of Kerala Agricultural University, Mannuthy. Sixty male piglets formed ten treatments with six replicates. Piglets were of one and eight weeks of age. Treatments allotted to one-week-old groups were handling for ear notching, performing ear notching, handling and performance of both ear notching and castration. One week and eight weeks old groups underwent the treatments of castration and 'sham castration' (restrained identically but not castrated). Piglets with no treatment were kept as control group in two age groups. Subjects selected for castration were restrained by local anesthesia.

Behaviour was observed by 'Focal animal sampling' method to observe each individual for a specified amount of time. The intensity, duration and frequency of vocalization were determined. The 'intensity' was measured from the graphs using 'Sound Forge' and 'SoX' Software. Observations were recorded during four different time frames *viz:* 30 minutes before the treatment; during the treatment; 30 minutes after the treatment and 24 hours after the treatment respectively. Recording was done using video camera cum videocassette recorder. Salivary cortisol concentration was determined by radio immuno assay.

In behavioural observation, control group piglets with no treatment were observed to be nosing, mouthing, biting, chewing or rooting the substrates. One-week-old piglets during the handling for ear notching, attempted to escape, twitched their tail and defecated. During ear notching, the animals trembled, twitched their tail, urinated and attempted to escape more appreciably than handled for the same. In piglets handled for castration, trembling, startle reaction (typical porcine reaction, with a 'woof and freezing' posture) were noticed, during handling. At the time of castration, piglets showed reduced attempts to escape and decreased tail-twitching activity. When the subjects handled for ear

notching were immediately handled for castration, they attempted to escape. Piglets subjected to combined performance of ear notching and castration showed pain related behaviours such as startle reaction, tail twitching, straightened tail and they wandered less. Eight-week-old ones, handled for castration, showed increased attempts to escape, trembled and twitched their tail frequently during handling. Castrated piglets depicted reduced attempts to escape, reduced tail twitching and also increased time spent on ventral recumbency, after 30 minutes of castration. Piglets castrated at one week of age were observed to be in ventral recumbency and with startle reaction, after 30 minutes of the treatment. Subjects castrated at eight weeks of age were observed to be nosing and mouthing the substrates after 30 minutes and 24 hours of the treatment.

In measuring the intensity of vocalization in one-week-old piglets, the following observations were made. Ear notched and castrated groups showed significant increase in intensity of vocalization than the groups handled for the same. 'Sham-castrates' showed higher intensity than castrates. One-week old castrates had higher intensity of vocalization than castrates of eight weeks.

In measuring the duration of vocalization, one-week-old control groups, with no treatment, produced longer duration of vocalization than eight weeks old control group. Ear notched groups vocalized for longer duration than the groups handled for ear notching at one-week-old, during the procedure and after 30 minutes of time period. At the same age group, duration of vocalization was longer in 'Sham-castrated' group than 'Castrated' group during the procedure, 30 minutes after and 24 hours after the treatments. The duration of vocalization was longer in week old piglets with combined performance of ear notching and castration, than the treatment group with only ear notching. The same result was obtained in one week olds, after 24 hours of the procedures. In eight weeks old piglets, during the treatment of 'Sham-Castration', the duration of vocalization was longer than the control group. After 30 minutes of castration, castrates vocalized for lesser duration than the 'Sham-castrates'. One-week-old 'Sham-castrates' had longer duration of vocalization than eight weeks old 'Sham-

castrates'. Also piglets castrated at one week of age produced vocalization for longer duration than castrated at eight weeks of age during the procedure, 30 minutes after and 24 hours after the application of treatments.

The frequency of vocalization was higher in control groups of one-week-old than in eight weeks old piglets. The frequency of vocalization was higher in ear notched group than the group handled for ear notching. This observation persisted for 30 minutes after the treatments at one week of age. In the same age group, the frequency of vocalization was higher in 'Sham-castrated' groups during the procedure compared to the 'Castrated' groups. Piglets handled for ear notching and castration simultaneously at one week, produced vocalization with higher frequency, during the procedure. At eight weeks, 'Sham-castrated' piglets produced higher frequency of vocalization than the 'Castrates' during the treatments. The frequency of vocalization was higher in one-week-old 'Sham-castrates' than eight weeks old 'Sham-castrates' during the procedure. Treatment group of piglets 'Castrated' at one-week age produced higher frequency of vocalization than those 'Castrated' at eight weeks of age. This observation was made during the procedure and after 30 minutes of the castration.

The cortisol value was higher in one-week-old control groups with no treatment, than eight weeks old control groups. At one week, the group handled for ear notching had higher cortisol level than ear notched group. At the same age group, 'Castrates' had elevated cortisol concentration than 'Sham-castrates'. Cortisol value was higher in treatment groups of combined ear notching and castration, than the groups handled for the same. At eight weeks old, 'Castrates' showed higher cortisol value than 'Sham castrates'. Cortisol concentration was higher in one-week old 'Castrates' than eight weeks old 'Castrates'.

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STRESS ASSESSMENT OF PIGLETS UNDERGOING ROUTINE SURGICAL PROCEDURES RELATED TO MANAGEMENTAL PRACTICES

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ABSTRACT

Stress in piglets undergoing routine surgical procedures related to managemental practices was assessed in the present study. The experiment was conducted at the Center for Pig Production and Research (CPPR) of Kerala Agricultural University, Mannuthy. Sixty male piglets formed ten treatments with six replicates. Treatments undertaken were ear notching, handling for ear notching, castration, sham castration (restrained identically but not castrated), combined handling and performance of ear notching and castration. Behaviour and vocalization were observed during four different time frames viz: 30 minutes before the treatment, during the treatment, 30 minutes after the treatment and 24 hours after the treatment respectively. Salivary cortisol concentration was determined by radio immuno assay technique.

In one-week-old piglets, though the stress related behavioural scores due to ear notching was higher during the procedure, the scores got decreased after 30 minutes and 24 hours of ear notching. The scores were intermediate in the combined performance of ear notching and castration compared to individual performance at one week. Castrates at eight weeks showed fewer stress related behaviours than the castrates at one week of age.

The intensity of vocalization in week old ear notched groups got reduced after 30 minutes and 24 hours of ear notching. Vocalization intensity persisted till 24 hours in one week aged castrates, but it got reduced after 30 minutes and 24 hours in eight weeks castrates. The groups with combined performance of ear notching and castration were intermediate in the intensity of vocalization compared to individual performance at one week.

There was reduction in the duration of vocalization in week-aged ear notched groups after 30 minutes and 24 hours of treatment. Castrates at eight weeks of age showed increased duration of vocalization after 30 minutes and 24

hours, but the level was lesser compared to one week old castrates. The groups with combined performance of ear notching and castration were intermediate in the duration of vocalization compared to individual performance at one week.

The frequency of vocalization in ear notched group got reduced in week old group after 30 minutes and 24 hours. Castrates at eight weeks of age showed increased frequency of vocalization after 30 minutes and 24 hours, but the level was lesser compared to one week old castrates. The groups with combined performance of ear notching and castration had longer duration of vocalization compared to individual performance at one week.

At one week, ear notched group had lower cortisol value than the group handled for ear notching. One-week old castrates had higher cortisol concentration than eight-week-old castrates. Cortisol level was higher in combined performance of ear notching and castration than individual performance of the same.

Individual performance of ear notching and castration was less stressful. Performance of ear notching at one week and castration at eight weeks of age is beneficial. This is the routine management related surgical procedure, practiced in the Center for Pig Production and Research (CPPR) of Kerala Agricultural University.