VETERINARY CLINICAL SCIENCE
Journal homepage: www.jakraya.com/journal/vcs

ORIGINAL ARTICLE

Comparative evaluation of analgesic and operative techniques for amputation of horn in buffaloes

K Rama Rao[1], Makkena Sreenu[2], KBP Raghavender[3] and PVS Kishore[4]

Department of Veterinary Surgery and Radiology, NTR College of veterinary Sciecne, Sri Venkateswara Veterinary University Tirupati, Gannavaram-521 101(AP), India.
[1]Veterinary Assistant Surgeon, Veterinary Dispensory, Gollapudi, Krishna (Dt) AP, India.
[2]Professor and Head, Teaching Veterinary Clinical complex, College of Veterinary Science, Korutla, Karim Nagar (TS), India.
[3]Professor and University Head, Dept of Veterinary Surgery and Radiology, College of Veterinary Science, Rajendra Nagar, Hyderabad-500 030 (TS), India.
[4]Professor and Head, Dept of Veterinary Anatomy and Histology, College of Veterinary Science, Korutla, Karim Nagar (TS), India.

*Corresponding Author:

Makkena Sreenu

Email: drmakkena@yahoo.co.in

Received: 14/08/2014

Revised: 19/08/2014

Accepted: 20/08/2014

Abstract

The buffaloes suffering with affections of horn and warranted horn amputation were divided as Standard method of horn amputation with and without sedation and modified method of horn amputation with and without sedation were grouped into I, II, III and IV respectively. The common pain symptoms like restlessness, twitching of ears, shaking of head, bruxism, rubbing against fixed objects, pawing at the affected site and evading the affected site were observed in horn affections. The pain symptoms and pain score index were more pronounced in the horn cancer and standard method of horn amputation without sedation when compared to the other affections and groups. Elevated physiological parameters and leucocytosis was noticed in all the groups up to 3rd and 5th postoperative day respectively. A significant increase in plasma cortisol and C-reactive protein levels was observed up to 3rd postoperative day in groups I, II and III; whereas in group IV it was observed only up to 1st postoperative day. The modified method of horn amputation with sedation proved to be best, due to its advantages like ease of flap rising, less duration of operation and less stress in terms of low plasma cortisol and C-reactive protein levels. The disadvantage of other groups includes more stress and more duration of operative procedure.

Key words: Horn amputation, pain, analgesia, operative technique, buffaloes.

Introduction

The horn is prone to various affections like avulsion, fracture, overgrowth, sepsis, fissures and cancer. Most of these affections do not respond to the routine medical management and demand amputation of the horn (Sreenu and Kumar, 2006). Horns of buffaloes are massive, angular and well developed with a wider base as compared to cattle. Amputation of the horn in adult buffaloes is considered to be a difficult task as compared to cattle (Tank *et al.*, 2006). The degree of physiologic and behavior response varies with the method of dehorning (Heinrich *et al.*, 2010). Anil *et al.* (2002) suggested that behavior is a more sensitive indicator of pain than cortisol and other physiological parameters. This paper compares the different analgesic and operative techniques for amputation of horn in buffaloes.

Materials and Methods

All the animals suffering with horn affections which warranted amputation were randomly divided into four groups of six animals each which includes standard method of horn amputation with sedation (G-I); standard method of horn amputation without sedation (G-II); modified method of horn amputation with sedation (G-III) and modified method of horn amputation without sedation (G-IV). Feed and water were withheld for 24 and 12 hours respectively before operative procedure. The operative site at the base of the horn region was prepared for aseptic surgery and restrained in standing position in the trevis. The animals of group I and III were sedated with xylazine hydrochloride (Xylaxin® Indian immunologicals, Hyderabad) at the rate of 0.05 mg/kg body weight intramuscularly 15 minutes before the operative procedure. For all the buffaloes analgesia of the operative site was achieved by using cornual nerve block and ring block with 2% lignocaine hydrochloride (Xylocaine ® Astra IDL Ltd , Bangalore). The degree of desensitization was assessed by pinprick method.

In group I, the affected horn of all the buffaloes were transected at the junction of the skin and base of the horn by using wire saw without damaging the dorsal and ventral surface of skin (Fig 1-2). A vertical cutaneous incision of 4-8 cm was made from the base of the horn exactly at the frontal crest and a horizontal cutaneous incision of 4-8 cm towards the nuchal crest. The skin over the dorsal and ventral aspect of the horn was raised as a full thickness flap to expose the bony horn (Fig 3-5). Haemostasis was achieved by applying pressure at temporal fossa and digital pressure. Ligature was applied to the bleeding vessel if any. The exposed bony horn along with a protruded frontal bone was transected using wire saw. The dorsal and ventral flaps were approximated with a series of horizontal mattress sutures using No.1 braided silk. Undermining of the skin edges on both dorsal and ventral aspects were performed to raise the flaps further if the flaps were not sufficient for suturing. The suture line was covered with a bandage applying retention sutures. In this group II, animal's amputation of horn was done without any sedation with the same procedure as described earlier.

All the buffaloes in group II were given a vertical cutaneous incision of 4-8 cm from the base of the horn exactly at the frontal crest and a horizontal cutaneous incision of similar length towards the nuchal crest. Then a cresent incision of 4-5 cm was made to connect the two previous incisions on the dorsal aspect of the horn exactly at the junction of skin and base of the horn. The skin over the dorsal aspect was raised as a full thickness flap to expose the bony horn. The exposed bony horn was transected with electrical oscillating saw up to the half of the horn diameter without causing damage to the ventral edges of the skin. The remaining horn was chiseled from one angle preferably from frontal crest towards the nuchal crest. After chiseling, the entire transected bone was lifted to expose the ventral aspect of the horn through the transected edge of a ventral skin flap. The dorsal and ventral flaps were approximated and a series of horizontal mattress sutures with No. 1 silk was carried to close the skin edges. Undermining of the skin edges on both dorsal and ventral aspects were performed to raise the flaps further if the flaps were not sufficient for suturing (Fig 6-11). The methods of control of hemorrhage were similar to the previous technique. In group IV amputation of horn was done without any sedation with the same procedure as described in the group III.

Following horn amputation the suture line was covered with bandage after applying retention sutures parallel to suture line on either side and/or all the buffaloes (Fig 12) were administered with parental injection of Streptopencillins 2.5 g/kg body weight for 5-7 days and Meloxicam 20 ml/day for next 2 days. Daily dressing of surgical wound was carried out with Povidone iodine. Sutures were removed between 10-14th postoperative day.

Post operatively all the buffaloes were observed for general condition, signs of pain like arched back, restlessness, twitching of ears, shaking of head, bruxism, rubbing against fixed object, pawing at the site and given pain score for each animal individually as per the procedure of Holton *et al.* (2001). In all the buffaloes various physiological parameters like rectal temperature, respiratory rate and pulse rate were recorded on 1, 3, 5, 7, 14th day of post operative period. Five milliliters of blood was collected from jugular vein of each animal and serum samples were prepared separately on 1st, 3rd, 5th, 7th and 14th post operative days. The hematological parameters like Packed Cell Volume (percentage), hemoglobin content (gm/dl), total leucocyte count (thousands/microlitre) were estimated using Auto Hematology analyzer. The biochemical parameters like plasma cortisol using cortisol kit with ELISA reader and C-reactive protein (ng/dl) with turbidometry CRP^2 using spectrophotometer were also estimated. All the data were subjected to statistical analysis with appropriate statistical method (One way ANOVA) using graphpad instat demo software.

Results and Discussion

Preoperative fasting of the animals provided easy handling of the animals. The procedures like shaving and scrubbing provided aseptic environment. Restraining the buffaloes in standing position in a trevis for amputation proved better handling. Sedation with Xylazine Hydrochloride at the dose rate of 0.05 mg/Kg bwt intramuscularly made the animal calm and facilitated easy restraint in standing position. Local infiltration anesthesia for cornual and ring block with 2% lignocaine hydrochloride was found to be suitable for desensitization of horn and subsequent amputation procedures.

Several researchers performed amputation of horn by administering sedatives and local infiltration techniques. The administration of xylazine helps in handling of the animal for injection of the local anesthetic, in addition to its analgesic and sedative effects during dehorning in calves as reported by Grondahl-Neilsen *et al.* (1999). Amputation of horn to

Fig 1: Photograph showing cutaneous incision for horn amputation at the junction of skin and base of horn.

Fig 2: Photograph showing standard method of horn amputation using wire saw.

Fig 3: Photograph showing transected horn at the junction of skin and horn base.

Fig 4: Photograph showing exposure of frontal bone after raising the dorsal flap by standard method of horn amputation.

Fig 5: Photograph showing exposure of both dorsal and ventral flaps by standard method of horn amputation.

Fig 6: Photograph showing a vertical and cutaneous incision in modified method of horn amputation.

Fig 7: Photograph showing undermining of dorsal aspect of horn to raise the dorsal flap of skin in modified method of horn amputation.

Fig 8: Photograph showing transection of frontal bone using oscillating electrical saw in modified method of horn amputation.

Fig 9: Photograph showing transection of frontal bone using chisel and mallet in modified method of horn amputation.

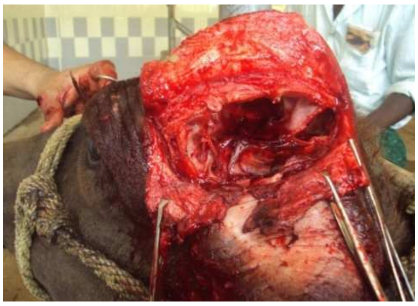

Fig 10: Photograph showing modified method of horn amputation after rising dorsal and ventral flaps.

Fig 11: Photograph showing approximation of dorsal and ventral flaps with a series of horizontal mattress sutures.

Fig 12: Photograph showing applied retention bandage after horn amputation.

treat various horn affections was performed under ring block (Kulbhushan *et al*., 1999), cornual nerve block (Kumar and Thilagar, 2000), administration of xylazine combined with a cornual block (Stafford and Mellor, 2005), xylazine sedation and ring block (Sreenu and Kumar, 2006), metamizol and cornual nerve block in calves (Schuszler *et al*., 2007), Xylazine sedation along with cornual nerve and ring block in buffaloes (Mistry, 2009), xylazine sedation and cornual nerve block (Mahida *et al*., 2010) with satisfactory results. The base of the horn was tested by pin prick method to assess extent of analgesia of the horn for amputation without any technical complicacy as observed by Kulbhushan *et al*. (1999).

The horn affections in buffaloes do not respond to the routine medical management and demand amputation of the horn (Sreenu and Kumar, 2006). Amputation of horn in adult buffaloes was considered to be a difficult task compared to cattle (Tank *et al.*, 2006). Amputation of horn at the base using wire saw (Kumar and Thilagar, 2000); amputation of horn at its base by rising ventral flap after resection of horn with hack saw blade half the way and remaining with chiseling in murrah buffaloes (Sreenu and Kumar, 2006) and amputation of horn at its base by making an elliptical incision joining frontal crest and nuchal crest with hack saw blade half the way and remaining with chisel and hammer in mehasani buffaloes (Mahida *et al*., 2010) are in literature.

Two surgical techniques followed in the present study effectively treated the clinical conditions with little variations. In group I and II the affected horn of all the buffaloes were transected at the junction of the skin and base of the horn by using wire saw without damaging the dorsal and ventral surface of skin which clearly visualized the horn as with the method of Sahu and Mohanty (1963) in cattle. A vertical cutaneous incision on frontal crest and a horizontal cutaneous incision towards the nuchal crest joining with an elliptical incision at the junction of skin and base of the horn helped in raising dorsal and ventral full thickness flaps to expose the bony horn as reported by Kumar and Thilagar (2000). The exposed bony horn was transected using electrical oscillating saw, chisel and mallet. These findings are in agreement with Sreenu and Kumar (2006) and Mahida *et al*. (2010). The idea of raising the ventral flap at a later stage was a convenient method in buffaloes due to curly massive horns which enabled sufficient visibility over ventral and dorsal flaps. Additionally the skin at the base of the horn is very thick which makes primary closure of wound difficult.

Manual assistance though limited to two to three people in group I and three to five persons in group II clearly indicate the effect of sedation to reduce the manpower to restrain the animal. The degree of analgesia at the site was adequate for the operative procedure. It took 40-45 minutes in group I and 50-55 minutes in group II for completion of operative procedure. The animals in group I were slightly depressed as it was sedated with xylazine hydrochloride and the animals were normal, as the operative site was infiltrated with local anesthesia in group II.

The technique of horn amputation was similar to previous technique in group III and IV except that the exposed bony horn was transected with electrical oscillating saw up to the half of the horn diameter without causing damage to the ventral edges of the skin. The remaining horn was chiseled from one angle preferably from frontal crest towards the nuchal crest. After chiseling, the entire transected bone was lifted to expose the ventral aspect of the horn through the transected edge of a ventral skin flap with and without sedation. The idea using electrical saw and chiseling for transecting the bone reduced the time and had advantages like amputation in standing position, less number of attendants to restrain the animal, convenient position to surgeon, minimum operating time, less haemorrhage and proper apposition of skin edges. These findings are in agreement with Sreenu and Kumar (2006) and Mahida *et al*. (2010).

The dorsal and ventral flaps were approximated in all the animals following horn amputation with a series of horizontal mattress using No. 1 braided silk. This yielded proper healing of wound with minimum complications. Undermining of the skin edges on both dorsal and ventral aspects to raise the flaps helped in proper suturing. Following amputation to close the skin some surgeons used metal clamps (Fioravanti *et al*., 1999), nylon by interrupted sutures (Yadav *et al*., 2002), vertical mattress sutures with No. 2 silk (Manjunath *et al*., 2007), modified mattress suture and horizontal mattress suture with thick non absorbable suture material (Sreenu and Kumar, 2006) with varying degrees of success.

In the present study modified method of horn amputation with sedation was considered best as these animals group showed less pain symptoms and score, duration of time and changes in biochemical parameters. It indicates that the procedure followed, maintained a greater level of well being during the horn amputation when compared to other procedures. The pain symptoms were also observed in group I and III following horn amputation which indicates that the sedatives had not a long lasting effect. These observations were in accordance with the findings of Vickers *et al.,* (2005). Grondahl-Nielsen *et al.* (1999) who mentioned that the main advantage of xylazine was it eliminates the animal response to the initial handling for injection of local anesthetic.

Skin sutures were completely healed in between 11-15 days in all the animals**.** The complications like suture dehiscence at the center of skin incision with discharge of pus were noticed in few buffaloes following horn amputation and they were treated with daily dressing with 5% povidone iodine and Topicure spray. Intramuscular administration of Dicrysticin-S for 5 days and Melonex for 2 days counteracted the bacterial infections and postoperative pain. Daily dressing of wound with povidone iodine facilitated the complete healing of wound without any complications in many of the buffaloes. In four animals postoperative dehiscence of suture at the center of the incision with discharge of pus was observed which might be due to self inflicted wound caused during the healing process.

In cattle many techniques have been developed for amputation of horn primarily to treat horn cancer. Amputation of horn at base by open method; flap method of horn amputation by cutting the horn at its bottom by seeing saw movements (Angelo and Das, 1970), an elliptical incision around the base of the horn to raise cranial and caudal skin flaps (Mohanty *et al.*, 1972), amputation of horn and frontal sinus trephining with a polyethylene tube in to sinus (Mohanty *et al* ., 1972) and amputation of horn along with the breakage of nasal septum (Chauhan *et al.*, 1981), amputation of horn by flap method with elliptical incision on anterior and posterior side at the base of the horns (Yadav *et al.*, 2002) are on record.

Pain evaluation

In group I, twitching of the ears was more pronounced followed by trying to rub the operated area against the fixed objects, evading the operated area on palpation, pilo erection and decreased appetite was recorded in the initial post operative period. Twitching of the ears, trying to rub the operated part against the fixed objects and decreased appetite and water intake were more pronounced in the group II followed by evading the operated area on palpation, pilo erection, restlessness, bruxism and dilatation of pupil in the initial post operative period. In group III twitching of the ears was the symptom recorded more followed by trying to rub the operated area against fixed objects, decreased appetite and water intake and evading the operated area on palpation were observed. Twitching of the ears and rubbing the operated area against fixed objects were recorded more in the group IV followed by evading the operated area on palpation, decreased appetite and water intake, restlessness, dilatation of pupil and pilo erection were noticed. The average pain score index was 7.12±0.29; 8.50±0.33; 4.75±0.25 and 7.25±0.25 in group I, II, III and IV respectively. The pain was graded as moderate in groups I, II, IV and low in group III.

Recognition and assessment of acute stress and pain following procedures such as dehorning is difficult, although a combination of physiological and behavioural measures have been recommended to measure an animal's reaction more accurately (Grandin, 1997). Amputation regardless of the method used has been shown to affect the animal's physiology and behavior in a manner typical of an acute stress response (Sylvester *et al.*, 2004). In the present study amputation by flap method was preferred in all cases, where communication to frontal sinus is exposed, to prevent empyema of frontal sinus. The typical acute stress response to amputation evidenced by increased heart rate and plasma cortisol concentration (physiological response) greater frequency of head shaking and rubbing (behavioral response).

In group I, twitching of the ears was more pronounced followed by rubbing the operated area against the fixed objects, evading the operated area on palpation. Piloerection and decreased appetite was recorded in the initial post operative period. Twitching of the ears, rubbing the operated part against the fixed objects and decreased appetite and water intake were more pronounced in the group II followed by evading the operated area on palpation, pilo erection, restlessness, bruxism and dilatation of pupil, which clearly indicating various degree of pain activities in animals with and without sedation.

In group III twitching of the ears was the symptom recorded more followed by rubbing the operated area against fixed objects, decreased appetite and water intake and evading the operated area on palpation. Twitching of the ears and rubbing the operated area against fixed objects were recorded more in the group IV followed by evading the operated area on palpation, decreased appetite and water intake, restlessness, dilatation of pupil and pilo erection due to pain reflexes as the animals in group IV did not receive any sedative.

The pain was graded as moderate in groups I, II, IV and low in group III. In the present study group I and III animals showed less pain symptoms and score when compared to other groups which indicate that the combination of xylazine and local anesthetic reduced the stress which could be evidenced by changes in biochemical parameters. Heinrich *et al.* (2010) opined that the degree of physiologic and behavior responses were varied with the method of dehorning. In groups II and IV more pain symptoms and pain score was noticed. Anderson and Muir (2005) mentioned that lignocaine hydrochloride was used to prevent incision pain during surgery. These drugs act locally or regionally when perineural anesthesia was performed, but had no systemic or behavioral effect.

The physiological parameters recorded in different groups of animals under gone horn amputation were recorded and presented in Table 1. The preoperative values of temperature of group I, II, III and IV were 99.92±0.09; 100.25±0.13; 00.85±0.07 and 100.67±0.16 °F respectively. There was a non significant increase in the values up to 3rd postoperative day and reached normal levels following postoperative period in all the groups. The preoperative values of pulse rate of group I, II, III and IV were 40.75±0.90; 43.12±1.20; 43.25±1.03 and 43.62±0.78 beats/min respectively. A non-significant increase in pulse rate was noticed from up to 3rd postoperative day and reached normal levels on following postoperative period in all the groups. The preoperative values of respiratory rate of group I, II, III and IV were 24.25±0.94, 24.87±1.37, 25.12±0.58 and 25.25±0.56 breaths/min respectively. A non significant increase in respiratory rate was noticed from up to 3rd postoperative day and reached normal levels on following postoperative period in all the groups.

The physiological parameters in different groups of animals that had undergone horn amputation were recorded. There was a non significant increase in the values of temperature, pulse rate and respiratory rate up to 3rd postoperative day and reached normal levels in subsequent postoperative period in all the groups. A non significant increase in temperature in the early postoperative period might be due to surgical trauma as suggested by Boddie (1969) or the surgical stress might have produced sympathetic nervous stimulation resulting in increased metabolic rate. Guyton (1981) suggested that the neurotransmitter liberated following surgical trauma induces glycogenolysis. This increases cellular activity, culminating in increased temperature.

The elevated pulse rate following horn amputation might be attributed to pain which reflexly accelerated pulse (Boddie, 1969). Following tissue damage, substances like histamine, bradykinin, prostaglandin, excess of potassium ions and proteolytic enzymes will be released (Guyton, 1981). The elevated pulse rate in the present study might be due to release of histamine and bradykinin which stimulate pain nerve endings. The transient rise in respiratory rate in the present study might be due to reflex stimulation of respiratory center (Boddie, 1969).

Hemato-biochemical studies

The haemato-biochemical changes recorded in different groups of animals under gone horn amputation were recorded and presented in Table 2. The preoperative values of hemoglobin of group I, II, III and IV were 8.60±0.09, 8.55±0.10, 8.50±0.06 and 8.55±0.09 g/dl respectively. Non-significant changes were noticed in the postoperative period and they were within the normal levels in all the groups. The preoperative values of hemoglobin of group I, II, III and IV were 5.56±0.02, 5.60±0.02, 5.60±0.02 and 5.56±0.02 thousands/cmm respectively. A non-significant increase was noticed up to 5th postoperative period and they were reached to normal levels within the following postoperative period in all the groups. Non significant changes of PCV, haemoglobin and total leucocyte count were noticed upto 5th postoperative day and they reached normal levels within the following postoperative period On the

Table 1: Changes in physiological parameters (mean±SE) of buffaloes following horn amputation. (N=8)

Parameter	Grou	Preoperati Values	Postoperative Days					
			1	3	5	7	10	14
Temperature (°F)	I	99.92±O.C	102.70±0.08	103.05±0.10	102.20±0.13	101.40±0.22	100.65±0.14	100.65±0.12
	II	100.25±0.1	102.70±0.11	103.10±0.08	102.12±0.15	100.95±0.12	100.72±0.08	100.82±0.12
	III	100.85±0.(	102.65±0.07	103.08±0.10	102.12±0.11	101.02±0.08	100.65±0.10	100.80±0.17
	IV	100.67±0.1	102.60±0.08	103.20±0.13	102.17±0.13	100.85±0.13	100.75±0.12	100.65±0.11
Pulse rate (beats/min)	I	40.75±0.9(	41.37±0.70	63.50±1.13	46.75±1.09	45.25±0.82	43.00±0.82	41.87±0.83
	II	43.12±1.2(	40.87±0.58	66.37±0.94	47.62±1.05	45.50±0.75	42.50±0.53	42.25±0.75
	III	43.25±1.03	41.37±0.49	63.12±0.95	47.75±0.86	44.75±0.90	43.37±0.46	42.12±0.74
	IV	43.62±0.78	41.12±0.79	63.87±0.44	48.87±0.89	45.12±0.85	43.62±0.49	43.25±0.59
Respiratory rat (breaths/min)	I	24.25±0.94	27.12±0.74	46.00±0.53	36.37±0.30	37.62±0.44	30.50±0.82	28.00±0.53
	II	24.87±1.37	25.88±0.58	45.75±0.72	35.37±0.59	32.75±0.53	30.37±0.88	27.63±0.46
	III	25.12±0.58	25.12±0.44	44.37±0.73	35.37±0.78	33.25±0.37	30.62±0.92	27.00±0.53
	IV	25.25±0.56	25.12±0.51	46.25±0.75	35.62±0.56	32.75±0.49	30.50±0.94	26.75±0.45

Table 2: Changes in haemato-biochemical parameters (mean±SE) of buffaloes following horn amputation. (N=8)

Parameter	Groups	Preoperative values	Postoperative days					
			1	3	5	7	10	14
Packed (volume (%)	I	27.44±2.13	28.00±0.89	25.75±0.53	27.00±0.87	26.00±0.46	28.37±0.73	27.37±0.59
	II	25.25±O.49	28.38±0.82	25.62±0.49	26.62±0.73	25.88±0.61	27.38±0.80	26.62±0.37
	III	25.25±0.56	28.75±1.01	25.88±0.44	26.88±0.44	25.38±0.46	26.25±0.49	27.12±0.71
	IV	25.00±0.33	28.88±0.77	25.63±0.42	27.50±0.63	26.00±0.46	27.62±0.73	27.75±0.77
Hemoglobin (g/dl)	I	8.60±0.09	8.35±0.05	8.80±0.11	8.82±0.13	8.90±0.16	8.95±0.12	9.00±0.09
	II	8.55±0.10	8.40±0.05	8.56±0.09	8.70±0.16	8.75±0.15	9.00±0.09	9.07±0.09
	III	8.50±0.06	8.47±0.06	8.51±0.06	8.78±0.14	8.90±0.14	9.02±0.10	9.00±0.10
	IV	8.55±0.09	8.40±0.06	8.58±0.10	8.75±0.14	9.00±0.09	9.10±0.10	8.90±0.11
Total leucoc count (thousands/cmm	I	5.53±0.04	5.72±0.02	7.35±0.04	8.52±0.02	7.46±0.02	6.75±0.04	6.45±0.03
	II	5.56±0.02	5.75±0.01	7.34±0.06	8.54±0.03	7.51±0.03	6.73±0.04	6.42±0.02
	III	5.60±0.02	5.71±0.02	7.33±0.06	8.47±0.03	7.48±0.02	6.71±0.03	6.45±0.02
	IV	5.56±0.02	5.74±0.01	7.39±0.05	8.48±0.03	7.53±0.02	6.76±0.03	6.42±0.01
Plasma cortisol (µg/dl)	I	0.58±0.04	0.86±0.06*	0.81±0.01*	0.60±0.01	0.59±0.03	0.58±0.01	0.57±0.01
	II	0.55±0.02	1.16±0.03*	0.91±0.01*	0.54±0.02	0.52±0.01	0.50±0.01	0.48±0.00
	III	0.55±0.01	0.64±0.01*	0.54±0.01	0.49±0.01	0.48±0.00	0.45±0.01	0.42±0.00
	IV	0.52±0.01	0.89±0.05*	0.79±0.02*	0.59±0.01	0.58±0.01	0.55±0.01	0.51±0.01
C-reactive prote (ng/ml)	I	3.92±0.24	4.80±0.27*	4.59±0.04*	3.53±0.02	3.48±0.11	3.33±0.03	3.27±0.01
	II	3.73±0.10	6.66±0.10*	5.64±0.07*	3.49±0.04	3.39±0.02	3.32±0.03	3.24±0.01
	III	3.43±0.14	3.63±0.08	3.56±0.04	3.45±0.03	3.36±0.02	3.29±0.01	3.23±0.02
	IV	3.64±0.07	4.93±0.16*	4.60±0.03*	3.58±0.05	3.41±0.02	3.34±0.01	3.29±0.01

*-significant at $P \leq 0.01$

contrary Mistry (2009) observed leucocytosis in septic horn of buffaloes. Leucocytosis was observed following horn amputation in all the groups. This could be attributed to the surgical trauma and inflammation.

The preoperative values of plasma cortisol of group I, II, III and IV were 0.58±0.04, 0.55±0.02, 0.55±0.01 and 0.52±0.01 µg/dl respectively. Significant increase was noticed on 1st and 3rd postoperative period in group I, II and IV; whereas in group III a non significant increase was noticed on 1st postoperative period only and they were reached to normal levels within the normal levels in the following postoperative period in all the groups. The preoperative values of serum C-reactive protein of group I, II, III and IV were 3.92±0.24, 3.73±0.10, 3.43±0.14 and 3.64±0.07 ng/ml respectively. Significant increase was noticed on 1st and 3rd postoperative period in group I, II and IV; whereas in group III a non-significant increase was noticed on 1st postoperative period only and they were reached to normal levels within the normal levels in the following postoperative period in all the groups. A significant increase in plasma cortisol was noticed on 1st and 3rd postoperative day in group I, II and IV; whereas in group III a non significant increase was noticed on 1st postoperative day only indicating less stress in this procedure and they were reached to normal levels in the following postoperative period in all the groups. Terlouw *et al.* (1997) mentioned that increased levels of circulating corticosteroids; reflects changes in the hypothalamus-pitutary-adrenal axis after dehorning. A rise in cortisol concentration represents a stress response due to activation of the hypothalamic-pitutary adrenal axis and is not directly indicative of pain (Weary *et al.,* 2006).

A significant increase in C reactive protein values was noticed on 1st and 3rd postoperative day in group I, II and IV; whereas in group III a non significant increase was noticed on 1st postoperative day only and they were reached to normal levels in the following postoperative period in all the groups. The acute phase response refers to a nonspecific and complex reaction of an animal that occurs shortly after any tissue injury. The origin of the response can be attributable to infectious, immunologic, neoplastic, traumatic, or other causes, and the purpose of the response is to restore homeostasis and to remove the cause of its disturbance. The acute phase response is considered a part of the innate host defense system, which is responsible for the survival of the host during the critical early stages of attack, and in evolutionary terms, it predates the acquired immune response. C-reactive protein can regulate the immune system during the early stage of an infection. C-reactive protein plays a role in destroying infectious agents, minimizing tissue damage and facilitating tissue repair and regeneration (Singh *et al.,* 1985).

Skin sutures were completely healed by 11-15 days in all the animals**.** The complications like suture dehiscence at the center of suture line with discharge of pus were noticed in four buffaloes (7.14%) in different groups following horn amputation and they were treated with daily dressing with 5% povidone iodine and Topicure spray.

Conclusion

The modified method of horn amputation with sedation proved to be best, due to its advantages like ease of flap rising, less duration of operation and stress.

References

Anderson DE and Muir WW (2005). Pain management in ruminants. *Veterinary Clinics in Food Animals*, 21: 19-31.

Angelo SJ and Das SC (1970). Uttar Pradesh Veterinary College, Mathura, Magazine.

Anil SS, Anil L and Deen J (2002). Challenges of pain assessment in domestic animals. *Journal of American Veterinary Medical Association,* 220: 313-319.

Boddie GF (1969). Diagnostic methods in veterinary medicine 6th edn. *Oliver and Boyd, Edinbourgh.* Pp: 109.

Chauhan HVS, Kalra DS, Kaul PL and Kumar R (1981). Studies on horn cancer-etiopathology. *Indian Veterinary Journal,* 58: 851-858.

Fioravanti MCS, Silva LAF, Leao MA, Juliano RS, Eurides D and Alves CD (1999). Use of metal clips for skin suture after cattle dehorning. *Ciencia Rural,* 29(3): 507-510.

Grandin T (1979). Assessment of stress during handling and transport. *Journal of Animal Science,* 75: 249-257.

Grondahl-Nielsen C, Simonsen HB, Damkjer LJ and Hesselholt M (1999). Behavioural, endocrine and cardiac responses in young calves undergoing dehorning with and without use of sedation and analgesia. *Veterinary Journal,* 158: 14-20.

Guyton AC (1981). Text book of Medical Physiology, 6th edn. *W B Saunders Co., Philadelphia,* Pp 259-293.

Heinrich A, Duffield TF, Lissemore KD and Millman ST (2010). The effect of meloxicam on behavior and pain sensitivity of dairy calves following cautery dehorning with a local anesthetic. *Journal of Dairy Science,* 93: 2450-2457.

Holton L, Pawson P, Nolan A, Reid J and Scott EM (2001). The development of a behavioural based pain scale to measure acute pain in dogs. *Veterinary Record,* 148: 525-531.

Kulbhushan, Varshney AC, Sharma DN, Singh M, Sharma SK and Nigam JM (1999). Analgesia of horn in yak. *Indian Veterinary Journal,* 76: 804-806.

Kumar R and Thilagar S (2000). An unusual case of bilateral horn cancer in a buffalo. *Indian Veterinary Journal,* 77: 48-49.

Mahida HK, Tank PH, Dhami MA, Vagh AA, Vala KB, Karle AS and Vedpathak HS (2010). Modified flap method for amputation of the horn in buffaloes. *Indian Journal of Veterinary Surgery,* 31(1): 47-48.

Manjunath SM, Dilip Kumar D, Vishwanath RH and Usturge SM (2007). Squamous cell carcinoma of eye and horn in Deoni cross bred cattle. *Indian Veterinary Journal,* 84: 399-400.

Mistry JN (2009). Surgical affections of horn in Mehsani buffaloes and their management. *Indian Journal of Veterinary Surgery,* 30(2): 112-113.

Mohanty J, Mitra AK and Das AC (1972). Modified surgical approach for treatment of horn cancer. *Indian Veterinary Journal,* 5(49): 726-729.

Schuszler L, Igna C, Mircu C, Brudiu I and Sabau M (2007). Multimodal approach of dehorning pain management using metamizol in 2 month old calves. *Lucrai Stiintifice- Medicina Veterinara, Universitatea de Stiinte Agricole si Medicina. Veterinara Ion Ionescudela Bradlasi,* 50(9): 446-449.

Singh US, Somvanshi R, More T and Singh GR (1985). Blood serotonin and cortisol levels in bovine horn cancer. *Indian Veterinary Journal*, 62: 13-15.

Sreenu M and Kumar NR (2006). Affections of horn in buffaloes. *Indian Veterinary Journal,* 83: 1206-1207.

Stafford KJ and Mellor DJ (2005). Dehorning and disbudding distress and its alleviation in calves. *The Veterinary Journal*, 169: 337-349.

Sylvester SP, Stafford KJ, Mellor DJ, Bruce RA and Ward RN (2004). Behavioural responses of calves to amputation dehorning with and without local anesthesia. *Australian Veterinary Journal,* 82(11): 697-700.

Tank PH, Kshama D, Shirodkare, Sunetra, Patil VN and Mankad MY (2006). Amputation of horn in Mehasani buffalo: A modified method. *National Seminar Organize at college of Veterinary Science and Animal Husbandry, S. D.A.U.*

Terlouw EMC, Schouten WGP and Ladewig J (1997). in Animal Welfare, (Ed. Appleby MC and Hughes BO). *CAB International, Wallingford, UK.*

Vickers KJ, NIel L, Kiehlbauch LM and Weary DM (2005). Calf response to caustic paste and hot iron dehorning using sedation with and without local anesthetic. *Journal of Dairy Science*, 88(4): 1454-1459.

Weary DM, Neil L, Flower FC and Fraser D (2006). Identifying and preventing pain in animals. *Applied Animal Behavioral Science*, 100: 64-76.

Yadav GU, Jadhao PT, Moregaonker SD, Bhikane AV and Mugale RR (2002). An unusual horn cancer in a cow-A case report. *Indian Veterinary Journal*, 79: 515-516.