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Zun Zun Wut Hmohn
Department of Surgery and
Theriogenology, University of
Veterinary Science, Nay Pyi
Taw, Myanmar

Haymar Kyaw
Department of Surgery and
Theriogenology, University of
Veterinary Science, Nay Pyi
Taw, Myanmar

Su Hlaing Phyu
Livestock Breeding and
Veterinary Department,
Myanmar

Chaw Lae Yee Hnin
Livestock Breeding and
Veterinary Department,
Myanmar

Saw Po Po
Department of Medicine,
University of Veterinary Science,
Nay Pyi Taw, Myanmar

Than Than Sint
Department of Surgery and
Theriogenology, University of
Veterinary Science, Nay Pyi
Taw, Myanmar

Min Bo
Department of Surgery and
Theriogenology, University of
Veterinary Science, Nay Pyi
Taw, Myanmar

Soe Win Naing
Department of Surgery and
Theriogenology, University of
Veterinary Science, Nay Pyi
Taw, Myanmar

Correspondence

Zun Zun Wut Hmohn
Assistant Lecturer, Department
of Surgery and Theriogenology,
University of Veterinary Science,
Nay Pyi Taw

Effect of toxic levels of *Leucaena leucocephala* on semen quality of goats in Myanmar

Zun Zun Wut Hmohn, Haymar Kyaw, Su Hlaing Phyu, Chaw Lae Yee Hnin, Saw Po Po, Than Than Sint, Min Bo and Soe Win Naing

Abstract

The aim of this study was to investigate the effects of toxic levels of *Leucaena leucocephala* (*L. leucocephala*) on semen quality in Myanmar goats. The study was done on nine male goats, and randomly divided into three groups. Group A was kept as control. Group B and C were given 40% and 60% *L. leucocephala* of total diet respectively for two months. All leucaena treated goats exhibited clinical signs such as alopecia, weight loss, dullness, appetite loss and salivation. There were significant difference on semen volume, live spermatozoa and percentage of motile spermatozoa among three groups. The concentration of spermatozoa was significantly lower in the Group C when compared to Group A ($p < 0.05$).

Keywords: *Leucaena leucocephala*, bucks, semen quality, spermatozoa

1. Introduction

Goats play an important socio-economic role and most of the resource for poor farmers in the rural areas (Anaeto *et al.*, 2009) [2] and form an integral part of the cultural life and system of Africa life (Ajala, 2004) [3]. To increase small stock productivity, improved goat nutrition appears to be a more critical factor. Leucaena is a vitally important source of protein for ruminants throughout south-east Asia (Shelton and Brewbaker, 1994) [28]. Although *L. leucocephala* has many positive nutritional benefits, it possesses the toxic non-protein free amino acid (Hegarty *et al.* 1964) [17, 18]. Typical signs of *L. leucocephala* toxicity are alopecia, anorexia, reduced weight gain and weight loss, excessive salivation, esophageal lesions, enlarged thyroid and low circulating concentrations of thyroid hormones (Jones, 1979) [23]. Chronic or acute toxicosis can occur and death rapid and removal of *L. leucocephala* supplementation is the best way for recovery (Blunt and Jones, 1997; Jones, Blunt and Nurnberg, 1978; Jones and Hegarty, 1984) [7, 22, 24]. Foetal abortions (Holmes 1980) [19], low bull fertility (Holmes 1981) [20] and death (Prasad and Paliwal, 1989; Dalzell *et al.*, 2012) [27, 11] were reported by feeding leucaena. The aim of this study was to find out the effects of leucaena feeding on reproductive performance especially on the semen quality of goats in Myanmar.

2. Materials and methods

2.1 Experimental design

Nine bucks were randomly divided into three groups and penned in well-ventilated sheds with free access of feed and fresh water (*ad libitum*). The animals were treated for internal parasites prior to the experiment. Before the experiment, the animals were fed rice straw, sesame and rice bran for four weeks to make sure the animals are free from mimosine toxicity. Experimental bucks were fed three percent of their body weight daily. Diets fed to the groups A, B and C were described (Table 2). All treatment diets were adjusted to be isonitrogenous containing 18% crude protein. Leucaena leaves were air-dried at room temperature (about 37 °C). The feeding trial (treatment) lasted for 8 weeks.

Table 1: Feeding Management.

Groups	Treatments
A	Rice straw + sesame + rice bran
B	Rice straw + sesame + rice bran + Leucaena (40% of the total diet)
C	Rice straw + sesame + rice bran + Leucaena (60% of the total diet)

2.2 Semen collection by electro ejaculator (EEJ) method

Semen was collected from the bucks by EEJ method. To collect the semen, goat was restrained under lateral recumbency. Before running the EEJ, 1ml of normal saline was used to clean the prepuce by irrigation. And then 3 ml of normal saline was infused into the anus by disposable syringe for better contact. Aseptically prepared probe was used to prevent the infection. Lubricants were used to insert the probe easily into the rectum. More than half of the length of probe was inserted. The nerves of reproductive tract of the bucks were stimulated by pressing the probe ventrally along the anus.

2.3 Semen colour

Gross appearance in colour was assessed and noted clear, thin milky (watery white), thick milky (milky white), creamy or thick creamy white (Cameron, 1977) [8].

2.4 Semen volume

The volume was measured by using graduated test tube. The measuring was done immediately after ejaculation (Chemineau *et al.*, 1991) [9].

2.5 Sperm concentration

A haemocytometer was used to count concentration. Apparatus using for assessment were microscope, haemocytometer, cover slip, red cell pipette and diluting fluid. Diluting fluid was prepared by dissolving 50 ml of 40% commercial formaldehyde with 450 ml of normal saline. To assure accuracy of the measurements of these diluted suspensions, the same pipette and tip were used for dilution. This dilution created a range of sperm concentrations, each of which was measured for light absorbance and counted by use of haemocytometer for sperm concentration.

2.6 Motility of spermatozoa

Table 2: Range of motility

Motility %	Forms of motion
0	Totally immobile
< 10	Very slow movement
30-60	General wave motion, small waves
65-80	Rapid waves, no eddies
85-95	Rapid waves with eddies

(Chemineau *et al.*, 1991,) [9]

2.7 Percentage of live spermatozoa

The percentage of live spermatozoa was determined from a smear stained by using Eosin and Nigrosin. Eosin was taken up by the already dead spermatozoa, thus staining pink. Nigrosin was used as the background stain in blue colour. The Nigrosin-Eosin stain is made up of 0.25 g of aqueous eosin,

1.25 g of aqueous nigrosin, 0.75 g of Tri-sodium citrate and 25 ml of double-distilled water (from Bearden and Fuquay, 1980) [6]. The stain was kept at room temperature.

2.8 Statistical analysis

The data were analysed using the SAS software system (Version 9, SAS). The comparison between treatments means were worked out by Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

2.9 Observing clinical signs

As the experimental animals were fed three times daily, body condition and presence of any abnormal signs were assessed while feeding.

3. Results

3.1 Animal health

Clinical signs of buck due to toxic effect of *L. leucocephala* were found. Alopecia, dullness or salivation was visually assessed. The bucks from groups B and C showed loss of body weight, alopecia, dullness and salivation.

3.2 Semen colour

Semen colour collected from bucks fed on different levels of *L. leucocephala* during the experiment was presented. Most of the semen sample were watery white and milky white in colour.

3.3 Semen volume (ml)

There was no significant difference for semen volume among three groups. Volume of semen collected from bucks fed on different levels of *L. leucocephala* were (0% level, 1 ± 0.06 ; 40% level, 1 ± 0.03 ; and 60% level, 1 ± 0.04 , $p > 0.05$) as shown in Table 3.

3.4 Sperm concentration (millions per ml)

The concentration of spermatozoa of bucks fed on different levels of *L. leucocephala* were (0% level, 2320 ± 170 , 40% level, 2200 ± 250 and 60% level, 1950 ± 158). The concentration of spermatozoa were not significant different between 0% and 40% leucaena-fed group (Table 3). The concentration of spermatozoa fed on 0% level of *L. leucocephala* was significantly higher than 60% level of *L. leucocephala* ($p < 0.05$).

3.5 Percentage of live spermatozoa

Percentage of live spermatozoa in 0%, 40% and 60% level of *L. leucocephala* were 76 ± 0.02 , 74 ± 0.02 and 70 ± 0.03 , respectively ($p > 0.05$). There were no significant differences in percentage of live spermatozoa among 0%, 40% and 60% leucaena-fed groups as shown in Table 3.

3.6 Motility of spermatozoa (%)

Percentage of motile spermatozoa fed on different levels of *L. leucocephala* was (0% level, 73 ± 0.04 ; 40% level, 81 ± 0.03 ; 60% level, 76 ± 0.04 ; $p > 0.05$). Percentages of motile spermatozoa are presented in Table 3. There were no significant differences in percentage of motile spermatozoa among three groups.

Table 3: Sperm concentration, semen volume, percentage of live sperm cell and motility of spermatozoa of bucks fed on different levels of *L. leucocephala*.

Item	Diet			p value
	<i>L. leucocephala</i> 0%	<i>L. leucocephala</i> 40%	<i>L. leucocephala</i> 60%	
Volume (ml) Motility (%)	1 ± 0.06 ^a 7	1 ± 0.03 ^a	1 ± 0.04 ^a	NS
	3 ± 0.04 ^a	81 ± 0.03 ^a	76 ± 0.04 ^a	NS
Concentration (millions per ml).	2320 ± 170 ^a	2200 ± 250 ^{ab}	1950 ± 158 ^b	<0.05
Live sperm percent (%)	76 ± 0.02 ^a	74 ± 0.03 ^a	70 ± 0.02 ^a	NS

Different script within same row indicates significant difference.

Average values of semen volume, motility, sperm concentration and live sperm percentage are summarized in table 3. Statistical analyses indicate no significant differences between treatments with regard to volume, motility and live sperm percentages. However, spermatozoa concentration exhibited between Group A and C at 0.05% level.

4. Discussion

L. leucocephala showed unfavourable effect on health of local bucks during the experiment. The earliest and most prominent signs were alopecia, loss of appetite and body weight loss. Aung Aung (2007) [4] reported that toxic signs were observed in local sheep one week after feeding 40% leucaena. The toxic effect of mimosine such as weight loss, alopecia and emaciation in bucks, rats and ruminants were observed by Harith *et al.* (1979) [13] and Halliday *et al.* (2013) [14]. Colour of semen ranged from white to creamy white in visual option (Chemineau *et al.*, 1991) [9]. In this experiment, most of the semen colour were white and milky white. Wildeus (1996) [30] described the volume of semen per ejaculate as between 1 and 1.5 ml and recommended 1-1.6 ml as wider range. Average volume of semen recorded in this experiment ranged from 1.0 to 1.4 ml per ejaculate. No significant difference was recorded. Chemineau *et al.* (1991) [9] reported that 65 – 80% was the stage of rapid waves without eddies. In this experiment, the percentage of motile spermatozoa were 73 ± 0.04 in Group A, 81 ± 0.03 in Group B and 76 ± 0.04 in Group C. Therefore, there was no significant difference among all groups. For the percentage of live spermatozoa, Chemineau *et al.* (1991) [9] stated that the ratio did not contain more than 20-30% of dead spermatozoa by staining method. In this study, 76 ± 0.02, 74 ± 0.02 and 70 ± 0.03 were group A, B and C respectively. Therefore, there were no significant difference among all groups on the percentage of live sperm cell ($p > 0.05$). Feeding of *L. leucocephala* at the level of 20% to rabbit bucks and male rat for 10 weeks decreased in percentage of live sperm cell (Herbert *et al.*, 2005) [16]. However, no significant differences in the effect of *L. leucocephala* on semen characteristic for 60 days were observed by Akingbade *et al.* (2002) [1]. Therefore, the longer experimental period (more than eight weeks) should be needed to confirm the toxic effect of *L. leucocephala* on motility, percentage of motile spermatozoa and percentage of live spermatozoa. The concentration of spermatozoa ranged from 1600 to 6000 million per ml (Ax *et al.*, 2000) [5]. The concentration of spermatozoa (millions per ml) in Group A, B and C ranged in this study were (2320 ± 170), (2200 ± 250) and (1950 ± 158) respectively. There were no significant differences in spermatozoa concentration between Group A and B. However, the concentration was significantly lower in Group C than in group A ($P < 0.05$). No adverse effect on the reproductive performances of bucks in Philippines fed on 75% of leucaena in the ration was reported by Girdhar (1991) [13]. These might be due to feeding adaptation of mimosine for

long time ago get in Phillipines (Franciso, 1988) [12]. Akingbade *et al.* (2002) [1] also reported that *Leucaena* species can be safely fed to South African Nguni goats without adverse effect on semen quality and reproductive performance of bucks since they have received the bacteria *Synergistes jonesii* once. The supplementation of *L. leucocephala* up to 50% can be fed for four weeks to Myanmar local bucks without deleterious effect on semen quality (Thet Su Myat 2010) [29]. However, poor semen quality occurred when feeding level of 50% leucaena in Murrah male goats in research of Lohan *et al.* (1987) [25]. They presented that this might be due to the mimosine toxicity, seasonal effect and breed of experimental animal. In this research, the feeding of toxic level of *L. leucocephala* did not effect on the semen quality (volume, motility and live sperm per cent) of local bucks in Myanmar. Herbert *et al.* (2005) [16] found that depressed spermatozoa production and semen output in the animal receiving the 20% leucaena leaves meal of total diet for the rabbits and bucks. They reported that this might be due to the mimosine toxicity. In this study, the finding indicated that feeding of *L. Leucocephala* containing 40% and 60% of total diet did not cause adverse effect on semen quality (exception sperm concentration) of bucks during the experimental period. The time required to produce a single fertile spermatozoon is 49 days, with little variation (Machado *et al.*, 2011) [26]. The process can be divided into three stages: thirteen one day are necessary for development of the spermatozoan from the first division of the germ cells until it reaches the centre of the seminiferous tubule. In the second stage, three and a half days are required to pass through the tubules, out of the testis and into the epididymis. Fourteen days are needed for the journey through the epididymis in the third stage. It is suggested that no changes on semen quality parameter such as volume, percentage of motile sperm and percentage of live spermatozoa of bucks fed on *L. Leucocephala* for eight weeks might be due to the storage of spermatozoa lasted about 49 days before this experiment was done. It is suggested that experimental duration was not long enough to produce adverse effect of *L. Leucocephala* on semen quality of Myanmar local bucks. Therefore, long term study on feeding of toxic levels of leucaena might give many explanations for semen quality.

5. Conclusion

Feeding leucaena leaves to the experimental goats of this study produced not significant differences in semen colour, semen volume, sperm motility and live sperm percentage of buck semen. A significant reduction in sperm concentration of bucks fed 60% leucaena was observed when compared to that of 0% group.

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