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Review Article

Application of Biotechnology for Augmentation of Productivity in Mithun (*Bos frontalis*)

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ABSTRACT

Mithun, also known as 'Cattle of Mountain" is an important bovine species of north-eastern hill region of India and also of China, Myanmar, Bhutan and Bangladesh. This magnificent massive bovine is presently reared under free-range condition in the hill forests at an altitude of 1000 to 3000 m above mean sea level. Mithun plays an important role in the socio-economic and cultural life of the local tribal population. Due to dwindling population of mithun over the years and gradual denudation of free range forest areas for mithun grazing along with the biotic and abiotic stress, there is urgent need of scientific intervention for proper management as well as conservation of this species. Application of various biotechnological tools like artificial insemination, estrus synchronization for timed-AI, multiple ovulation and embryo transfer, rumen microbial manipulation and modern breeding techniques may be of great use for faster multiplication and propagation of this species in near future.

Keywords: mithun productivity, biotechnology, conservation, meat, milk, rare species

INTRODUCTION

Livestock improvement is a continuous process. This can be achieved through application of various scientific methods of breeding, feeding and management. In the recent times, due to tremendous development in the field of biotechnology, especially in the areas such as functional genomics, pharmacogenomics, proteomics, nutrigenomics, stem cell etc., we are being able to cope up ourselves with the modern techniques in those fields for improvement of our livestock species. These technologies are of great use for augmenting the productivity of our livestock.

Mithun *(Bos frontalis)*, a pride animal of Northeastern hills of India, is a rare species of semi-domesticated bovine (Simoons, 1984; Mondal and Pal, 1999; Mondal *et al.*, 2004; 2005a-e; 2006a-f; 2008; 2010; 2014). This animal is mainly confined in the North Eastern Hill region of India and also found in lesser numbers of few selected tracts of Myanmar, Bhutan, and Bangladesh as well as Yunan province of China (Mondal *et al.*, 2014). Mithun is well adapted in steep jungles at an elevation ranged from 1000 to 3000 meter above mean sea

level and has got important place in economic, social, cultural and religious life of tribal people (Simoons, 1984; Mondal and Pal, 1999; Mondal *et al.*, 2004; 2005a-e; 2006a-f; 2008; 2010; 2014). This is an underutilized animal and has got a great potential for quality meat, milk and leather production. Mithun meat, milk as well as leather are of very good quality and there is a great scope to promote this species as an organic meat and milk producer (Mondal *et al.*, 2014). At present, farmers rear this animal under free grazing condition without much emphasis on scientific rearing methods. At this perspective, there is an immediate need to implement the scientific mithun rearing system as well as to utilize the optimum production capabilities in terms of meat, milk and other related value added products.

Distribution and Habitat

Mithun (*Bos frontalis*), is considered as the domesticated form of wild gaur (Mondal *et al.*, 2014). This species has a limited geographical distribution. It is mainly found in the rain forests of Arunachal Pradesh, Nagaland, Manipur and Mizoram States of the Northeastern Hill Regions of India. It is also found in small numbers in Myanmar, China, Bangladesh and Bhutan (Simoons, 1984; Mondal and Pal, 1999; Mondal *et al.*, 2004; 2005a-e; 2006a-f; 2008; 2010; 2014). At present, the population of mithun in India is approximately 0.26 million. As per the quinquennial All India Livestock Census (1997), India had a total population of 1, 76, 893 mithuns. Of the total population, the Arunachal State alone had 70.25% (1, 24, 194 heads). The Nagaland State had 18.86% (33,445 heads) followed by Manipur (9.42%; 16,660 heads) and Mizoram States (1.47%; 2,594 heads). It was seen from the census of the year 2003 that the country possessed 2, 46, 315 numbers of mithun, which registered a growth rate of only 6.5% per year over the population reported in 1997 census and this growth rate is far below than those recorded during last census in 1997 over 1991 (growth rate: 24.56%) indicating declining trend of population of mithun growth rates over the decades. In the recent census (2007), it has been noted that except Arunachal Pradesh, in all other three mithun-inhabited States, percent contribution declined very drastically, particularly in Mizoram State, where the total number of mithuns are less than 2000 heads (Table 1; Mondal *et al.*, 2014). These data suggest an immediate developmental and research attention to this species of animal through proper scientific rearing.

Table 1. Recent trend of Mithun population in India (Mondal *et al.*, 2014)

State	Year		
	1997	2003	2007
Arunachal	1,24,194	1,84,343	2,18,931
Nagaland	33,445	40,452	33,385
Manipur	16,660	19,737	10,024
Mizoram	2,594	1,783	1,939
Total	1,76,893	2,46,315	2,64,279

Currently there are four defined mithun strains, namely Arunachal, Nagaland, Manipur and Mizoram strains (Mondal *et al.*, 2014). These strains are named after the North Eastern states, where they belong to. The contributions of different mithun strains to the total mithun population of India are 75%, 16%, 8% and 1% for Arunachal, Nagaland, Manipur and Mizoram strains, respectively. Among these four different strains, Arunachal strain is the biggest in size, while Mizoram strain is the smallest in size.

Economic Utility

Mithuns are mainly reared for meat purpose. This animal is popularly used as marriage gift and sacrificial animal for different social, cultural and religious ceremonies. At present

farmers do not consume its milk but the milk of this animal is highly nutritious (Mondal *et al.,* 2014).

Being a beef animal, the growth rate of mithun is the prime concern to the farmers. The birth weight of Mithun calves varies from 17 to 22kg. With adequate feeding the growth rate of this animal varies from 266 to 733 g per day, which is comparable with cattle and buffalo. However, the plasma growth hormone concentration (30-90 ng/ ml) is much higher in mithun than any other domesticated animals (Mondal *et al.,* 2006d, 2014). There is a heavy demand for this meat and tribal consumers consider this meat as more tender and superior over the meat of any other species except pork. The dressing percentage in mithun varies from 48 to 54% in different age groups. To achieve an optimum dressing percentage, it is suggested that the Mithun should be slaughtered at the age of 4 to 5 years. This animal produces around 1 to 1.5 kg milk per day (Mondal *et al.,* 2014). However, mithun milk is nutritionally superior to any other domesticated species as it contains high fat (8 to 13%), solid-not-fat (18 to 24%) and protein (5 to 7%). Hence, mithun has a great scope to be exploited as moderately good milk animal for home consumption in these hilly areas. As milk contains high fat and protein, there is great scope for the preparation of different value added products like paneer, various sweet products, ghee, cream, curd etc. Due to high protein content in mithun milk, there is scope to utilize this milk for cheese production also.

As that of milk, the quality of mithun meat is also very good as the protein content in muscle and organs varies from 14 to 19 percent and crude fat and carbohydrate in mithun carcass have been found within the range of 0.4 to 3.58 percent and 0.06 to 4.97 percent, respectively (Das *et al.,* 2011). As mithun is grown organically, it can be promoted as organic meat in the international market. Moreover, various value added meat product can also be promoted in the international market. Some value added meat product like meat powder, nuggets, patties and meat block has already been prepared. There is ample scope to utilize some of the modern biotechnological procedure available in the field of food technology for producing quality value added meat and milk products.

The quality of mithun hide is found to be superior in comparison to the traditional cow hides (Das *et al*., 2011). Mithun hide has been found to be very good for the production of shoe leather, bag leather as well as garment leather. Bag leather has been found to be much superior quality than cow leather (Das *et al.,* 2011). Besides, mithun hide with hairs could be promoted as exotic stuff as sofa cover as well as carpet material (Das *et al*., 2011). The finished product like shoes, jacket, ladies bag etc., which have been prepared from mithun leather, got a very good acclaim.

Genetic Improvement of Mithun

Traditional breeding approaches like selection of mithun for desired trait and nominated mating has been the only method for improvement of livestock so far. However, advancement of science particularly in the field of molecular genetics has given a number of powerful tools in the hand of breeders and scientists for faster genetic improvement. Markers assisted selection approach is one these powerful techniques that is currently being utilized for this purpose. Marker assisted selection is based on the genetic polymorphism at the DNA level, which is identified and utilized for selection of animals having desired genotype. The various applications of molecular markers can be parentage determination, estimation of genetic distance, embryo and semen sexing, gene mapping and selection of animals for disease resistance, and traits with very low heritability.

Marker-assisted selection provides accurate selection of specific DNA variations that are associated with a measurable difference or effect on complex traits of animals. However, marker assisted selection should be considered as a powerful technique to assist with conventional selection techniques and not as its total replacement. Potential benefits from marker assisted selection may be achieved for traits that are: a) simply inherited traits (coat

color, genetic defects), b) carcass quality and palatability traits, c)fertility and reproductive traits, d)carcass quantity and yield, e) milk production and maternal ability, and f) growth performance.

Even if the potential benefit is enormous, still there is long way to go to reap the rich benefit of marker assisted selection in livestock improvement including mithun and yak, as the technology is complex and their applications are widely variable under existing situations. However, marker assisted selection technique has completely revolutionized the system of genetic improvement of livestock, which was thought to be impossible even a decade before. DNA-based technologies are developing at a rapid pace. It is likely that these technologies will play a progressively more important role in animal improvement. However, future application of marker assisted selection technique will depend on how much they are profitable, their economics of use and the rate of genetic gains and their sustainability in long run.

Biotechnological Interventions for Better Production

Though at present the farmers are rearing mithun under free grazing system without any additional managemental inputs, but to utilize this animal with optimum production potential, there is urgent need to adopt this animal with the scientific rearing systems. The various components of scientific mithun rearing are discussed below.

Nutritional Biotechniques

Antibiotic compounds have been employed as feed additive for livestock and poultry for nearly 50 years. There has been a general believe that use of low concentration of antibiotics may favour proliferation of antibiotic resistant microorganisms, which in turn may have serious consequences for disease control in human and animals. Probiotics or feeding of microbial feed additive is the alternative to antibiotics, which benefit the animal by establishing a favorable intestinal microbial balance, nutrition, growth and health. These are nonpathogenic and non toxic to animals and human being. The probiotics like yeast (*Sacharomyces cerevisiae*) and lactic acid bacteria (*Lactobacillus acidophilus*) have successfully been tried in cattle for increasing growth and production. The same type of probiotics can also be used in Mithun for increasing the growth rate and feed conversion efficiency as a consequence of economizing the Mithun production. The overall health condition like rumen fermentation pattern of Mithun was also improved after feeding yeast.

Genetic manipulation of rumen in Mithun can also be initiated for increasing the Mithun production. The population of fiber degrading microbes in the Mithun rumen can be increased for degradation of lignocellulosic feeds by genetic manipulation. These lignocellulosic by-products contain 60-70% of gross energy in the form of either cellulose or hemicellulose, which is otherwise good sources of energy for the ruminants. Due to presence of anti-nutritional factors like lignin, tannin, silica etc. the animals are able to extract only up to 50 to 60% of potential energy from such feeds. So, enhancement of extraction of this energy by genetic manipulation techniques may result in increasing livestock productivity with the available feed resource in the country.

Another area of interest for biotechnological intervention is the microbial diversity of Mithun. The microorganisms playing key role in degradation of lignocellulosic feed in the rumen of Mithun must be identified and different chemicals/plant secondary metabolites must be tried for their potential to stimulate the activity of identified microbes and improvement in fiber degradation. The work on genetic characterization of the mithun rumen microbes should also be carried out. These microbes can also be maintained and refined for quality and fast production for meeting the demand of the market.

Breeding management

Mithun is a polyestrus animal. A healthy adult female mithun shows repeated estrous cycle at an interval of 19 to 24 days unless it is pregnant (Mondal *et al.*, 2004; 2005a-e; 2006a-f; 2008; 2010; 2014). The mithun bred throughout the year and no definite breeding season is observed in this species. The length of gestation period, service period and calving interval in mithun varies from 270 to 290 days, 50 to 100 days and 350 to 400 days, respectively. Whereas, the age at puberty and age at first calving varies form 18 to 24 months and 35 to 40 months, respectively (Mondal *et al.;* 2004; 2005a-e; 2006a-f; 2008; 2010; 2014). The mithun bulls become matured to breed at 3 to 4 years of age. Under free-range system, a practical approach to rear a good stock of animal will be selection breeding through introduction of superior and tested bulls (1 bull for 10 breedable females) in the herd and simultaneous culling of the unwanted bulls from the herd. There should be simultaneous effort to replace breeding bulls preferably once in five years to avoid inbreeding depression. Under semi intensive system, the detection of heat in female is another important step as this animal very often expresses silent heat so that breeding can be done with superior bull either through natural service or through artificial insemination (Mondal *et al.*, 2014).

Reproductive Biotechniques

Artificial Insemination

Artificial insemination is a very efficient method to propagate this animal and also a tool to give the farmers a way of increasing quality animals through using quality semen collected from proven mithun bull for artificial insemination. Semen collection and preservation of commercially important livestock may be a viable venture in the field of biotechnology for young entrepreneurs for augmenting the productivity status of livestock.

Unlike other bovine, mithun bulls do not mount cows that are not in estrus (heat) thereby problem to get semen regularly using artificial vagina (AV) method (Mondal *et al.,* 2010). Here, we tried to use urine from estrus cows stored at refrigerated temperature to attract bull to mount. This urine samples were sprinkled over the perineal region of mithun cow not in estrus and bull reacted as an estrus cow and semen was collected successfully. Urine collected from estrus cows and stored at refrigerated temperature was effective till day 7 post-collection (Mondal *et al.,* 2010).

To get more quantity of semen, we stimulated the bull (which were not capable of mounting the mithun cow properly) with artificial vagina in presence of an estrus/urine sprinkled cow. Massage followed by stimulation produced more quantity semen and there was no need of centrifuge the semen samples to get more concentrated semen for cryopreservation. After suitable evaluation of mithun semen, we have successfully cryopreserved mithun semen for AI and in situ conservation of this valuable germplasm (Mondal *et al.,* 2010).

Estrus synchronization

Estrus synchronization is the manipulation of reproductive process so that female can be bred with normal fertility during a short, predefined interval. It facilitates breeding in two important ways: it reduces and in some cases eliminates labour of detecting estrus, and it allows the producers to schedule the breeding. Considering the importance of synchronization of estrus in mithun, estrus synchronization protocols for timed-AI in mithun has been developed using $PGF_{2\alpha}$, GnRH-$PGF_{2\alpha}$-GnRH (Ovsynch) and controlled intravaginal drug (progesterone) releasing device (CIDR).

a) Estrus synchronization using $PGF_{2\alpha}$

Two injections of $PGF_{2\alpha}$ were given at 11 days apart in cyclic mithun cows. Animals were observed for signs of estrus after second injection of $PGF_{2\alpha}$ and found that mithun cows responded to this treatment. The time from onset of estrus to ovulation was 27.7±0.61 hr with

a range of 26 to 31 hour in $PGF_{2\alpha}$ treated group compared to 26.9 ± 0.31 hour with a range of 26 to 29 hr in control group (Mondal *et al.,* 2014; unpublished data).

b) Estrus synchronization using Ovsynch protocol

Cyclic mithun cows irrespective of any day of estrous cycle were subjected to Ovsynch protocol of estrus synchronization. All mithun cows responded to this treatment. The ovulation time and its relation with LH characteristics were recorded. After developing this protocol in mithun, in the next step we went for fixed time artificial insemination following synchronization. Initially, a total of 16 animals were inseminated artificially (AI) with the cryopreserved mithun semen, 12 cows were conceived (Mondal *et al.,* 2014; unpublished data).

c) Estrus synchronization using CIDR

Experiments were conducted to synchronize estrus using CIDR in cyclic and post-partum mithun cows. In both categories of animal, synchronized estrus using CIDR showed more prominent behavioural signs of estrus than spontaneous heat. More interestingly, application of CIDR on day 45-50 after parturition induced first postpartum estrus immediately after uterine involution (day 53-58 post parturition). Unlike other bovine, mithun cows exhibit first postpartum estrus at around day 102±19.6 postpartum. Use of CIDR is therefore advantageous in terms of a) prominent behavioural signs of estrus thus ease detection of estrus and b) increased productive life span of around 50 days (Mondal *et al.,* 2014; unpublished data).

Multiple ovulation and embryo transfer (MOET)

Superovulatory treatments are widely used in embryo transfer programs to increase the supply of embryos from animals of superior genetic merit. Mithuns suffer from many inherent reproductive problems and these problems coupled with poor production potential due to high inbreeding depression are making mithun husbandry less profitable and accounting for rapid dwindling of mithun population. Through successful application of multiple ovulation and embryo transfer program many problems of mithun reproduction could be solved by faster multiplication of superior germplasm, reducing inbreeding depression by disseminating superior quality male germplasm to mithun pockets and by conserving mithun germplasm pertaining to ex-situ conservation procedure.

Superovulation and embryo transfer technology (ETT) has been standardized for mithun. The first mithun calf, Bharat, was born through embryo transfer technology in 2012 at National Research Centre on Mithun, Nagaland, India. Cryopreservation of mithun embryos has also been standardized. Mohan, the first mithun calf was born from transfer of a 100-day old cryopreserved embryo in 2012 at National Research Centre on Mithun, Nagaland, India (Mondal *et al.,* 2014; unpublished data).

CONCLUSIONS

International Union for Conservation of Nature and Natural species (IUCN, 2000) has categorized mithun (*Bos frontalis*) as the most “vulnerable” facing extinction. It is therefore, the responsibility of the scientific community to save this precious species of Asian origin before being extinct from the nature. As the concept of mithun husbandry is still in developing stage in almost all the mithun rearing areas of our country, more emphasis should be given on scientific rearing of mithun using modern biotechnological tools beginning with application of reproductive biotechniques, feed technology to use of modern technologies of diagnosis of disease at very early stage to exploit the production potential of this unique

species, which can ultimately cater the nutritional needs of poor tribal people of hilly areas of mithun inhabited countries in a sustainable way. Modern biotechnological tools as described herein may be used extensively to increase productivity of livestock in general and mithun in particular.

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