वार्षिक प्रतिवेदन

Annual Report

2012-13

भाकृअनुप
ICAR

राष्ट्रीय मिथुन अनुसंधान केन्द्र

(ाारतीय कृषि अनुसंधान परिषद)

झरनापानी, मेड्जीफेमा, नागालैन्ड - 797 106 ाारत

I.C.A.R. N.R.C. MITHUN-NAGALAND

NATIONAL RESEARCH CENTRE ON MITHUN

(Indian Council of Agricultural Research)

Jharnapani, Medziphema, Nagaland - 797106, India

www.nrcmithun.res.in

Glimpses of our previous Annual Reports

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Hindi Translation

Dr. (Mrs.) Anupama Mukherjee

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प्राक्कथन

संस्थान के 2012-13 के मूल्यवान दस्तावेज वार्षिक प्रतिवेदन जिसमें वि िन्न प्रक्रियों को दर्शाया गया है को प्रस्तुत करते हुए मुझे अत्यंत गर्व महसूस हो रहा है।

इस वर्ष में हमारे संस्थान में मिथुन पाले जाने वाले किसानो की कई गतिविधियो जैसे संस्थान का स्थापना दिवस, कृषि नवाचार दिवस, शिक्षा दिवस, उद्योग इत्यादि, जिसमें काफी प्रति ागियों ने हिस्सा लिया। हमारे वैज्ञानिकों ने मणिपुर नागालैंड, मिजोरम, एवं अरूणाचल प्रदेश के दूरस्थ एवं अत्यंत दूरवर्ती क्षेत्रों में जाकर मिथुन किसानों एवं मिथुन धारकों के साथ सीधा संपर्क करने में सहायता साबित हुआ है।

वर्ष 2012-13 में मिथुन पालन के क्षेत्र में वि िन्न विषयों के वैज्ञानिको कि टीम बहुमूल्य जानकारी एकत्रित है।

मिथुन आनुवांशिक एवं प्रजनन में फ्लोरोसेंट इनसीटू संकरण के उपयोग के काम निश्चित रूप से मिथुन गुणसूत्रों को समझने के लिए एक नई अंतरदृष्टि होगी। जो मिथुन के गुणसूत्रीय

PREFACE

It is my proud privilege to place this valuable document of Annual Report of our institute depicting the activities of the institute for the year 2012-13

In this year we had the opportunity to do some activities like institute foundation day, Farm innovation day, Education day, Industry day etc where there was large number of participant's n from various Mithun rearing areas. Our scientists have even visited remotest of remote area of Manipur, Nagaland, Mizoram and Arunachal Pradesh for empowering the Mithun farmers with recent technologies. These programmes have helped us for making direct contact with the stake holders.

The team of scientists from various discipline generated valuable information in the field of mithun husbandry during 2012-13.

The work initiated in mithun genetics and breeding through the application of FISH will definitely give a new insight to the

इवाल्यूशन का पता लगाने के लिए सहायक होगी।

दूध प्रोटीन जीन पर किया कार्य मिथुन दुध प्रोटीन के मुख्य गुणों को पहचाने एवं केसीन जीन सं ाावित बहुरूपता को पहचानें में सहायता देगी।

एफएओ की सूची अनुसंधान माइक्रोसेटेलाइट मार्करों मिथुन एवं गाउर में जैव विविधता अध्ययन के लिए रोचक जानकारी प्राप्त किया जा सकता है।

व्यय अनाज को फीड ब्लॉक के रूप में मिथुन के आहार में मिश्रण किये जाने से एक सस्ता आहार मिश्रण बनाया गया है। कुछ जीवाणु प्रतिरोधी लक्षणों का अध्ययन ाविष्य में इस क्षेत्र में पाये जाने वाले प्र ाावी चिकित्सीय उपलब्ध एजेंट के रूप में किया जा सकता है।

मिथुन भ्रूण के हिमशीतलन की विधि को मानकीकृत करके एक मिथुन बछड़े जिसका नाम मोहन रखा गया क जन्म 12 मई 2012 में गतिविधि में शामिल स ाी वैज्ञानिकों को बधाई देता हूँ।

पशु कार्यिकी वि ाग में अनुसंधान के क्षेत्र में मिथुन में वि िान्न गतिविधियों से शारीरिक क्षमता को पता लगाने में सहायता मिल सकती है। यह एक बहुत रोचक अनुसंधान हो सकता है।

पशु स्वास्थ्य समूह में पशुओं के स्वास्थ्य की स्थिति के बारे में आँकडे एकत्रित करना एक बहुत ही आशाजनक कदम हो सकता है एवं मैदानी स्थिति में वि िान्न बीमारियों के बारे में वास्तविक जानकारी प्रदान करने में सहायता देती है, जिसके स्वास्थ्य से संबंधित वि िान्न विषय के बारे में योजना बनाई जा सके।

इस संस्थान की प्रगति एवं विकास के लिए डॉ. एस.अय्यप्पन,सचिव डेयर और महानिदेशक,

understanding of mithun chromosome and will also help us to find out the karyotypic evolution of mithun.

The work of milk protein gene will give us a way for determining the major characteristic of mithun milk protein and will also help us to detect possible polymorphism of casein gene.

The testing of FAO listed 30 numbers of microsatellite markers in mithun and Gaur will help us to generate interesting information in the biodiversity study in mithun and Gaur.

The use of spent grain in the preparation of total mixed ration in the form of fed block has shown us an alternative cheap source of feed ingredients. The studies on certain herbs for their anti-microbial properties may help us to identify locally available effective therapeutic agent in future.

The standardization of the method of cryopreservation of embryo has already proved to be successful with the birth of a mithun calf named "Mohan" on May 12, 20012. I must congratulate the scientists engaged in the activities related to this achievement.

The initiation of research in the field of work physiology will help us to know the physical capability of mithun for carrying out different physical activities. This will be a very interesting field of research.

The initiative of the scientists of animal health group to generate the data in respect of health status of animal in field condition is a very encouraging step and will help us to

ाारतीय कृषि अनुसंधान परिषद एवं डॉ. के. एम. एल. पाठक, उप महानिदेशक (पशु विज्ञान) एवं डॉ. बी एस. प्रकाश, सहायक महानिदेशक (पशु पोषण एवं कार्यिकी) के आर्शीवाद, सहायता मार्ग दर्शन एवं निरंतर मदद का परिणाम है, और मैं, इन स ाी का ह्रदय से आ ाारी हूँ और धन्यवाद देता हूँ।

मैं, डॉ. राजन गुप्ता, प्रधान वैज्ञानिक (पशु पोषण) एवं डॉ. विनीत ासीन, प्रधान वैज्ञानिक (पशु आनुवांशिकी प्रजनन) एवं डॉ. (श्रीमती) नीलम गुप्ता प्रधान वैज्ञानिक (पशु जैव तकनीकी) को धन्यवाद देता हूँ, जिन्होंने इस संस्थान की निरंतर प्रगति के लिए हर सं ाव मदद की है।

मैं, इस वार्षिक प्रतिवेदन के सम्पादकों को विशेष धन्यवाद देना चाहता हूँ, जिन्होंने इस प्रतिवेदन को वर्तमान समय में इस रूप में लाने के लिए अथक परिश्रम किया है। मैं, अध्यक्ष डॉ. सब्यासाची मुखर्जी वरिष्ठ वैज्ञानिक, को विशेष धन्यवाद देना चाहता हूँ, जिन्होंने इस प्रतिवेदन को सही समय पर प्रकाशित करने के लिए अत्यंत मेहनत की है। मैं, डॉ. (श्रीमती) अनुपमा मुखर्जी एवं श्रीमती कामिनी वर्मा को कार्यकारी सारांश एवं प्राक्कथन 2012-13 का हिन्दी में अनुवाद एवं टंकण करने के लिए विशेष धन्यवाद देता हूँ।

अंत में, मैं, ागवान सर्वशक्तिमान के कमल चरणों में आर्शीवाद के लिए प्रार्थना करता हूँ कि, वे स ाी वैज्ञानिकों एवं स्टाफ को प्रेम एवं आर्शीवाद प्रदान करें जिससे वे स ाी अपने अथक प्रयास द्वारा इस संस्थान के ाविष्य के कार्यक्रमों को सफलतापूर्वक पूर्ण कर सके।

"जय हिन्द"

चन्दन राजखोआ

(चन्दन राजखोआ)

know the authentic information related to various diseases in field condition so that appropriate strategies can be drawn to address various issues related to health condition.

The progress and development of the institute wouldn't have been possible without constant support, guidance and blessings of Dr. S. Ayyappan, Honorable Secretary DARE and DG, ICAR; DR. K.M.L. Pathak, Honorable DDG (Animal Science); Dr. B.S. Prakash ADG (AN&P). I offer my deep sense of gratitude to all of them.

The help and advice rendered by Dr. Rajan Gupta, PS (Animal Nutrition), Dr. Vineet Bhasin, PS (Animal Genetics and Breeding) and Dr. (Mrs.) Neelam Gupta, PS (Animal Biotechnology) is also acknowledged with gratitude.

Special thanks to Dr. Sabyasachi Mukherjee, Sr. Scientist, and Chief Editor, with other members of the editorial board for their painstaking effort to bring this document in the present shape. I must express thanks to Dr. (Mrs.) Anupama Mukherjee and Mrs. Kamini Verma for the preparation of the hindi portion of the report.

Lastly I pray to the Lotus feet of Lord Almighty for blessings and love to all the scientists and other staff members of the institute so that they can put their tireless effort for successful implementation of the programmes of the institute in future.

(Chandan Rajkhowa)

कार्यकारी सारांश

ाारतीय कृषि अनुसंधान परिषद के तत्वावधान में राष्ट्रीय मिथुन अनुसंधान केन्द्र के साथ उत्तर पूर्वी पर्वतीय क्षेत्र में आवश्यक वैज्ञानिक जानकारी में मिथुन पालन के संर्वागीण विकास के लिए प्रतिबद्ध है। इस बारे में वैज्ञानिक तरीके से उनकी बेशकीमती पशु पालन के माध्यम से जनजातीय मिथुन पालकों के लिए आर्थिक ला ा हेतु यह संस्थान प्रयासरत है। पशु आनुवांशिकी एवं प्रजनन, पशु शरीर क्रिया विज्ञान, पशु पोषण, पशु स्वास्थ्य, पशु प्रबंधन एवं विस्तार आनुवांशिक सुधार के लिए संरक्षण के क्षेत्र में अनुसंधान कार्य को बाहर ले जाने के लिए वैज्ञानिक एवं अन्य स्टाफ की एक टीम ने विकसित किया है। मूल्यवान जननद्रव्य मिथुन की बेहतर पोषण एवं गुणवत्ता, स्वास्थ्य देख ााल प्रदान के लिए प्रचार के माध्यम से पशुओं के इन स ाी, पूर्ववर्ती वर्ष में किए गए कार्यों को आसानी से समझने के लिए यहाँ संक्षेप में है।

फ्लोरोसेंट इनसीटू संकरण के अनुप्रयोग से मिथुन गुणसूत्रों में अनुठी विशेषता का पता लगाने के लिए शुरू किया गया है। वि ाि न्न प्रजातीयों के बीच सबसे पहले संबंधित गोपशुओं के साथ मिथुन के केरियोटाईप की तुलना द्वारा अध्ययन किया गया। वि ाि न्न बैंडिंग तकनीक द्वारा मेटाफेस गुणसूत्र में गुणसूत्र बिंदू की स्थिति मिथुन गोपशु एवं गाउर में की गयी है। मेटाफेस गुणसूत्र में गुणसूत्र बिंदू की स्थिति मिथुन गोपशु एवं गाउर में पर्याप्त साइटोजेनेटिक होमोलोजी (आकारिकी संख्या, एवं गुणसूत्र बैंडिंग नमुना) के साथ संबंधित प्रजातियों आणविक स्तर पर निकट संबंध मान रहे है। एफ आई एस एच तकनीक मिथुन की मेटाफेस गुणसूत्र के रूप में अच्छी तरह से पैतृक संबंधित प्रजातियों पर मिथुन केरियोटाईप विकास को खोजने के लिए लागू किया गया था। इन प्रजातियों के लिए एफ आई एस एच प्रोटोकॉल मानकीकृत किया गया है एवं प्रारं ाि क परीक्षण के रूप में टीलोमेरिक प्राइमरों गुणसूत्र बिंदू का उपयोग कर आयोजित किया गया।

एक अन्य अध्ययन में विकास,पोषण प्रदर्शन एवं मिथुन के वि ाि न्न प्रकारों के शव लक्षण पर आँकड़े संकलित है। विश्लेषण के लिए उपयुक्त नवीनतम सॉफ्टवेयर का उपयोग कर सांख्यिकीय तकनीक के साथ किया गया है। प्रयोगात्मक परिणाम से पता चला है कि मिथुन मांस में औसत वसा मोटाई, मारबलिंग साधारण एवं प्रचुर मात्रा में है। जब कि रिब आँख क्षेत्र अपेक्षाकृत बड़ा एवं बेहतर मिथुन का मांस गुणवत्ता का संकेत है। मिथुन में वि ाि न्न शव गुणवत्ता गुणों का आनुवांशिक सह संबंध शव गुणवत्ता गुणों के साथ किया गया एवं यह पाया गया कि शव ााग आर ईए के साथ ऋणात्मक है वर्तमान अध्ययन में यह पाया गया एवं यह निष्कर्ष निकलता है कि मिथुन में रिब आँख क्षेत्र अधिक वसा मोटाई कम एवं मिथुन मांस मारबलिंग अधिक है जिसकी वजह से यह अपेक्षाकृत अधिक रसदार है।

एक अन्य अध्ययन में मिथुन दूध कि गुणवत्ता जांच के लिए दूध प्रोटीन जीन एवं मुख्यतः केसीन जीन में विविधता को जांचा गया है एवं अन्य गौपशुओं में दूध की महत्व को मध्य नजर रखते हुए मिथुन के मुख्य दूध प्रोटीन जीन में आनुवांशिक गुणवर्णन निश्चित ही एक अत्यंत महत्वपूर्ण जानकारी होगी दूध प्रोटीन जीन अध्ययन चालीस वर्ष पहले शुरू किया गया था एवं अ ाी ाी निरंतर जारी है। यह इस बात को दर्शता

है, कि दूध की गुणवत्ता संघटन एवं तकनीक गुणवत्ता अत्यंत जरूरी है। उत्तर पूर्वी पर्वतीय क्षेत्र में पाये जाने वाले मिथुन प्रजाति के दूध उत्पादन की आनुवांशिक विविधता मिथुन दूध के उपयोग के बारे में महत्वपूर्ण जानकारी है। गौपशु दूध के मुख्य प्रोटीन के केसीन जीन के घटक α_1, α_2, β κ- बीटा कप्पा केसीन के अघुलनशील, लेक्टा एल्व्यूमीन बीटा लैक्टोग्लॉब्यूलिन जो पोषण के दृष्टि से महत्वपूर्ण नही है, किन्तु मुख्यतः रूप से बायोएक्टीव पेप्टाईड का निर्माण शरीर में जाने के बाद करते है।

मिथुन में केसीन जीन के आनुवांशिक गुणवर्णन परियोजना संपूर्ण हो गई है। मिथुन के समान कप्पा केसीन जीन आनुवांशिक विविधता गाउर में Hind III इन्जाइम के प्रयोग के द्वारा पाई गई। इस अध्ययन में पाये गये मिथुन कप्पा केसीन घटकीय जीन अनुक्रमण एवं जीन बैंको में उपस्थित अन्य संबंध प्रजातियां के अनुक्रमण का इस्तेमाल करते एक फाइलोंजेनेटिक पेड़ का निर्माण किया गया। यह पाया गया, कि मिथुन अन्य गौपशु प्रजातियों की अपेक्षा बायसन बोनासस तथा बोस टोरस के अधिक निकट है।

एक अन्य अध्ययन में तीस गौपशु माइक्रोसेटेलाइट मार्कर जो कि (MoDAD) एफएओ की सूची के आधार पर मिथुन एवं गाउर में जांचे गये कुल 135 नमूनें (120 मिथुन, 7 गाउर तथा 8 थो-थो गाय) में माइक्रोसेटेलाइट जीनो टाइपिंग की गई एवं आँकड़ो को पॉपजीन सॉफ्टवेयर की सहायता विश्लेषण किया गया।

एफएओ (MoDAD) की सूची के आधार पर 30 गौपशु माइक्रोसेटेलाइट मार्कर में से 19 मार्कर (63%) मिथुन एवं गाउर में जीनोमिक डीएनए में से 14 मार्कर (74%) सफलतापूर्वक परिलक्षित किए गए। इनमें से 19 मार्कर मिथुन एवं गाउर में अत्यधिक विविध रूपी जिसमें मूल्य (> 0.50) काफी अधिक तथा अलील का नम्बर 10 सो 26 मिथुन में एवं 2 से 7 गाउर में पाया गया। इसी लिए यह 14 माइक्रोसेटेलाइट मार्कर (BM1818, HAUT27, LSTS030, ETH185, HEL1, ETH3, BM1824, LSTS034, MM 12, HEL 135, ETH152, BM2113, LSTS006, ILSTS011 जो कि एफएओ के गौपशु विविधता अध्ययन के लिए ाी मानक पाये गये है। गौपशु माइक्रोसेटेलाइट मार्कर मिथुन एवं गाउर परिलक्षित नहीं हो पा रहे है। मिथुन एवं गाउर में जाँची गई हेट्रोजायगोसीटी (H_o) 0.15 to 0.94 एवं 0 से 1.00 से मिथुन में तथा गाउर में पाई गई है। जब कि नाइ की अस्तित्व हेट्रोजायगोसीटी (H_o) मिथुन में 0.31 से 0.89 एवं गाउर में 0.36 से 0.83 पाई गई है एवं मूल्य मिथुन में 0.29 से 0.88 एवं गाउर में पाया गया।

राइट के अनुसार प्रजनन गुणांक मिथुन में 30.00% तथा गाउर में 6.53% पाया गया, जो कि मिथुन में गाउर की अपेक्षाकृत धनात्मक एवं काफी उच्च यह इस बात को दर्शता है कि मिथुन जनसंख्या में शायद बाहरी परिगमन द्वारा जीन के बहाव में कमी, बाधित प्रजनन एवं शायद कम मिथुन साँड़ो का प्रजनन के लिए इस्तेमाल का उपयोग में लाना है।

मिथुन में साइटोक्रोम b जीन तथा माइटोकांड्रयल जीनो के मिथुन में साइटोक्रोम जीन तथा माइटोकांड्रयल जीनो के डी -लूप की अनुक्रमण का कार्य प्रगति पर है। साइटोक्रोम जीन के परिलक्षिन के लिए प्राइमर जोड़ी के एक सेट का नमूना गौपशु माइटोकोनड्रीयल जीन की तुलना में बनाया गया।

प्रथम एवं उल्टा पी सी आर प्रक्रिया 5'-1x प्रतिक्रिया बफर प्रथम एवं उल्टा प्राइमर एवं प्रतिक्रिया परिस्थित 95 डिग्री सेल्सियस 5 मिनट के लिए, 30 डिग्री सेल्सियस 45 सेकेन्ड, 57 डिग्री सेल्सियस 45 सेकेन्ड, 72 डिग्री सेल्सियस 1 मिनट के लिए एवं अंतिम विस्तार 10 मिनिट के लिए 72 डिग्री सेल्सियस परिलक्षित प्रोडक्ट को अनुक्रमण किया गया। बायोसरफ की सहायता से पीसीआर एमप्लीकोन 10 मिथुन में पाये गये।

एक अन्य चल रही परियोजना में मिथुन में लेप्टीन जीन SNPs को परिचय करने के लिए 25 मिथुन का जो कि नागालैंड एवं अरूणाचल प्रदेश से थे का चयन किया गया एवं उनका शरीर ाार (वृद्धि) शरीर माप को निर्धारित अंतर पर मापा गया। इन पशुओं में से डीनएनए को आइसोलेट किया गया एवं लेप्टीन के लिए 422 बी पी, 588 बी पी एवं 1820 बी पी पर प्राइमर के सहायता से पाये गये 422 एवं 528 एमप्लीकोन की अनुक्रमण का कार्य 25 प्रायोगिक पशुओं पर संपूर्ण किया गया। मिथुन लेप्टीन जीन 422 बी पी एमप्लीकोन में 17 SNPs पाये गये। जब कि 588 बी पी एमप्लीकोन में 6 SNPs पाये गये।

मिथुन लेप्टीन प्रोटीन जीन के सहयोगी गुणवर्णन के लिए रक्त नमूनो को एकत्रित किया गया, सिरा को बाहर निकला गया और सहयोगी केन्द्र में लेप्टीन प्रोटीन की आगे की प्रक्रिया के लिए ोजा गया। मिथुन के उत्तक को नागालैंड एवं अरूणाचल प्रदेश से एकत्रित किया गया। मिथुन सीफाक्रयल S-200 के इस्तेमाल से जेल क्रिस्टल क्रोमेटोग्राफी तकनीक द्वारा क्रूड एडीपोसाइट मिथुन प्रोटीन के घटकों का अध्ययन, तत्पश्चात DEAE सेल्युलोज के इस्तेमाल द्वारा परिवर्तित द्वारा किया गया। इनमें से एक घटक (DIV) जिसमें 15-16 kDa जिसमें कोली पेप्टाइट पाया गया, जो मिथुन लेप्टीन हो सकता हालाँकि इसका निर्धारण करना अ ाी बाकी है। यह घटक लेप्टीन प्रोटीन का आंशिक स्वच्छ घटक हो सकता है एवं जिसमें तीन से चार और पोली पेपटाईट जिनका अधिक ाग 40 से 60 kDa के बीच होगा। इसका इसलिए स्वच्छ करने की प्रक्रिया निरंतर की गई जिससे प्राकृतिक स्वच्छ लेप्टीन प्रोटीन प्राप्त किया जा सके, यह कार्य प्रगति पर है। लेप्टीन प्रोटीन प्रगति को जानने के लिए SDS-PAGE तत्पश्चात वेस्टन बालोट विश्लेषण क्रिया को लेप्टीन प्रतिरोधी प्रतिरोधक का इस्तेमाल बकरी एडीपोसाइट प्रोटीन में जांचा गया। इस तरह से 16 kDa की रेंज में स्वच्छ बैंड पाया गया हालाँकि इस प्रतिरोधी द्वारा मिथुन एडीपोसाइट प्रोटीन में लेप्टीन प्रोटीन नहीं पाया गया। जो इस बात को दर्शता है, कि मिथुन लेप्टीन तथा बकरी के लेप्टीन में कोई संकर प्रतिक्रिया नहीं है और इस बात का अनुमान ाी था। इस तकनीक को मानकीकृत करने के लिए गौपशु एवं बकरी के एडीपोसाइट प्रोटीन के नमुनों में आइसोइलेक्ट्रिक फोकेसिंग तत्पश्चात 2-D जेल इलेक्ट्रोफोटेसस की विधि अपनाई गई इस तकनीक को मिथुन एडीपोसाइट प्रोटीन में अपनाया गया है। यह कार्य प्रगति पर है।

पशु पोषण अनु ाग में बीव्रीज से प्राप्त व्यय अनाज का प्रयोग सघन प्रणाली में मिथुन पालने के लिए आर्थिक रूप से सं ाव पोषण योजना विकसित कि गई है। एक प्रयोग में मिथुन में ऊर्जा के इस्तेमाल विधि के नमूने का अध्ययन कांगों सिंग्नल घास पर आधारित राशन जिसमें व्यय अनाज के साथ- साथ गेहूँ की ूसी एवं चावल की ूसी के इस्तेमाल करके अध्ययन किया गया। इस प्रयोग से यह निष्कर्ष निकलता है,

कि व्यय अनाज के साथ-साथ चावल एवं ूसी के मिथुन आहार में इस्तेमाल मिथुन में शुष्क ाार बाहरी ऊर्जा का ग्रहण, पाचन क्षमता के आधार पर मिथुन में पारंपरिक दाने के मिश्रण के बदले में इस्तेमाल किया जा सकता है।

एक अन्य प्रयोग मिथुन में व्यय अनाज से बने फीड ब्लॉक के इस्तेमाल से ऊर्जा के इस्तेमाल का अध्ययन किया गया इस प्रयोग के आधार पर यह निष्कर्ष निकला गया कि मिथुन के आहार में व्यय अनाज का उपयोग कर बनाये गये फीड ब्लॉक बेहतर राशन साबित हुआ है।

एक अध्ययन में यह निष्कर्ष निकाला गया है कि आरट्रीनिसीया वलगेरीस में उपस्थित आवश्यक तेल (AVEO) प्रतिरोधी क्षमता को इस पौधे को सुखाने के पश्चात जांचा गया (AVEO) को इस पौधे के ताजे नमूनो में से काट के सुखाया गया एवं जीवाणुओं के 1199 नस्लें 113 जो प्रजातियों की ाी एवं यह काफी पेथोजेनिक एवं प्रकृति रूप से पाये जाने वाले जीवाणुओं जो कि 33 वि िन्न प्रजाति 1172 जीवाणुओं और 27 यीष्ट एवं मोल्ड की थी 114 क्लिनिकल नमूनों में से जो कि मनुष्य एवं पशुओं में बीमारी फैलाते है) एवं जो 25 जीवाणु की प्रजाति में से 23 (20.2%) AVEO के लिए संवेदनशील पाई गई क्लिनिकल नमूने स्वस्थ्य मनुष्य एवं पशुओं में से पाये गये इस तरह से कि अंतिम प्रतिरोधी क्षमता प्रकृति रूप से प्राप्त ताजे पौधे से निकाले गये AVEO में पाई गई ाविषय के लिए एक प्र ाावी चिकित्सीय एजेंट हो सकता है एवं नूतन प्रतिरोधी अणु विशेषता अक्सीडेज घनात्मक जीवाणु का आधार हो सकता है।

पशु चिकित्सा प्रकार टाइप कल्चर कार्य में रूमेण द्रव्य को दो नर फीसचुलेटेड मिथुन जिनको चावल की ूसी हरा चारा एवं दाने का मिश्रण खिलाया गया था से एकत्रित किया गया इन मुक्त चारण में वितरित मिथुन से गोबर के नमूने लिए गये जीवाणु के 41 शुद्ध कल्चर को रोल टयूब विधि द्वारा निकाला गया, इन जीवाणुओं का पहचान एवं गुणवर्णन का कार्य प्रगति पर है।

पशु कार्यिकी अनु ाग में भ्रूण के हिमशीत एवं हिम संरक्षण की विधि को मानकीकृत किया गया 4 मिथुन भ्रूण को दो दाता गाय मिथुन गायों FSH, v- द्वारा सुपरओब्यूलेटेड से फलेसिंग द्वारा गर्मी के 6 दिन में एकत्रित. किया गया एवं विट्रई फिकेशन विधि द्वारा हिमशीत किया गया। इन भ्रूण को जूम स्टीरिया सूक्ष्मदर्शी द्वारा जांचा गया एवं मोरला स्टेज को ग्रेड 1 एवं 2 में वि ाजित कर विट्रोई फिकेशन विधि हिमशीत किया गया। वर्तमान अध्ययन में चार भ्रूण में से दो भ्रूण मोरला स्टेज के ग्रेड वन के पाये गये (अतिउत्तम- संपूर्ण,गोलाइयों बराबर एवं कोशिका यूनिफाम आकार, रंग एवं टेक्चर) तथा ग्रेड 2 (अच्छा-एक भ्रूण में ब्लॉटोमियर अनश्चित आकार कुछ वेसिकल्स) क्रमशः पाये गये इन भ्रूण को विट्रफिकेशन विधि द्वारा हिमशीत किया गया। 100 दिनों के हिमशीतन के पश्चात इन भ्रूण ग्रहण पशुओ में स्थानांतरित किया गया । जो कि दाता पशुओं के गर्मकाल से सींक्रोनइज की गई थी और इस में से एक नर मिथुन बछड़े का जन्म मिथुन बछड़े (मोहन का जन्म) 12 मई 2012 को हुआ मोहन विश्व का प्रथम 100 दिन के हिमशीत भ्रूण का प्रथम स्थानांतरण तकनीक द्वारा पैदा हुआ है।

मिथुन साँडो में कार्य के दौरान प्राकृत ,रक्त के पैरामीटर तथा परिवहन थकान हारमोन का अध्ययन

किया गया औसत नाड़ी दर प्रयोग में 54.93 ± 2.07 प्रति मिनट थी। जो कि प्रयोग के अंत में 90.80 ± 2.16 प्रति मिनट थी इस प्रयोग में मिथुन को खेतों में 3 घंटे जोता गया जिससे उपापचय दर की वृद्धि हुई एवं जो मांस पेशीओं को अतिरिक्त ऊर्जा देने के लिए थी। अतिरिक्त तथा गर्मी का ाार विचरित करने के लिए थी। खेत में जोतने के पहले औसत श्वसन दर 27.50 ± 1.88 औसत जो कि 3 घंटे खेत जोतने के पश्चात बढ़कर 76.43 ± 2.83 प्रति मिनट हो गई । इस तरह से शरीर का तापमान शुरूआत में 100.80 ± 0.15 डिग्री सेल्सियस से बढ़कर 3 घंटे खेत जोतने के पश्चात 104.52 ± 0.12 डिग्री सेल्सियस हो गया था।

औसत प्लाज्मा NEF 168.45±15.34 प्रयोग के शुरूआत मे था जो तीन धंटे खेत जोतने के पश्चात बढ़कर 310.71±25.39 प्रयेग के अंत मे पाया गया था । इसी तरह से औसत प्लाज्मा α- अमाइनो नाइट्रोजन प्रयोग 25.55±4.66 से बढ़कर 41.75±8.21mg प्रयोग के अंत में पाई गई औसत प्लाज्मा कर्टिसोल का स्तर प्रयोग के शुरूआत 7.82±0.89 में नैनो ग्राम पर से बढ़कर 13.21±1.67 नैनो ग्राम प्रति प्रयोग में पाई गई। पशु स्वस्थ्य अनु ाग में अम्वारलोमा टोस्टू डीनाईयम कोच का पूर्वीय मिजोरम जो कि म्यांमार तट पर स्थित है मिथुन वस्यक मिथुन में तथा ट्राईसोविजोरम का मिथुन बछड़े में संक्रमण पाया गया है। अरूणाचल में पाये एक मिथुन में थीलेजियेसेस का संक्रमण पाया गया है।

एक और अन्य अध्ययन में रसायनिक एवं आर्युवैदिक परिजीवी रोधी दवा की क्षमता मिथुन बछड़ो में प्रकृति रूप से पाये जाने वाले पेट एवं आँख के परजीवी पर अध्ययन किया गया। आईवरमैक्टिन दवा का प्र ाव टोक्सोकारा वियूलोरमा पर देखा गया जबकि आर्युवैदिक जैसे की वेटवम तथा फाइकस हीरटा पर इनका प्र ाव क्रमशः 88% एवं 84.88% एवं पाया गया जो कि निर्धारित प्र ाव क्षमता 99.59% से काफी कम थी हालाँकि नीम (आजह डीरा चिटा इंडीका) टोक्सोकारा वियूलोरमा संक्रमण पर काफी प्र ावशाली (98.52%) तत्व पाया गया। अंत परजीवी प्रतिरोधी दवा जैसे कि एलवेंड़ा जोल फेनवेंड़ाजोल और फेनवेंड़ाजोल तथा परजीवी किवंटल के सहयोग का प्र ाव उपचार के 14 दिन के पश्चात 100% प्र ावी पाया गया।

मिथुन की सामान्य बीमारियों का निरीक्षण किया गया नागालैंड, अरूणाचल एवं मिजोरम के करीब 16 गांव में 750 मिथुन पशुओं का निरीक्षण किया गया तथा रक्त के नमुनें मुक्त चरण स्थित मिथुन से िान्न बीमारियों से सीरोमोंट्री किया गया सघन प्रणाली में पाले गये 150 मिथुन का निरीक्षण किया गया रक्त नमूने लिए गये तथा वि िान्न सामान्य बीमारियों जैस ब्रुसीलोसिस और BVD के लिए जांचा। मुक्त चरण स्थिति में का ब्रुसीलोसिस 13.33% (300 में से 40 पाया गया IBR के लिए 9.33% (300 में से 28 पशु) पाये गये तथा BVD प्रतिरोधी के विरूद्ध 12.66% (300 में से 38) मुक्त चरण स्थिति में मिथुन में पाये गये जब कि BVD एंटीजन के विरूद्ध व्यवसाय का किट के द्वारा 1.33% (300 में से 4) पाये गये।

एक और अध्ययन में त्वचा में पाये जाने वाले वार्ट में 2.28 % (750 में से 16) मिथुन में पाया गया तथा BPV2 मिथुन के वर्ट मे PCR विधि द्वारा पहचाना गया त्वचा पर पाये जाने वाले वार्ट को फाइब्रोपेप्लोमा हीस्टोपेथोलोजिकल अध्ययन द्वारा पहचाना गया।

EXECUTIVE SUMMARY

The National Research Centre on Mithun under the aegis of Indian Council of Agricultural Research is committed for all round development of Mithun rearing in the North Eastern Hilly region with necessary scientific inputs. This in turn is sure to bring economic benefits for the tribal Mithun owners through rearing of their prized animals in scientific way. This Institute has developed a team of scientists and other staff for carrying out research work in the fields of Mithun Genetics, Nutrition, Physiology, Health, Reproduction for genetic improvement, conservation, propagation of valuable Mithun germplasm through providing better nutrition and quality health care for the animals. All these works carried out in the preceding year are summarized here for easy understanding.

Application of Fluoresecent In-situ Hybridization was initiated to find out unique feature in mithun chromosomes. The homologies between the different species were studied by firstly comparing the Karyotype of Mithun with related bovids. Different banding techniques were used to study the band homology among these species; C-banding was carried out to localize the position of centromere in the metaphase chromosome. Mithun, Cattle and Gaur are very closely related species with sufficient cytogenetic homologies (morphology, number, and chromosomal banding pattern) to assume a close relationship at the molecular level. FISH technique was applied on the metaphase chromosome of mithun as well as ancestral related species for finding out Karyotypic Evolution of mithun. FISH protocol is standardized for these species and initial trials were conducted using centromere as well telomere primers.

In another study, data on growth, nutritional performance and carcass traits of various strains of mithun is compiled and the same is subjected for analysis with suitable statistical technique using latest software. Experimental results showed that the marbling and average fat thickness in mithun meat is moderately abundant, whereas the Rib eye area is comparatively larger indicating the better meat quality of mithun. Genetic correlation among the various carcass quality traits in mithun were generated and it was found that the Carcass weight (CWT) was highly and significantly correlated with fat thickness, rib eye area and dressing % and is desirable association and similarly fat thickness was found to be negatively correlated with that of Rib eye area. It was observed and can be concluded from the present investigation that the rib eye are was more in mithun thus resulting in less fat thickness and more marbling and more juiciness of the mithun meat.

One pilot study on milk protein gene in Mithun was taken up to evaluate the characteristics of Mithun milk and to detect possible polymorphisms of casein gene in mithun milk. Considering the importance of milk from other bovine species the genetic characterization of milk protein genes will provide the information of nature of major milk proteins of Mithun. Studies on milk protein started more than 40 years ago and still is continuing because of their important role in milk quality, composition and technological characteristics (Martin et al 1999). Being an important bovine species in the north Eastern States exploring the genetic potentials of this unexplored bovine species will definitely provide the knowledge about their milk constituents and their genetic variations further

usability of Mithun milk. The bovine milk specific proteins include casein fraction, α1, α2, β κ- caesins (insoluble fractions), α -lactalbulin and β -lactoglobulin which are not only useful for nutrient requirement purpose but also they specifically provide useful bioactive peptide once these casein proteins broken down into smaller fragments inside our body.

The project on Genetic Characterization of kappa casein gene (CSN3) of Mithun was completed. Similar genetic polymorphism in kappa casein gene of gaur was identified using Hind III – restriction fragment length polymorphism (RFLP) studies. A phylogenetic tree was prepared taking mithun sequences of our study and the available related species sequences from Genbank. It was found that mithun was more closely related with bison bonasus and Bos taurus than other bovine species in respect of kappa casein partial gene sequence.

In one landmark study, 30 bovine microsatellite markers from FAO list of Measurement of Domestic Animal Biodiversity (MoDAD) were tested in mithuns and gaurs individually. Microsatellite genotyping of total 135 samples (120 mithun, 7 gaur and 8 tho tho cattle) were done and data analysed by Popgene software.

Out of 30 bovine microsatellite markers from the list of FAO MoDAD (1996) tested, 19 markers (63%) successfully amplified mithun and wild gaur genomic DNA. 14 markers (74%) out of these 19 were highly polymorphic with high PIC value (> 0.50) in mithun and gaur with allele numbers ranging from 10 to 26 in mithuns and two to seven in guar, respectively. Therefore, these 14 microsatellite markers (BM1818, HAUT27, LSTS030, ETH185, HEL1, ETH3, BM1824, LSTS034, MM 12, HEL 135, ETH152, BM2113, LSTS006, ILSTS011) which are also in the FAO standard panel for cattle diversity studies will be most suitable for diversity studies in Indian mithuns and gaur as well. 11 bovine microsatellite markers failed to amplify in mithun and guar. Observed heterozygosity (H_o) ranged from 0.15 to 0.94 and 0 to 1.00 in mithun and gaur, respectively while Nei's expected heterozygosity (H_e) ranged from 0.31 to 0.89 and 0.36 to 0.83 in mithun and gaur, respectively. PIC value ranged from 0.29 to 0.88 in mithun and 0.32 to 0.82 in gaur, respectively.

Estimate of inbreeding coefficient (F_{IS}) as per Wright (1978) was 30.00% and 6.53% in mithun and gaur population, respectively which was positive and quite high particularly in mithun population compared to gaur. This indicates non-random mating, possibly extensive breeding with very less number bulls and absence of gene flow from external migration in the mithun population.

Sequencing of the Cytochrome b gene and D-loop of the mitochondrial genome of mithuns. was in progress. To amplify the entire Cytochrome b gene we designed a set of primer pair with respect to the cattle mitochondrial genome - forward 5'–CGAAGCTTGATATGAAAAACCATCG TTG-3' and reverse 5'-GGAATTCATCTCTCCGGTTTACAAGAC-3'. PCR reaction was performed using 1x reaction buffer, 1.5 mM $MgCl_2$, 5 pmol forward and reverse primer with following reaction condition 95 ºC for 5 min. 30 cycles of 94ºC for 45 sec, 57ºC for 45 sec, 72ºC for 1 min and final extension at 72ºC for 10 minute. Amplified product was treated with ExoSAP-IT, as per manufacturer's instructions. Sequencing of the PCR product was performed by (BioServe). Finally, 838 bp amplicons of the 10 mithuns were obtained. In one ongoing project for

identification of SNPs in lepting gene of mithuns, 25 Mithuns calves belonging to Nagaland and Arunachal Pradesh strains were selected and their body weight (growth), body measurements in regular intervals were monitored. DNA was also isolated from these animals and PCR amplification using primers for Leptin 422bp, 588bp and 1820 bp PCR products of Mithuns. Sequencing of 422 bp and 588 bp amplicons in respect of 25 experimental mithuns were completed. 17 SNPs detected in the 422 bp amplicons of mithun leptin gene, while nine SNPs detected in the 588 bp amplicons of mithun leptin gene. For characterization of leptin protein of mithun, blood samples were collected, sera separated and sent to collaborating centre for further processing for leptin protein. Mithun adipose tissue samples also collected from Nagaland and Arunachal Pradesh. Fractionation of crude adipocyte protein of mithun was done by gel-filtration chromatography using sephacryl S–200 followed by anion exchange chromatography using DEAE–Cellulose. One of the fractions (DIV) contained polypeptide of 15-16 kDa, supposed to be of mithun leptin. However, it needs confirmation. The said column chromatographed fraction may be considered as semi- purified fraction of leptin protein having 3-4 more polypeptides in the molecular weight range of 40-60 kDa. Hence, further purification steps to be followed to have purified native leptin protein. This work is in progress. SDS-PAGE followed by Western blot analysis was also performed to detect leptin using anti-leptin antibody in goat adipocyte protein preparation. It showed clear band in the range of 16 kDa. However, leptin protein couldn't be detected in mithun adipocyte protein preparation using this anti- goat leptin antibody, indicating no cross reactivity of mithun leptin with goat leptin as anticipated. Iso-electric focusing followed by 2-D gel electrophoresis was performed with adipocyte proteins using cattle and goat samples to standardize the technique. The same protocol will be followed for mithun adipocyte protein sample. Further work was in progress. In Animal Nutrition section, development of economically viable feeding strategy for rearing mithun in intensive system using spent grains from breweries industries is in progress. Experiment on energy utilization pattern in mithun fed on Congo Signal grass based diet supplemented with spent grain with wheat bran or rice bran was studied. It was concluded that supplementation of spent grain and wheat bran / rice bran in the diet of mithun increased dry matter and gross energy intake and digestibility and supplementation of spent grain and wheat bran / rice bran in the diet of mithun can replaced traditional concentrate mixture.

In another experiment, energy utilization in mithun fed on spent grain based feed block was studied. It was concluded that feeding spent grain based rations in the form of feed block is better than mixed form.

The study was conducted to determine antimicrobial activity of Artemisia vulgaris essential oil (AVEO), and to see the effect of drying of herb for AVEO extraction on its antimicrobial activity. AVEO was extracted from fresh chaffed herb and dried powdered herb and tested on 1199 strains of 113 species of pathogenic, potentially pathogenic and environmental microbes belonging to 33 different genera, 1172 were bacteria and 27 were yeast and moulds. Of the 114 clinical isolates (associated with illness in human and animals) belonging to 25 bacterial species, 23 (20.2%) were sensitive to AVEO. Clinical isolates were significantly ($p < 0.03$) more sensitive than

isolates from healthy human and animals (12.6%). Thus for better antimicrobial activity AVEO should be extracted from fresh herb. The AVEO may be an effective therapeutic agent of future either as such or as the source of some novel antibacterial molecule(s) particularly against oxidase positive bacteria.

In the Veterinary type culture work, rumen liquor has been collected from two adult male fistulated mithun fed on paddy straw, green grass and concentrate mixture. Faecal samples from freely browsing mithun have also been collected. Forty one pure cultures of bacteria have been isolated using roll tube method. Characterization and identification of those bacteria is under progress.

In the Animal Physiology section, standardization of cryopreservation/ embryo freezing protocol for mithun was completed. In order to do cryopreservation of mithun embryos by vitrification method, four embryos were recovered from two donor (superovulated by using FSH, Folltropin-v-Bovine- 400mg) animal by flushing on 6th day of oestrous cycle. Embryos were evaluated under stereo-zoom microscope and morula classified as Grade 1 and 2 were used for freezing by vitrification method. In the present study out of the four embryos, two embryos were morula stage and found to be classified as Grade 1 (Excellent - an ideal embryo, spherical, symmetrical and with cell of uniform size, colour and texure) and Grade 2 (Good- an embryo with few extruded blastomeres, irregular shape and few vesicles), respectively. These embryos were used for freezing by vitrification method. After 100 days of preservation, the embryos were transferred into two recipient animal, whose cycle well synchronised with the donor and out of that one male calf (MOHAN) was born on May 12, 2012. MOHAN, is the world's first ETT born mithun calf from 100-day cryopreserved embryo.

Investigating the changes in behaviour, blood parameters and circulatory stress hormone during the course of work in mithun bull was also studied. The overall mean of pulse rate prior to start of the experiment was 54.93 $\pm$ 2.07 per minute. The pulse rate rose to 90.80 $\pm$ 2.16 per minute at the end of three hours of ploughing, which might be due to increased metabolic rate to provide more energy to muscle and to dissipate the extra heat load. The mean respiration rate prior to ploughing was 27.50 $\pm$ 1.88 per minute. The respiration rate rose to 76.43 $\pm$ 2.83 per minute at the end of three hours of ploughing. Similarly, the body temperature of mithun bulls rose from 100.80 $\pm$ 0.15^{o} C per minute at the beginning of ploughing to 104.52 $\pm$ 0.12^{o}C per minute at the end of three hours of ploughing, respectively.

The overall mean of plasma NEFA rose from 168.45$\pm$15.34 mEq/l at the beginning of the ploughing to 310.71$\pm$25.39 mEq/l at the end of three hours of ploughing. Similarly, the overall mean of plasma α-amino nitrogen (AAN) prior to start of ploughing was 25.55$\pm$4.66 mg/dl and it rose to 41.75$\pm$8.21mg/dl at the end of three hours of ploughing. The mean plasma cortisol level also increased from 7.82$\pm$0.89 ng/ml prior to start of ploughing to 13.21$\pm$1.67 ng/ml at the end of three hours of ploughing, respectively.

In Animal Health section, prevalence of Amblyomma testudinarium Koch, 1844 in mithun (Bos frontalis) of eastern Mizoram (India) near Myanmar border and Trichobezoars in mithun calves were recorded. A case report of Thelaziasis was also reported in mithuns from Arunachal Pradesh.

In another study, efficacy of chemical and herbal

Anthelmintic drugs against naturally infested gastrointestinal helminthiasis in Mithun calves (Bos frontalis) was tested. The drug, Ivermectin showed 99.59% efficacy against Toxocara vitulorum. While the herbal drugs i.e. Vet worm and Ficus hirta have shown efficacy of 88% and 84.88% respectively, which was below the recommended standard efficacy of 98%. Nevertheless the Neem (Azadirachta indica) was found to be effective (98.52%) to some extent against Toxocara vitulorum infection. The efficacy rate of anthelmintics drugs like Albendazole, Fenbendazole and a combination of Fenbendazole and Praziquantel was 100% and for all the treatment was observed to be effective from day 14th of treatment.

Survey for common diseases of mithuns was conducted. A total of 750 animals have been surveyed in 16 villages in Nagaland, Arunachal Pradesh and Mizoram and about 300 serum/blood samples were collected form free ranging Mithuns for sero-monitoring of different diseases. 150 reared under intensive system of rearing were surveyed, sampled and screened for common diseases of bovine including brucellosis, IBR, and BVD. In free range condition a prevalence of brucellosis was observed to be 13.33% (40 of 300). Sero-prevalence studies for IBR revealed a prevalence of 9.33% (28 of 300 animals) while sero-prevalence of BVD antibody was found to be 12.66% (38 of 300) in free mithun and that of BVD antigen was observed to be 1.33% (4 of 300) by using commercial ELISA Kits.

In another study, cutaneous warts were found to be prevalent in 2.28 % (16 of 750) of Mithuns and BPV2 has been identified from cases of warts in mithun by PCR. The cutaneous warts have been diagnosed as fibropapilloma by Histopathological studies.

"Damsels of Mithun Country during Reh Festival, Rowing, Arunachal Pradesh"

INTRODUCTION

The National Research Centre on Mithun was established in June, 1988 with the main objectives of conservation and genetic improvement of Mithun germsplasm, development of Mithun nutrition, management, health and products processing technologies related to Mithun. The Centre, which started its functioning from Barapani near Shillong during the initial days, grew considerably over the years in its present location of Nagaland despite some socio-political and logistic difficulties. The Institute has made inroad deep inside the Mithun habitat among the tribal rearers of this unique bovine speice found only in the North Eastern Hilly regions of this country through its commitment of service for overall benefit of tribal populace.

Mithuns which are thought to be originated in the Indo-Myanmar border area are now restricted only in the hilly parts of four North East States (Nagaland, Arunachal Pradesh, Manipur and Mizoram) and found a loving relation with their tribal owners since antiquity. Inspite of this, decreasing population of Mithuns in three of the four states (Nagaland, Manipur and Mizoram) has been a concern even if there is increasing trend of Mithun population in Arunachal Pradesh. We are deeply concerned for this declining trend of Mithun population even though this is a fact to be realized and similar to other well-known livestock breeds in Inda. Mithuns are part of tribal life in their rugged and hilly terrains and hence, any improvement programme for Mithuns will be a natural and correlated sign of benefit for tribal population because Mithun is a source of food as well as companions of tribals.

The National Research Centre on Mithun under the aegis of Indian Council of Agricultural Research is committed for all round development of Mithun rearing in the North Eastern Hilly region with necessary scientific inputs. This in turn is sure to bring economic benefits for the tribal Mithun owners through rearing of their prized animals in scientific way. This Institute has developed a team of scientists and other staff for carrying out research work in the fields of Mithun Genetics, Nutrition, Physiology, Health, Reproduction and Extension.

The Annual Report of this Institute is not only the treasure of information related to different aspects of research on Mithun (Bos frontalis) in a particular year, but also this contains all those nitty-gitty news in nutshell. Every effort is made to show case the whole panorama of work carreid out by this Institute in all its hues. It will be a matter of satisfaction for us if these efforts could benefit the people in any way in this Mithun country!

जनादेश

- देश में उपलब्ध मिथुन के जननद्रव्य की पहचान, मूल्याँकन एवम गुणवर्णन करना।
- दुग्ध एवं मांस उत्पादन के लिए मिथुन का गुणवर्धन एवम संरक्षण करना।
- मिथुन के जननद्रव्य का संग्रह एवम सूचना केन्द्र के रूप में कार्य करना।

MANDATE

- Identification, evaluation and characterization of Mithun germplasm available in the country.
- Conservation and improvement of Mithun for meat and milk.
- To act as repository of a germplasm and information centre on Mithun.

FINANCIAL STATEMENT (2012-13)

Plan

(₹ in lakh)

Sl.no	Head of Account	Revised Estimate	Expenditure Incurred
1	Esstt. Charges	-	-
2	OTA	-	-
3	TA	8.0	8.0
4	Contingency	300.00	299.96
5	Equipments	6.00	5.86
6	Works	74.00	73.97
7	Library	17.00	17.00
8	Vehicle	-	-
9	HRD	6.00	5.96
10	Furniture and fixtures	2.00	2.00
11	Livestock	1.00	1.00
12	Maintenance	11.00	11.00
	TOTAL	425.00	424.75

Non Plan

(₹ in lakh)

Sl.no	Head of Account	Revised Estimates	Expenditure Incurred
1	Esstt. Charges	240.00	226.46
2	Wages	31.44	28.93
3	OTA	1.00	0.00
4	TA	4.59	4.59
5	Other charges	19.97	19.91
6	Works -annual repair & maintenance		
	i. Office building	4.00	4.00
	ii. Residential building	4.00	4.00
	iii. Minors works	6.00	5.99
	TOTAL	311.00	293.88

RESOURCE GENERATION (2012-13)

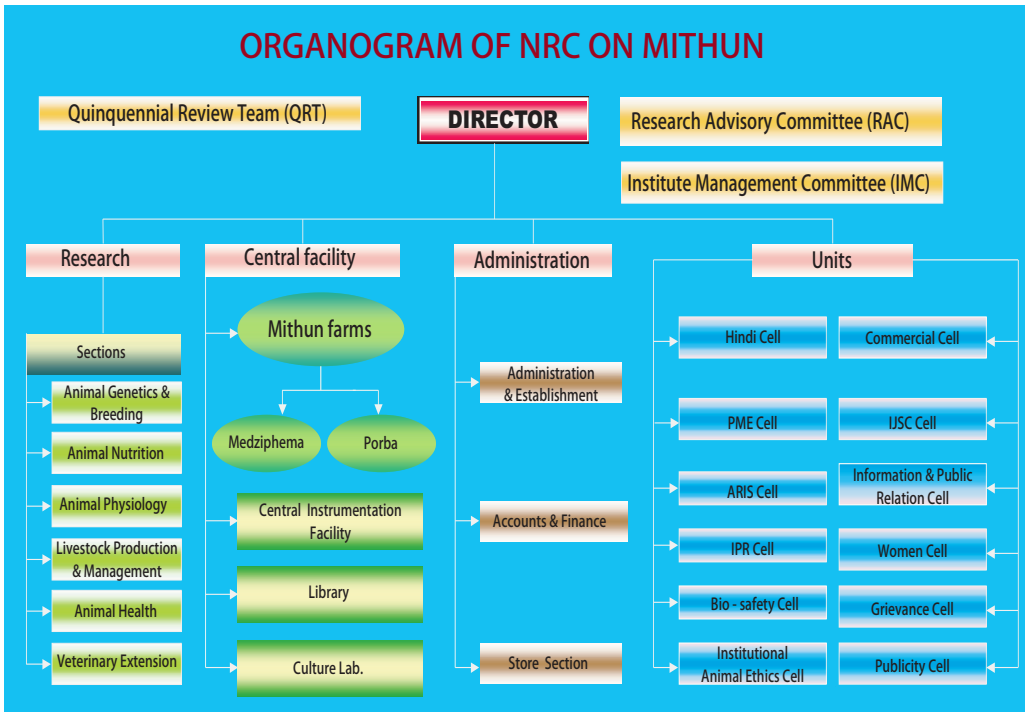
(₹ in lakh)

Sl. no.	Items	Resource Generation	
		Target	Actual
1.	Sale of farm produce, others sales	16.00	19.93

STAFF POSITION

Category	VIIth	IXth	Xth	XIth	Redeployment/ Revision of cadre strength	Present Strength after redeployment' Revision	Present Position	Vacant
RMP	1	-	-			1	1	0
P.S	1	-	-			1+1***	2	1
Sr.Scientist	3	-	-		1	5	2	3
Scientist	7	-			3	11	5	6
T6	3	-	-			3	3	0
AO	-	-	-		-	1	-	1
AAO	1	-	-		-	2	1	1
AFAO	-	1	-		-	1	1	0
Assistant	1	-	-		-	4	4	0
P.A	-	-	-		1	-	-	1
UDC	1	1*	-		-	1	-	1
LDC	1	1*	-		1	4	3	1
Jr.Steno	1	-	-		-	1	1	0
T2	-	5*	-		-	-	-	0
T1	2	5*	-		-	2	2	0
Supporting	8	7*	3**		-	8	8	0
Total	30	20(19*+1)	3**		6	46	33	51

*IXth Plan post not created. **Xth Plan post not created. *** In the redeployment stage, the sanctioned post of Principal Scientist in Animal Physiology has been redeployed to Animal Nutrition.



Research Achievements

The empiricists are like the ant; they only collect and use. The rationalists resemble the spiders, who make cobwebs out of their own substance. The scientist is like the bee; it takes a middle course; it gathers material from the flowers, but adapts it by a power of its own. [*Novum Organum, XCV*, 1620]

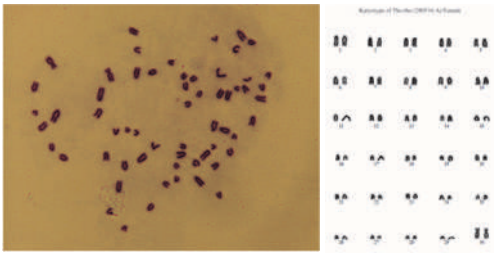
...Francis Bacon

ANIMAL GENETICS AND BREEDING

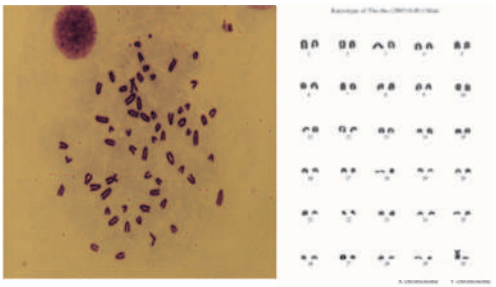
Application of FISH to find out unique feature in mithun chromosomes

The homologies between the different species were studied by firstly comparing the karyotype of mithun with related bovids. The results indicated that mithun chromosome number and morphology was similar to that of mithun cattle crossbred and Gaur whereas in cattle it is different with diploid chromosome number as 60 as compared to 58 in earlier two species. Among the autosomes chromosome number 1 is submetacentric in mithun, mithun cattle crossbred as well as gaur; however it is acrocentric in cattle. Similarly among the sex chromosome Y chromosome in mithun, mithun cattle crossbred and Gaur is metacentric whereas it is acrocentric in cattle.

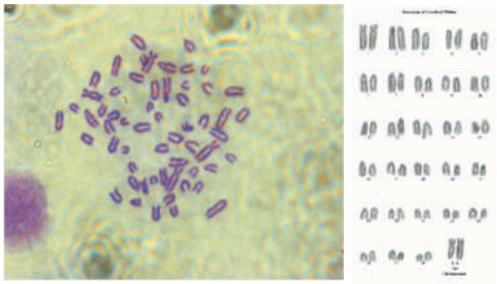
Species	2n	Autosomes		Sex Chromosome	
		SM	A	X	Y
Cattle	60	-	29	SM	A
Mithun	58	1	28	SM	M
Mithun cattle crossbred	58	1	28	SM	M
Gaur	58	1	28	SM	M



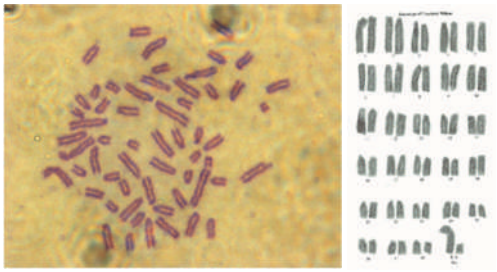
Metaphase Spread and Karyotype for Female Tho-tho cattle



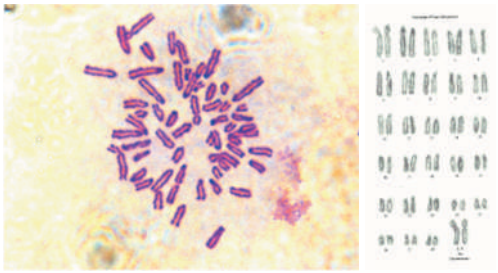
Metaphase Spread and Karyotype of Male Tho-tho cattle



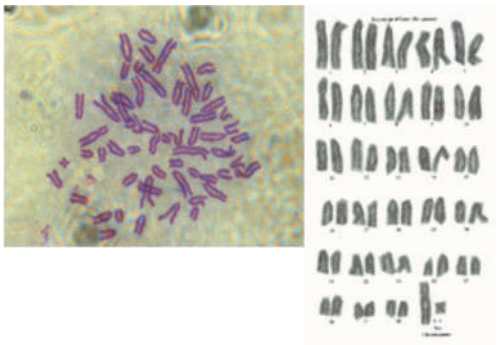
Metaphase Spread and Karyotype of Female Mithun X cattle crossbred



Metaphase Spread and Karyotype of Male Mithun X cattle crossbred

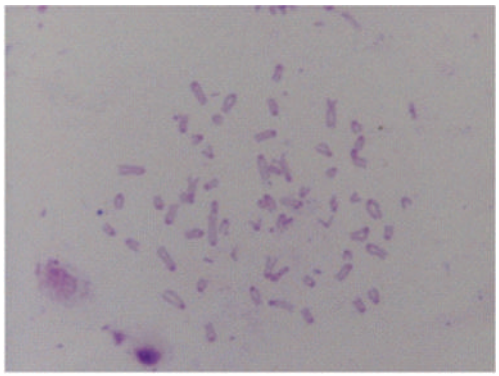


Metaphase Spread and Karyotype of Female Gaur (Bos gaurus)



Metaphase Spread and Karyotype of Male Gaur (Bos gaurus)

Different banding techniques were used to study the band homology among these species; C-banding was carried out to localize the position of centromere in the metaphase chromosome



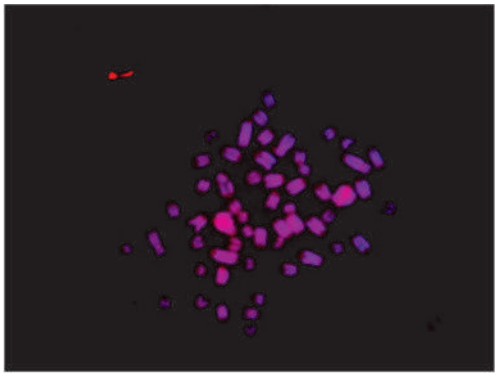
C-banding in Tho-Tho cattle

Highly repetitive DNA represents a large fraction of most eukaryotic genomes. In mammals, these DNA components are either dispersed throughout the genome or arranged in tandem in large blocks known as satellite DNA, which often localise to pericentromeric areas. Probes containing such sequences are considered as powerful tools for detecting numerical chromosome abnormalities in eukaryotic cells. In situ hybridization is used to localize DNA sequences on chromosomes to confirm the linear differentiation of the chromosomes. After *Fluorescent in situ hybridization* (FISH), they display distinct bright signals in metaphase as well as interphase cells.

In the bovine species, very few repetitive DNA probes are available. This is partly due to the fact that, in animal research programmes, FISH is mainly used for physical genome mapping which involves unique sequence probes to localise genes or genetic markers on metaphase plates. The telomeric sequence is a conserved sequence among vertebrates and is repetitive in nature. The telomeric region located at the end of chromosomes is required for replication and stability of the chromosome (Lavoie et al. 2003).

Mithun, Cattle and Gaur are very closely related species with sufficient cytogenetic homologies (morphology, number, and chromosomal banding pattern) to assume a

close relationship at the molecular level. FISH technique was applied on the metaphase chromosome of mithun as well as ancestral related species for finding out Karyotypic Evolution of mithun. FISH protocol is standardized for these species and initial trials were conducted using centromere as well telomere primers.

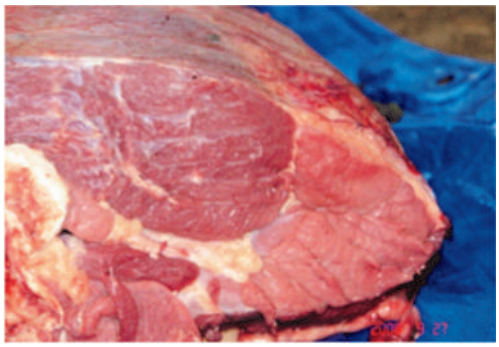


Genetic Study of Leptin Gene and its Association With Growth And Nutritional Performance of Mithun (*Bos frontalis*)

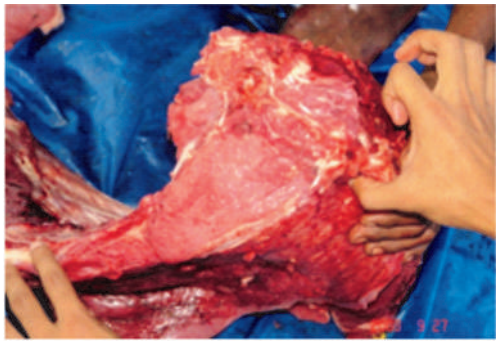
Data on growth, nutritional performance and carcass traits of various strains of mithun is compiled and the same is subjected for analysis with suitable statistical technique using latest software. Experimental results showed that the marbling and average fat thickness in mithun meat is moderately abundant, whereas the Rib eye area is comparatively larger indicating the better meat quality of mithun.

S.No.	Performance Indicators	Mean ± SE
1.	Live Weight	367.66 Kg
2.	Carcass Weight	198.33Kg
3.	Slaughter Weight	349.33Kg
4.	Carcass Length	49.66 Inches
5.	Carcass Oblique Length	52.16 Inches
6.	Round Weight	16.33Kg
7.	Round Length	16.66Inches
8.	Round Width	16.5 Inches
9.	Round Circumference	37.5 Inches
10.	Fat Thickness	0.74
11.	Rib Eye Area	84.29 cm. square
12.	Dressing Percentage	56.42%
13.	Shrinkage	5.00%
14.	Degree of marbling	Moderately abundant
15.	Texture of Marbling	Fine
16.	Colour of lean	Dark Red
17.	Firmness of lean	Slightly soft
18.	Texture of lean	Fine
19.	Average daily gain	0.396 gm /day
20.	Body condition score	2.5

Genetic correlation among the various carcass quality traits in mithun were generated and it was found that the Carcass weight(CWT) was highly and significantly correlated with fat thickness, rib eye area and dressing % and is desirable association and similarly fat thickness was found to be negatively correlated with that of Rib eye area. It was observed and can be concluded from the present investigation that the rib eye area was more in mithun thus resulting in less fat thickness and more marbling and more juiciness of the mithun meat.

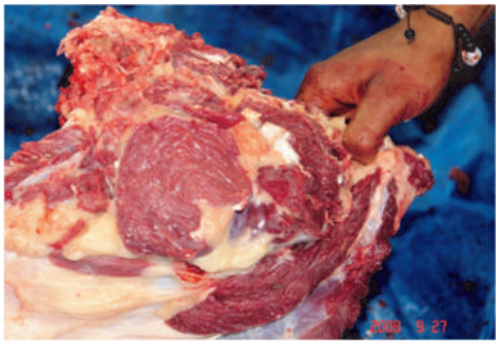


Round of Mithun showing Marbling



Rib Eye Area in mithun

	CWT	AFT	REA	SHRINKAGE	DP
CWT		0.39	0.83	-0.76	0.88
AFT			-0.53		0.49
REA					0.48



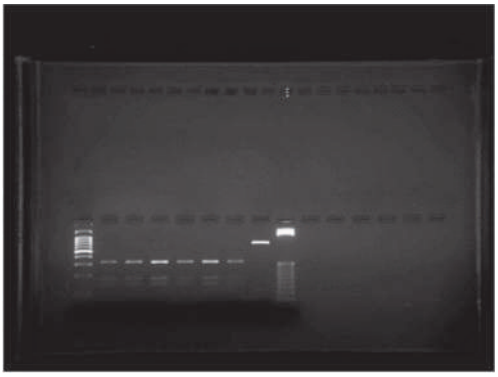
Fat Thickness in mithun carcass at 12 and 13 rib section

Pilot study on milk protein gene in Mithun

Mithun is although mainly raised for meat production, however in recent days attempts are started to explore the probability for developing mithun farming as an enterprise with more economic profit. Out of the four strains of mithun except Mizoram, rest of other is being traditionally raised for meat consumption, whereas Mizoram strain is popular for milk production also.

In one pilot study investigations was carried out to evaluate the characteristics of Mithun milk and to detect possible polymorphisms of casein gene in mithun milk. Considering the importance of milk from other bovine species the genetic characterization of milk protein genes will provide the information of nature of major milk proteins of Mithun. Studies on milk protein started more than 40 years ago and still is continuing because of their important role in milk quality, composition and technological characteristics (Martin et al 1999). Being an important bovine species in the north Eastern States exploring the genetic potentials of this unexplored bovine species will definitely provide the knowledge about their milk constituents and their genetic variations further usability of Mithun milk. The bovine milk specific proteins include casein fraction, α_1, α_2, β κ- caesins (insoluble fractions), α lactoglobulin and β lactoglobulin which are not only useful for nutrient requirement purpose but also they specifically provide useful bioactive peptide once these casein proteins broken down into smaller fragments inside our body.

The study was conducted to find out the genetic variation and polymorphism in α_1, α_2, caesins (insoluble fractions) and butryophyllin gene. The PCR RFLP technique was used to study the polymorphism among various strains of mithun.



Ethidium bromide stained Agarose gel of PCR amplified region of mithun casein gene

Cheese prepared from mithun milk

It will be helpful in better understanding of molecular basis of mithun milk. This will generate the possibility of using the mithun milk for producing value added product mainly cheese of superior quality. It will finally help in converting mithun farming to an economic enterprise, which will add commerce to this unique bovine species farming. The research on mithun's milk polymorphisms will generate the new impulse in the mithun rearing community and will definitely help in exploring the means for economic profit with mithun rearing.

Genetic Characterization of kappa casein gene (CSN3) of Mithun

The same bovine primers used for mithun were utilized to successfully amplify 271 bp and 874 bp regions of the part of exon IV and intron IV of gaur (*Bos gaurus*) kappa casein. Similar genetic polymorphism in kappa casein gene of gaur was identified using Hind III – restriction fragment length polymorphism (RFLP) studies.

A phylogenetic tree was prepared taking mithun sequences of our study and the available related species sequences from Genbank. It was found that mithun was more closely related with bison bonasus and Bos taurus than other bovine species in respect of kappa casein partial gene sequence.

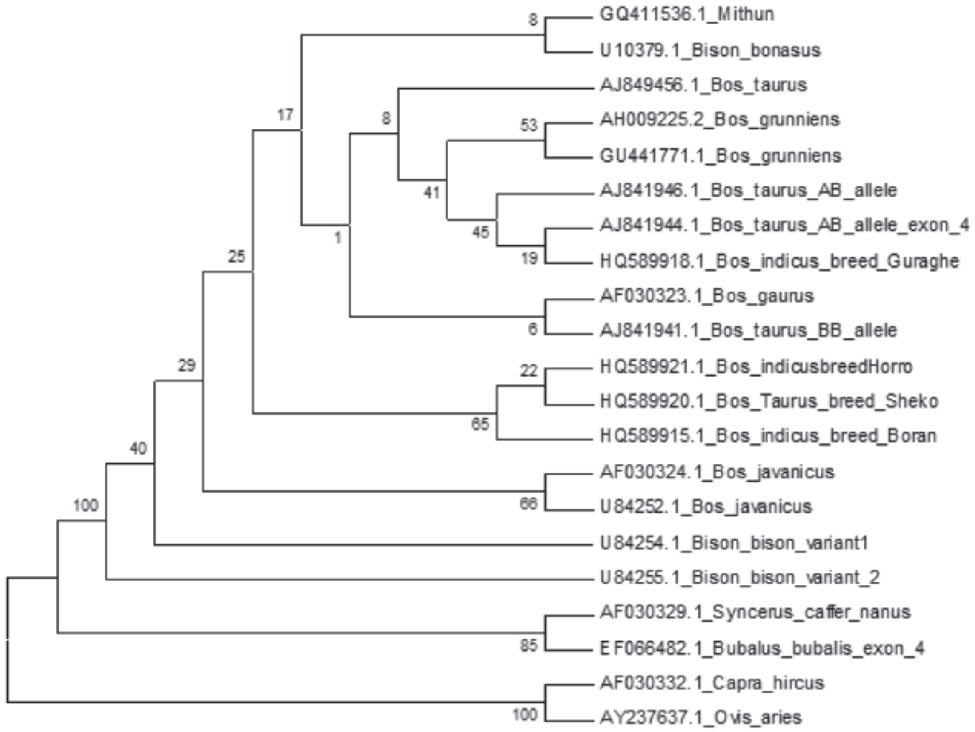


Figure. Evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Tamura 3-parameter method and are in the units of the number of base substitutions per site. The analysis involved 21 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 265 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 software.

Morphometric and Genetic characterization of Mithun

30 bovine microsatellite markers from FAO list of Measurement of Domestic Animal Biodiversity (MoDAD) were tested in mithuns and gaurs individually. Microsatellite genotyping of total 135

samples (120 mithun, 7 gaur and 8 tho tho cattle) were done and data analysed by Popgene software.

Out of 30 bovine microsatellite markers from the list of FAO MoDAD (1996) tested, 19 markers (63%) successfully amplified mithun and wild gaur genomic DNA. 14 markers (74%) out of these 19 were highly polymorphic with high PIC value (> 0.50) in mithun and gaur with allele numbers ranging from 10 to 26 in mithuns and two to seven in guar, respectively. Therefore, these 14 microsatellite markers (BM1818, HAUT27, LSTS030, ETH185, HEL1, ETH3, BM1824, LSTS034, MM 12, HEL 135, ETH152, BM2113, LSTS006, ILSTS011) which are also in the FAO standard panel for cattle diversity studies will be most suitable for diversity studies in Indian mithuns and gaur as well. 11 bovine microsatellite markers failed to amplify in mithun and guar.

Observed heterozygosity (H_o) ranged from 0.15 to 0.94 and 0 to 1.00 in mithun and gaur, respectively while Nei's expected heterozygosity (H_e) ranged from 0.31 to 0.89 and 0.36 to 0.83 in mithun and gaur, respectively. PIC value ranged from 0.29 to 0.88 in mithun and 0.32 to 0.82 in gaur, respectively.

The average estimates of H_o, H_e and PIC across all the 19 microsatellite loci were 0.48, 0.66 and 0.63 in mithun, and 0.62, 0.66 and 0.61 in gaur, respectively. The average observed heterozygosity values (H_o = 0.48 and 0.62) were significantly lower than the average expected heterozygosity values (He = 0.66 and 0.66) in mithuns and gaur, respectively. This indicates presence of overall low to medium genetic diversity in Indian mithun and gaur population, respectively indicating presence of low heterozygosity in Indian mithun and gaur which was similar to Yunan mithuns (the corresponding estimates were 0.53, 0.63 and 0.60, respectively) (Qu et al., 2012).

Level of inbreeding

Estimate of inbreeding coefficient (F_{IS}) as per Wright (1978) was 30.00% and 6.53% in mithun and gaur population, respectively which was positive and quite high particularly in mithun population compared to gaur. This indicates non-random mating, possibly extensive breeding with very less number bulls and absence of gene flow from external migration in the mithun population.

At the same time, inbreeding was lower in gaur population of the zoo, even if positive as compared to mithun population, which was possibly due to introduction of gaur bulls from outside into the herd from time to time.

Genetic And Biodiversity Studies on Mithun (*Bos frontalis*)

The phenotypic characters of Mithuns belonging to different strains were recorded in the proforma developed for this purpose. The recorded data tabulated and analyzed statistically using SAS package.

Sequencing of the Cytochrome b gene of the mitochondrial genome.

To amplify the entire Cytochrome b gene a set of primer pair was designed with respect to the cattle mitochondrial genome - forward 5'–CGAAGCTTGATATGAAAAACCATCG TTG-3' and reverse 5'-GGAATTCATCTCTCCGGTTTACAAG AC-3'. PCR reaction was performed using 1x reaction buffer, 1.5 mM $MgCl_2$, 5 pmol forward and reverse primer with following reaction condition 95 ºC for 5 min. 30 cycles of 94ºC for 45 sec, 57ºC for 45 sec, 72ºC for 1 min and final extension at 72ºC for 10 minute. Amplified product was treated with ExoSAP-IT, as per manufacturer's instructions. Sequencing of the PCR product was performed by (BioServe). Finally, we obtained 838 bp amplicons of the 10 mithuns.

>Cytochrome b – *Bos frontalis*

CCAGCTCCATCAAACATCTCCTCATGATGAAATTTCGGCTCCCTCCTGGGAGTATGCTTAATCCTACAAATCCTCA
CAGGCCTATTCCTAGCGATACACTACACATCCGATACAACAACAGCATTCTCCTCCGTTACCCATATCTGC CGAGA
CGTAAACTACGGCTGAATTATCCGATACATACACGCAAACGGAGCTTCAATGTTTTTTATTTGCTTATATATGCAC
GTAGGACGAGGCCTATATTACGGGTCTTACACCTTCCTAGAAACATGAAACATTGGAGTAATCCTTCTACTTACAG
TAATAGCTACAGCATTCATAGGGTATGTACTACCATGAGGGCAAATGTCATTTTGAGGAGCAACAGTCATCACCAA
CCTCCTATCAGCAATCCCTTACATCGGCACAAATTTAGTCGAATGAATCTGAGGTGGATTCTCAGTAGATAAAGCA
ACCCTTACCCGATTTTTTGCCTTCCACTTTATCCTTCCATTCATCATCACAGCAATTGCCATAGTCCACCTATTAT

Figure. Representative Cytochrome b sequence from Bos frontalis

To investigate the relationship of the *Bos frontalis* with other closely related *Bos* species we performed neighbor – joining analysis of 10 sequenced Cytochrome b gene of the *Bos frontalis* with known sequences of closely related *Bos* species. Values over the node is percentage of the re samplings of 1000 bootstrap values. Analysis revealed *Bos frontalis* does have not close relationship with any other known *Bos* species.

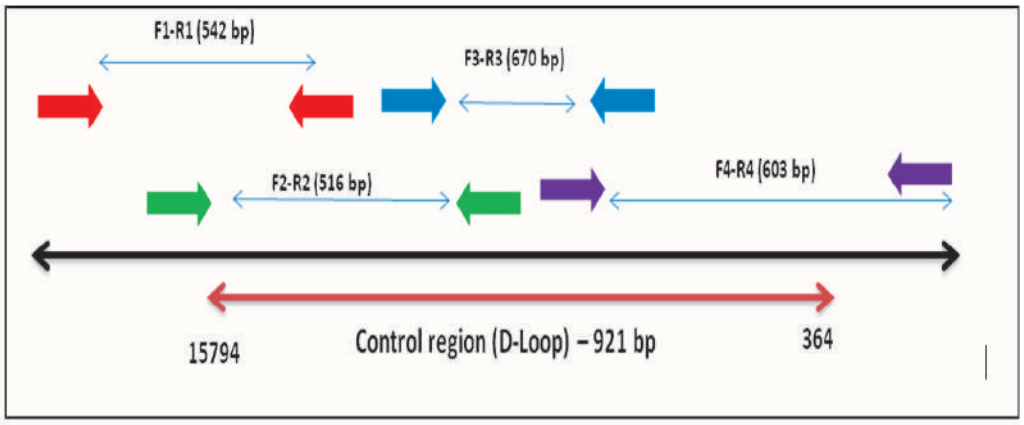
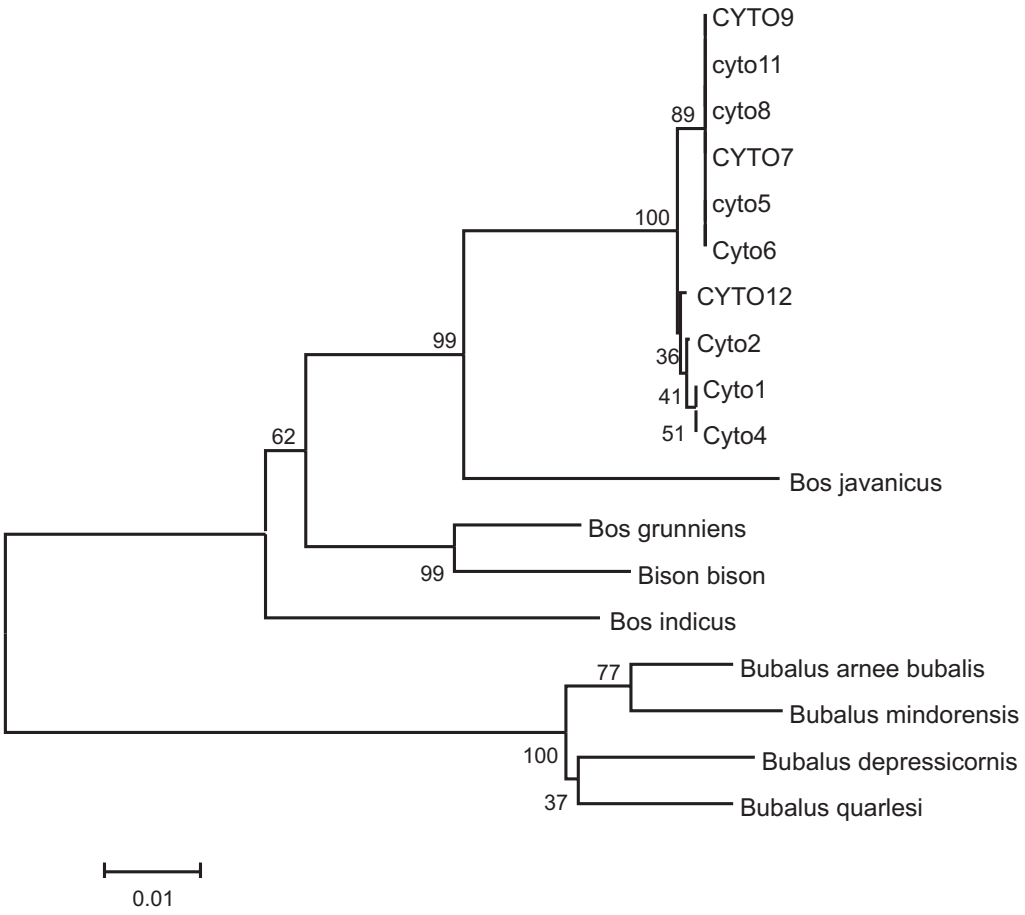


Figure. Schematic representation showing the alignment of primers used for D-Loop amplification

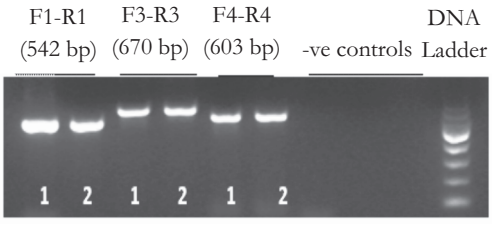
Amplicons were obtained from 2 Mithun samples using the F1-R1, F3-R3 and F4-R4 primer sets.

Figure. *Neighbor joining analysis of Cytochrome b gene from Bos genus*



Sequencing of the mitochondrial D - loop of the mitochondrial genome.

To amplify the entire displacement region (D-loop) of the mitochondrial genome, four set of overlapping primers were designed. *Bos indicus* (16339 bp) whole mitochondrial genome sequence (JN817305.1) was used as the reference sequence for primer designing. PCR reaction was performed using 1X reaction buffer, 1.5 mM $MgCl_2$, 5 pmol forward and reverse primers, with following reaction condition 95 C° for 5 min; 35 cycles of 94 C° for 1 min, Annealing temperature for 45 sec, 72 °C for 1 min and final extension at 72 C° for 10 minute.



Identification of SNPs In Leptin Gene for Selection Of Mithun (*Bos frontalis*) for Higher Growth Traits And Characterization of Leptin Protein

Blood samples were collected from Mithun (*Bos frontalis*) in heparinized vacuutainer tubes and genomic DNA was isolated from this whole blood using Promega Wizard DNA isolation kit as per manufacturer protocol. Quality and quantity was checked for each DNA samples and nanodrop rating (260/280) between 1.7-1.8 for each DNA was considered to be of good quality for further work.

Adipose Tissue samples of mithuns (Bos frontalis) were collected and kept in freezing condition (- 80^{0}C) till processing of these samples.

The PCR amplification of exon 3 and intron 3 of leptin 588 bp amplicon were carried out at the annealing temperature of 59°C. The PCR-RFLP was done with 5U of NruI restriction enzyme at 37^{0}C for 1 hour. The RFLP product was run at 3% agarose gel electrophoresis; however no cut were observed for leptin 588 bp.

Leptin 588 bp amplicon, which gave no cut with NruI restriction enzyme was later tried with HphI which showed three cuts of 588 bp, 397 bp and 191 bp respectively.

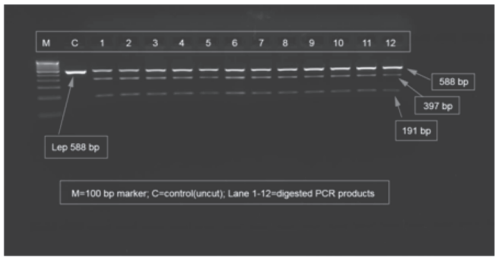


Figure. PCR-RFLP product of leptin 588 bp using HphI

The PCR amplification of exon 2 and intron 2 of leptin 422 bp were carried according to Liefers *et al.* (2002) and the annealing temperature was fixed at 57°C. PCR-RFLP was carried out using ClaI restriction enzyme. The PCR product was digested with 5U ClaI at 37°C for 1 hour. The digested product was run at 3% agarose gel electrophoresis and a product with single cut with two bands (about 272 bp and 150 bp) were observed.

99 animals were screened for leptin polymorphism using PCR-RFLP study taking 422 bp amplicons and all were found to be BB genotypes having two RFLP products (272 bp and 150 bp)

SNP identification in leptin gene of mithun

The raw sequences were alligned into consensus sequences using a number of softwares viz. DNASTAR, Blastn, Megalign etc. These consensus sequences of all the 25 animals were further analyzed through Megalign, Mega4, Phylip software and checked for SNPs.

A total of 17 SNPs have been identified in

the 422 bp amplicons sequenced from 25 experimental mithuns. Out of these 17 SNPs, five are detected in coding region (exon 2 - 115-258 bp). These are as below -

Sl No.	SNP	Location	Frequency
1	A/G	26	7/25
2	C/T	52	6/25
3	G/A	54	7/25
4	C/T	59	7/25
5	T/C	129	7/25
6	C/T	167	1/25
7	C/T	189	1/25
8	A/T	189	6/25
9	G/A	227	7/25
10	G/A	308	6/25
11	T/C	320	1/25
12	C/T	359	7/25
13	T/C	368	7/25
14	C/T	379	6/25
15	T/G	380	6/25
16	T/A	380	1/25
17	G/A	384	7/25

In another PCR amplicon of 588 bp, nine SNPs have been identified sequenced from 25 experimental mithuns. Out of these nine SNPs, six are detected in coding region (exon 3 - 87-446 bp). These are as below -

Sl No.	SNP	Location	Frequency
1	G/A	34	1/25
2	T/A	114	1/25
3	C/T	115	1/25
4	G/A	304	12/25
5	C/T	340	1/25
6	T/C	355	12/25
7	C/T	370	5/25
8	C/T	477	5/25
9	G/T	513	1/25

Leptin protein isolation from adipose tissues and characterization

Adipose tissue samples were collected from Mithun (*Bos frontalis*), Goat (*Capra hircus*) and Cattle (*Bos Indicus*) from different localities of Nagaland and West Bengal. Tissues were kept in deep freezer (-80^{0}C) until use.

Ultrasonication of adipose tissue and protein estimation

Adipose tissue samples were ultrasonicate with the help of ultra sound sonicator (Hysel Japan) in Phosphate Buffer Solution (pH - 7.4). The samples were centrifuged and concentrated using sucrose. The total protein concentration was determined using Lowry's method (1951).

Protein extraction by Chemical method

Total adipose tissue and mature adipocytes were thawed in 0.4 ml of cold Urea/thiourea buffer (7M urea, 2M thiourea, 4% CHAPS, 45mM Tris, pH 7.4, 60mM DTT) and complete protease inhibitors (one tablet/20 ml, Roche, Barcelona, Spain) supplemented with 0.1 mM NaCl. Cells were mechanically disrupted and briefly sonicated. Samples were adjusted to 900µl with lysis buffer (20 mM Tris, pH 7.4; 100 mM Nacl; 1% Triton and protease inhibitors) and incubated for 15 min at 35^{0}C. After cooling on ice (10 min),100µl of 0.1M Tris, pH 7, and 50 mM $MgCl_2$ were added to the homogenate, which were then incubated with DNase-1 (30 U Sigma) on ice (10 min). The homogenate was centrifuged (15 min, 10000

X g, 4^{0}C) and the aqueous phase between the upper lipid phase and the lower cellular debris phase was collected. Finally the extract was separated by chloroform/methanol precipitation. The protein estimation was done by Bradford assay for protein quantification.

SDS-PAGE

The crude adipose tissue proteins obtained from peritoneal, sternal and cardiac adipocytes were analyzed by sodium dodecyl sulphate polyacrylamide electrophoresis (SDS-PAGE) according to Laemmli using 12.5% polyacrylamide gel in a vertical slab gel electrophoretic apparatus. The samples were mixed with sample buffer in a proportion of 1:1 and subsequently the solution was heated at 100^{0}C for 3 min. The amount of protein applied was 50µg per track. Proteins were run at 20 mA for 150 min. The bands were visualized using silver staining method. Standard molecular weight markers were run parallel along with sample proteins to determine the relative molecular weights of the polypeptides. Following the same procedure, separately goat and cattle adipocyte and fish liver crude protein preparations were used to perform SDS-PAGE.

Gel filtration chromatography

The crude protein obtained from peritoneal adipocytes of mithun was subjected to gel permeation chromatography as per Joardar and Ram (1999). It was done using a 1.1 cm X 65 cm column with Sephacryl S-200 bed (Sigma, USA). The Void volume (24 cm) of the column bed was determined by blue dextran elution. The flow rate was 24ml/hr. The sample was eluted with 30 mM Tris buffer (pH-7.5). Absorbance of elutes was monitored at 280 nm. Protein concentration of the gel permeated fractions was estimated by Lowry's method (1951).

Ion- exchange Chromatography

The fractionated (gel permeated) mithun protein GPII was subjected to ion exchange chromatography as per Joardar and Ram (1999). It was done using a 1.1 cm X 30 cm column with DEAE-cellulose CL-6B (Sigma, USA). The flow rate was 18ml/hr. The sample was eluted with Buffer used-30mM Tris-HCl containing 6M urea, pH 8.0. Absorbance of elutes was monitored at 280 nm. Protein concentration of the ion-exchanged fractions was estimated by Lowry's method (1951).

Detection of leptin by anti-leptin antibody using Western blot analysis

The resolved polypeptides (done by SDS-PAGE) of crude goat adipocyte protein preparation were electro-blotted to nitrocellulose membrane (NCM, Sigma) from gel by a semi-dry Western blot apparatus (Atto, Japan) as per Towbin *et al.* (1979). After blocking and thorough washing, the NCM was incubated in the anti-goat leptin antibody, raised in chicken for 2 h at 37C. The antibody was diluted to

1:50 in PBS, pH 7.4. After washing, NCP was transferred to a small trough containing anti-chicken HRPO immunoconjugate (1:4000 dilutions in PBS, pH 7.4) for 2 h at 37C. The blot was then developed by immersion in diaminobenzydine substrate solution (40l H_2O_2, 0.025g diaminobenzydine in 10ml Tris-HCl, pH 7.5). Similar blotting analysis was performed with mithun, cattle and fish adipocyte protein preparations to determine cross-reactivity of mithun leptin with goat, cattle and fish.

Isoelectric focusing and 2-D PAGE

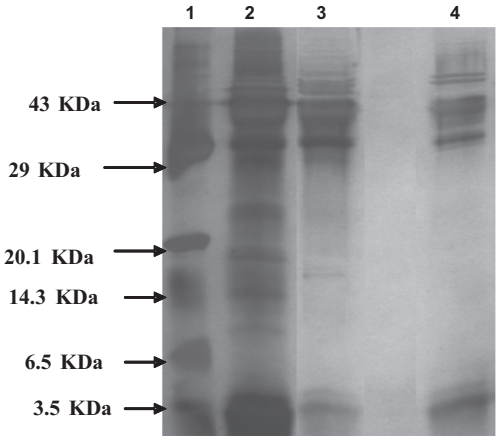
Isoelectric focusing and 2D-PAGE of proteins obtained from chemically treated adipocyte tissue was performed as per Peinado *et al.* (2010). Briefly, immobilized pH gradients strips (18 cm, pH 3-10 non linear) were processed, followed by equilibration of strips with SDS equilibration buffer (75 mM Tris, pH 8.8, 6M urea, 30% glycerol, 2% SDS) containing 2% DTT for 15 min. It was followed by 15 min wash. Gels were stained by SYPRO Rubi dye.

The stained gel was washed with washing solution (10% methanol, 7% glacial acetic acid) and the final washing was done by MilliQ water for 10 min according to the manufacturer's instructions. All gels were scanned with the help of Fuji scanner in the fluorescence mode to visualize the stained gels. The spot volume was measured and reported as percent volume of the spot (normalized volume of spots, % volume) in relation to the sum of all detected spot and this provided normalized spot volume. The change in % volume is basis of detection of differential protein identification. A cut-off value for selection of differential protein spot is set at a 1.2-fold increase or decrease in spot intensities in relation to the reference group by student t-test with $p<0.05$ (with visual inspection of the result).

SDS-PAGE of different adipocyte tissue proteins

Polypeptide profile of mithun adipocyte proteins of different sources (tissues) varied as observed in SDS-PAGE analysis. Maximum polypeptides (n=29) were observed in peritoneal adipocyte protein preparation. The molecular weight of the polypeptides varied between 2.5 to 55 kDa. Sternal adipocytes showed 14 polypeptides in the molecular weight range of 2 to 51 kDa. Cardiac adipocyte protein revealed 10 polypeptides in the molecular weight range of 3 to 46 kDa. Four polypeptides in peritoneal adipocyte protein preparation and one that of sternal adipocyte protein preparation were observed in the molecular weight range of 14 to 19 kDa. The peritoneal adipocyte protein preparation was selected as a starting material for leptin isolation due to more protein content and presence of more polypeptides.

Figure. Polypeptide profile of Mithun adipocyte proteins as assessed by SDS - PAGE using silver staining method

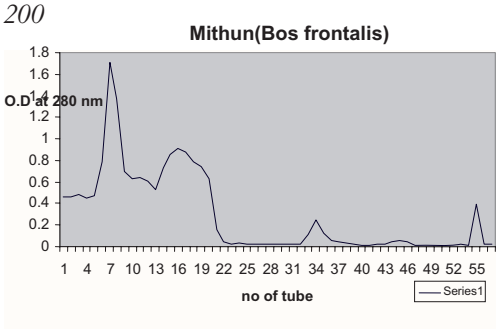


Lane 1- Standard molecular weight marker
Lane 2 - Peritoneal adipocyte Protein
Lane 3 - Sternal adipocyte Protein
Lane 4 - Cardiac adipocyte protein

Gel filtration chromatography

When the peritoneal adipocyte crude protein preparation of mithun was subjected to gel filtration chromatography using Sephacryl S-200, two prominent peaks (GP1 and GP2) were obtained (Figure).

Figure. Fractionation of Mithun adipocyte proteins by gel filtration chromatography using Sephacryl S-200

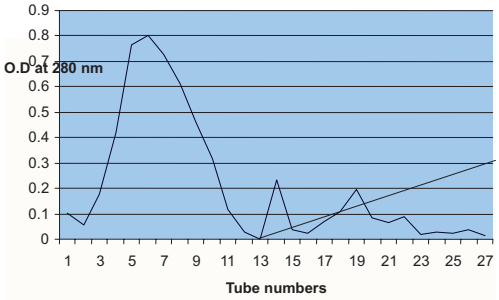


Buffer used - 30mM Tris - HCl, pH - 7.5, Flow rate - 24ml/hr

Ion-exchange chromatography

The proteins under GPII were subjected to ion-exchange chromatography (DEAE-cellulose). Unbound proteins revealed one major peak (DI). When bound proteins were subjected to continuous elution buffer having different molarities of sodium chloride, ranging from 0.15 M to 0.3 M, four further protein peaks (DII, DIII DIV and V) were observed.

Figure. Ion-exchange chromatography of gel permeated fraction-II (GP-II) of Mithun adipocyte proteins unsing DEAE-cellulose CL-6B

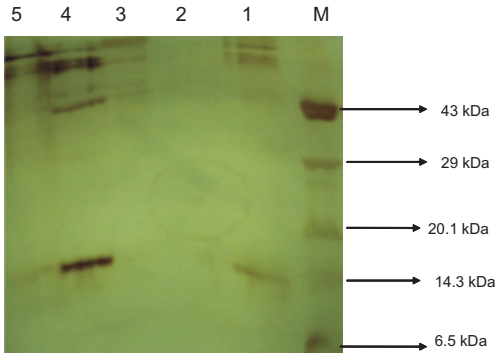


Buffer used - 30 mM Tris-HCI containg 6M urea, pH 8.0; Flow rate - 18 m/hr

SDS-PAGE of fractionated proteins of Mithun peritoneal adipocytes

When the fractionated proteins (DI through DV) obtained from DEAE-cellulose chromatography was subjected to SDS-PAGE, it was observed that polypeptide profiles of the fractions varied. Six polypeptides were observed in DIV out of which one prominent polypeptide was noticed in the range of 16 kDa upon silver staining method.

Figure. Polypeptide profile of fractionated adipocyte protein of Mithun as assessed by SDS - PAGE using silver staining method



Lane M - Molecular weight marker (medium), Lane 1 - Fraction-I, Lane 2 - Fraction-II, Lane 3 - Fraction-III, Lane 4-Fraction-IV, Lane 5 - Fraction-V

Attempts were made to purify and characterize mithun leptin protein from adipocyte protein preparation. Fractionation of crude adipocyte proteins was done by gel filtration (using Sephacryl S-200) in the first step and subsequently by ion-exchange chromatography (using DEAE-cellulose). One of the semi-purified fractions (DIV) possessed one prominent polypeptide band around 15-16 kDa. It could be leptin protein as from the literature it is known that, the molecular weight of leptin protein of mammal is 16-18 kDa (Taniguchi *et al.*, 2002). However, the observation needs confirmation by western blot analysis or other tests. Moreover, further purification steps are needed to have a single pure polypeptide (protein). The work is in progress in this direction.

Western blot analysis

To standardize the technique as also to identify leptin in animal adipocyte protein preparation, goat adipocyte protein preparation was subjected to western blot analysis using anti-goat leptin antibody. A prominent band corresponding to 16 kDa was detected indicating presence of leptin in the said protein preparation.

To identify leptin in mithun adipocyte protein preparation and to assess cross-reactivity of leptin in different animals (mammals and fish) SDS-PAGE was performed with mithun, goat, cattle and fish adipocyte proteins (Figure), followed by western blot analysis using anti-goat antibody. Anti-goat leptin antibody could detect goat leptin only, indicating no cross-reactivity between goat leptin and Mithun leptin (and with leptin of other animals).

Figure. Identification of leptin in goat adipocyte crude protein preparation by Western blot analysis using anti-goat leptin antibody

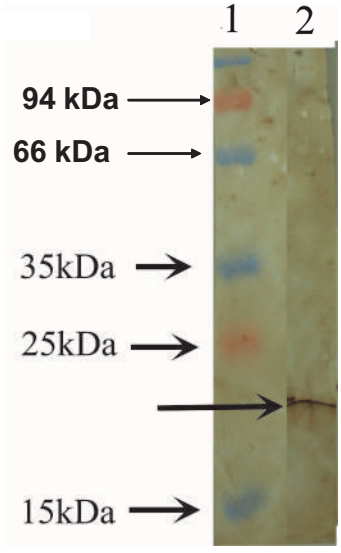
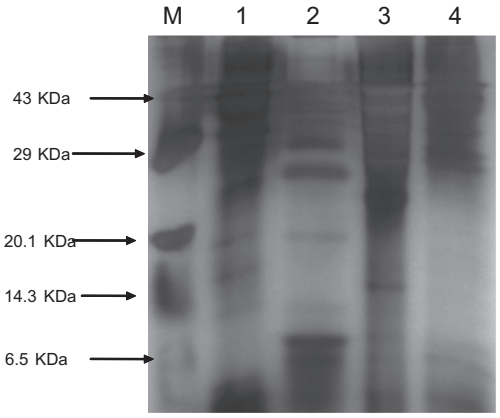
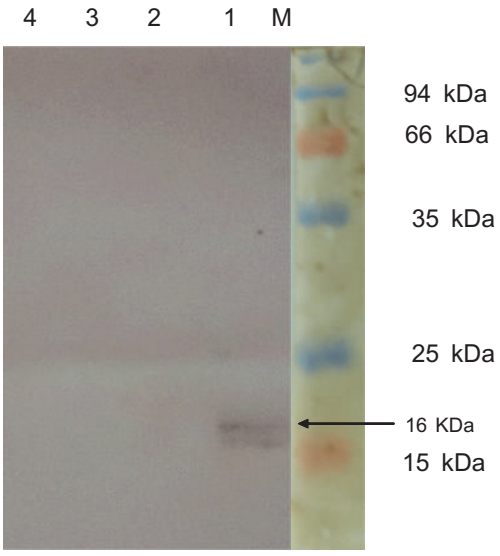


Figure. Polypeptide profile of different animal adipocyte proteins as assessed by SDS-PAGE using silver staining method



Lane 1- Standard molecular weight marker, Lane 1 - Goat protein, Lane 2 - Mithun protein, Lane 3 - Cattle protein, Lane 4 - Fish protein

Figure. Identification of leptin in adipocyte crude protein preparation of different animals by Western blot analysis using anti goat leptin antibody



Lane M - pre-stained marker, Lane 1 - Goat protein, Lane 2 - Mithun protein, Lane 3 - Cattle protein, Lane 4 - Fish protein

2D- PAGE

Two Dimensional Gel electrophoresis of crude adipocyte tissue after chemical treatment by urea/thiourea buffer showed several proteins in the range of molecular weight 10-22 5kDa. The proteins were mainly present in 4-9 pI range. To standardize the technique and to characterize Mithun leptin, cattle and goat adipocyte protein preparations were subjected to 2D-gel electrophoresis. In cattle adipocyte preparation, proteins were mainly present in 10-75 kDa range. Very few proteins were present outside this range. Proteins were largely found in the molecular weight range of 10-45 kD and pI range of 4-7 (Figure). Protein spot in the molecular weight range of 16 kDa and pI 5.8 was 'red encircled' (supposed to be of leptin). Likewise in goat adipocyte preparation, proteins of low molecular weight were less abundant. Proteins were mainly visible in the range of 15-60 kDa range (Figure). Here also, Protein spot in the molecular weight range of 16 kDa and pI 5.8 was 'red encircled' (supposed to be of leptin). However, this needs confirmation with the help of Western blot analysis or by other ways (viz. with the help of 2D- software analysis). 2D-gel electrophoresis with Mithun adipocyte protein preparation is in progress.

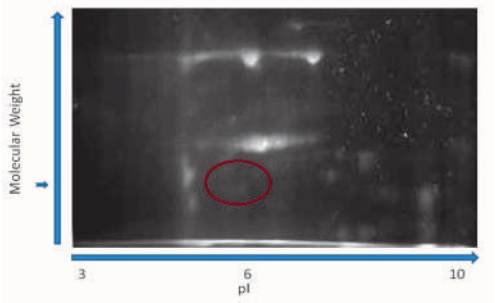


Figure. 2D- gel electrophoresis of cattle adipocyte derived crude proteins. X-axis represent pI range3-10 where Y-axis represent molecular weight of 10-224 kDa range

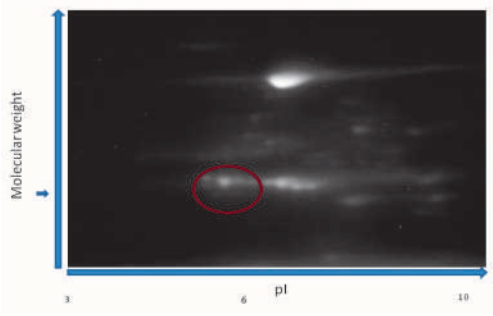


Figure. 2D- gel electrophoresis of goat adipocyte derived crude proteins. X-axis represents pI range 3-10 whereas Y-axis represents molecular weight of 10-224 kDa range.

ANIMAL NUTRITION

Development of economically viable feeding strategy for rearing mithun in intensive system using spent grains from breweries industries

Experiment - I: Energy utilization pattern in mithun fed on Congo Signal grass based diet supplemented with spent grain with wheat bran or rice bran

21 mithun with an average live weight of 331 ± 4.45 kg were randomly divided into 4 groups fed on Congo Signal based diet. In group I animals were fed Congo Signal grass without supplementation of concentrate mixture. However, in group II animals were supplemented with concentrate mixture composed of spent grain 3 parts and wheat bran 1 part on as such basis; in group III spent grain 3 parts and rice bran 1 part; and in group IV traditional concentrate mixture composed of crushed maize 50%, mustard cake 30% and wheat bran 20% to determine energy utilization pattern in mithun maintained in intensive system.

The dry matter intake per kg metabolic body size was 49.98, 95.59, 94.39 and 89.87 and dry matter digestibility was 49.46, 65.60, 64.34 and 65.50 percent in groups I, II, III and IV, respectively. The dry matter intake in groups II, III and IV was significantly ($P<0.05$) higher than group I. However, there was no significant difference between groups II, III and IV. The dry matter digestibility in groups II, III and IV was significantly ($P<0.05$) higher than group I. However, there was no significant difference between groups II, III and IV. The gross energy intake per kg metabolic body size per day was 203, 404, 402 and 366 kcal and digestible energy was 106, 271, 274 and 247 kcal in groups I, II, III and IV, respectively. The gross energy as well as digestible energy in take in groups II, III and IV was

significantly (P<0.05) higher than group I. However, there was no significant difference between groups II, III and IV. The digestible energy as percentage of gross energy was 51.9, 67.1, 67.8 and 67.8 in groups I, II, III and IV, respectively where it was significantly (P<0.05) higher in groups II, III and IV than group I. However, there was no significant difference between groups II, III and IV. The digestible energy content was 2.11, 2.84, 2.90 and 2.76 Mcal/kg DM in groups I, II, III and IV, respectively. The digestible energy content in groups II, III and IV was significantly (P<0.05) higher than group I. However, there was no significant difference between groups II, III and IV. It was concluded that supplementation of spent grain and wheat bran / rice bran in the diet of mithun increased dry matter and gross energy intake and digestibility and supplementation of spent grain and wheat bran / rice bran in the diet of mithun can replaced traditional concentrate mixture.

Experiment - II: Energy utilization in mithun fed on spent grain based feed block

Eight adult uncastrated male mithun weighing 308 ± 19.10 kg were divided in 2 groups of 4 animals each in a completely randomized design. The animals in group I was fed on a feed mix composed of paddy straw 54 parts and spent grain 46 parts with moisture content 49% and microbial load 110 x 10^5 CFU/ml, however, group II offered with same feed composition but in the form of feed block prepared after drying to determine comparative feed intake and nutrient utilization. However, after sun-drying and keeping in the form of feed block up to 10 months the microbial load was 46 x 10^5 CFU/ml.

The feed intake per kg metabolic body size per day was 63.4 and 86.0 g and dry matter digestibility was 53.4 and 65.6 percent in groups I and II, respectively which was significantly (P<0.05) different from each other. The gross energy intake was 298.7 and 388.0, digestible energy intake was 166.9 and 261.6 kcal per kg metabolic body size per day in groups I and II, respectively which was significantly (P<0.05) different from each other. The DE as percent of GE was 55.8 and 67.5 and DE content was 2.50 and 3.05 in groups I and II, respectively which was significantly (P<0.05) different from each other. It was concluded that feeding spent grain based rations in the form of feed block is better than mixed form.

Isolation and characterization of botanicals from NEH region for their antibacterial or antimethanogenic activities

Experiment – I. Determination of total gas and methane production pattern in Mithun

The study was conducted to determine antimicrobial activity of *Artemisia vulgaris* essential oil (AVEO), and to see the effect of drying of herb for AVEO extraction on its antimicrobial activity. AVEO was extracted from fresh chaffed herb and dried powdered

herb and tested on 1199 strains of 113 species of pathogenic, potentially pathogenic and environmental microbes belonging to 33 different genera, 1172 were bacteria and 27 were yeast and moulds. Although more number of strains was sensitive for AVEO extracted from fresh herb (23%) than AVEO from dried herb (21%), difference was statistically insignificant (p, 0.40) between AVEOs extracted from fresh or dried herb. About 19.9% of bacterial and 25.9% of fungal isolates were sensitive to AVEO. Interestingly, oxidase positive strains (63.7%) including those of pseudomonads (60%), aeromonads (53.6%), spore forming bacilli (71.6%), *Pastuerella* (83.3%) and micrococci (66.7%) were comparatively more sensitive (p, <0.001) than oxidase negative bacteria (8.3%) to AVEO. Of the 114 clinical isolates (associated with illness in human and animals) belonging to 25 bacterial species, 23 (20.2%) were sensitive to AVEO. Clinical isolates were significantly ($p < 0.03$) more sensitive than isolates from healthy human and animals (12.6%). Thus for better antimicrobial activity AVEO should be extracted from fresh herb. The AVEO may be an effective therapeutic agent of future either as such or as the source of some novel antibacterial molecule(s) particularly against oxidase positive bacteria.

Name of the project: Veterinary type culture

Rumen liquor has been collected from two adult male fistulated mithun fed on paddy straw, green grass and concentrate mixture. Faecal samples from freely browsing mithun have also been collected. Forty one pure cultures of bacteria have been isolated using roll tube method. Characterization and identification of those bacteria is under progress.

ANIMAL PHYSIOLOGY

Standardization of cryopreservation/ embryo freezing protocol for mithun

Cryopreservation of embryo is a means of long-term storage of valuable strains. This technique can be used to safeguard different valuable strains and it also reduces animal housing costs. Embryos are preserved in a cryoprotectant then slowly frozen and then transferred to liquid nitrogen tanks for long-term storage. Cryopreserved embryos can be recovered at any time as and when required and transfer it in to recipient or surrogate mother. Post-thaw embryo survival is dependent on several factors like the initial embryo quality, developmental stage, and species. Vitrification is one of the potential alternative methods to traditional slow cooling method. It does not induce intracellular ice crystal formation and also reduces the damage to embryos. Till now, successful results based on pregnancy rates have been obtained with cryopreserved cow, sheep, goat, and horse embryos but no work was done on Mithun. As the population of this animal (Mithun) is not in a comfortable status, the technology like ETT followed by cryopreservation of embryos will definitely help to propagate quality germplasm of this

magnificent species of animal. Additionally, the standardized protocol for cryopreservation of mithun embryos and its successful implication in ETT will be of great help in conservation and propagation of mithun in the field level. Embryo transfer technology is the conglomeration of use of the best quality of sperm and oocyte (unlike AI where only the best quality of sperm is used) and can produce 6-8 calves at a time from a single cow thereby useful for faster multiplication of the quality mithun germplasm.

In order to do cryopreservation of mithun embryos by vitrification method, four embryos were recovered from two donor (superovulated by using FSH, Folltropin-v-Bovine- 400mg) animal by flushing on 6th day of oestrous cycle. Embryos were evaluated under stereo-zoom microscope and morula classified as Grade 1 and 2 were used for freezing by vitrification method.

Vitrification and thawing

Two vitrification solutions were prepared in media consisting of TCM-199 with 10% FBS. Vitrification solution I (VS I) consisted of 10% ethylene glycol (EG) + 10 % dimethyl sulfoxide (DMSO) and vitrification solution II (VS II) consisted of 20 % EG + 20 % DMSO + 0.6M sucrose. The collected embryos were exposed to VS I for equilibration upto 3 minutes followed by 25-30 seconds in VS II at room temperature (22-25^{O}C). The embryos in VS II were immediately loaded to a 0.25 ml French straw preloaded with 0.6 M sucrose in holding medium with air gap in between and plunged into Liquid Nitrogen (LN_2). The straws were stored for a period of 100 days and then thawed in 37^{o}C water bath for 30 seconds. After immersion in the water bath, embryos were gradually rehydrated in sucrose solution. Embryos were kept into the medium containing 0.6M of sucrose in basic solution for 1 minute. Then, transferred successively into a holding medium in stepwise dilution containing 0.3M and 0.15 M of sucrose for one minute in each. Following rehydration, embryos were washed three times in holding medium. Morphological integrity of post thaw vitrified embryo was assessed under sterozoom microscope for transfer. The embryo was loaded in 0.25 ml insemination straw under sterozoom microscope with the aid of a 1 ml syringe. The straw was placed in a stainless steel insemination gun and passed through cervix. The ovary was palpated to know the status of the Corpus Luteumm and then the tip of the rod was allowed to slide into the uterine horn on the same side of the ovary with functional Corpus Luteum in order to expel the embryo in the tip of the horn.

In the present study out of the four embryos, two embryos were morula stage and found to be classified as Grade 1 (Excellent - an ideal embryo, spherical, symmetrical and with cell of uniform size, colour and texure) and Grade 2 (Good- an embryo with few extruded blastomeres, irregular shape and few vesicles), respectively. These embryos were used for freezing by vitrification method.

After 100 days of preservation, the embryos were transferred into two recipient animal, whose cycle well synchronised with the donor and out of that one male calf (MOHAN) was born on May 12, 2012. MOHAN, is the world's first ETT born mithun calf from 100-day cryopreserved embryo.

MOHAN, world's first ETT born mithun calf from 100-day cryopreserved embryo

Investigating the changes in behaviour, blood parameters and circulatory stress hormone during the course of work in mithun bull

In the North-East hill region, cattle and buffalo bullocks are the animals of choice for ploughing and other related agricultural activities. However, considering some of the unique features of mithun like excellent climbing capacity on steep hilly slope, huge body size and well physiological adoptability in the North-East hill ecosystem, this species can be used as a valuable draught animal. Currently, limited information is available on the work capability of this valuable species. Therefore, the expected outcome of the proposed project will help for introducing mithun as a draught animal in the region. Moreover, the project outcome will also help for possible utilization of mithun for agro processing and electricity generation using simple rotary system in the mithun inhabited areas where the access to electric power is poor. Therefore, the research work was conducted to evaluate the draught capability of mithun and also to investigate the changes in behaviour, blood parameters and circulatory stress hormones during the course of work.

The experiment was conducted on 4 mithun bulls (Aged ~ two years and body weight ~220 kg) from the herd maintained at the N. R. C. on Mithun, Jharnapani. A suitable harness was designed based on the model that used locally for draught buffalo for ploughing paddy field. The experimental animals were put together for work using the harness. Preliminary training was given to the experimental animals for obeying different movement commands, initially to the individual animal and then to the animals together. The mithun bulls were put to ploughing continuously for a period of three hours in a day. The physiological observations i.e. pulse rate, respiration rate and body temperature by standard techniques. The recording of physiological observations and collection of blood samples were done 15 minutes prior to the start of the experiment and after giving three hours of exercise to the animals. Blood metabolites namely NEFA, AAN (Alpha Amino Nitrogen) and stress hormone (Cortisol) were estimated.

Effect of ploughing on pulse, respiration and rectal temperature in mithun bull

The overall mean of pulse rate prior to start of the experiment was 54.93 ± 2.07 per minute. The pulse rate rose to 90.80 ± 2.16 per minute at the end of three hours of

ploughing, which might be due to increased metabolic rate to provide more energy to muscle and to dissipate the extra heat load. The mean respiration rate prior to ploughing was 27.50 ± 1.88 per minute. The respiration rate rose to 76.43 ± 2.83 per minute at the end of three hours of ploughing. Similarly, the body temperature of mithun bulls rose from 100.80 ± 0.15° C per minute at the beginning of ploughing to 104.52 ± 0.12° C per minute at the end of three hours of ploughing, respectively.

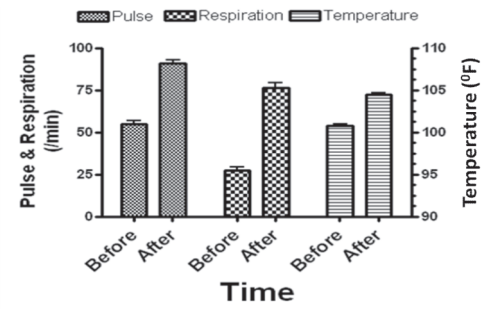


Figure. Pulse rate, Respiration rate and body temperature of mithun bull before and after work

Effect of ploughing on blood metabolites (NEFA, AAN) and stress hormone (Cortisol) in mithun bull

The overall mean of plasma NEFA rose from 168.45±15.34 mEq/l at the beginning of the ploughing to 310.71±25.39 mEq/l at the end of three hours of ploughing. Similarly, the overall mean of plasma α-amino nitrogen (AAN) prior to start of ploughing was 25.55±4.66 mg/dl and it rose to 41.75±8.21mg/dl at the end of three hours of ploughing. The mean plasma cortisol level also increased from 7.82±0.89 ng/ml prior to start of ploughing to 13.21±1.67 ng/ml at the end of three hours of ploughing, respectively.

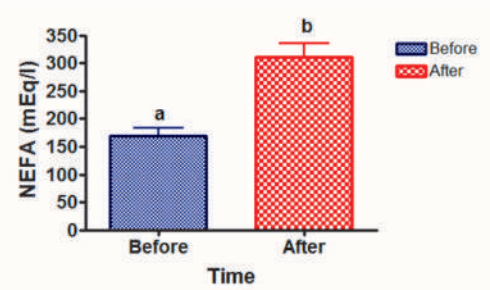


Figure. Plasma NEFA (mean±SEM) level before and after work in mithun bull. Different superscripts indicate statistical significance (P<0.05)

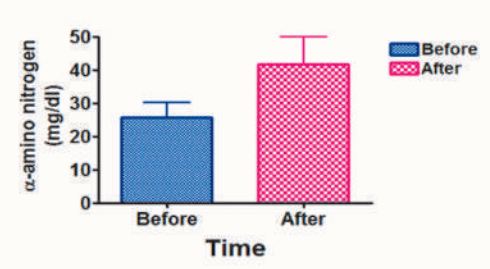


Figure. Plasma a-amino nitrogen (mean±SEM) level before and after work in mithun bull. Different superscripts indicate statistical significance (P<0.05)

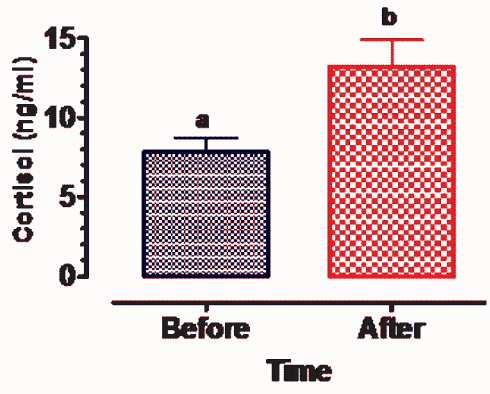


Figure. Plasma cortisol (mean±SEM) level before and after work in mithun bull. Different superscripts indicate statistical significance (P<0.05)

ANIMAL HEALTH

Prevalence of *Amblyomma testudinarium* Koch, 1844 in mithun (*Bos frontalis*) of eastern Mizoram (India) near Myanmar border.

The present investigation formed a part of the animal health check-up and treatment at a camp organized in Khuwangleng village under Champhai district of Mizoram, India. Among others, a total of 33 adult free ranging mithuns of both sex and having frequent access to Myanmar were presented in the camp. During the health check-up, three animals were found to have unusually large ticks attached to the skin of the dewlap and underside of the thigh. These ticks three in numbers were carefully collected in specimen vial and brought alive to the laboratory. Two specimens engorged with blood were first examined under stereoscopic microscope and thereafter processed in 10% KOH to study morphological characteristics for taxonomic identification with the help of available keys

Record of 3/33 mithuns positive for *A. testudinarium* infestation in the present study showed 9.09% prevalence in eastern region of Mizoram bordering Myanmar. Grossly, the ticks were shining golden yellow coloured with presence of numerous dark punctuations on the whole dorsum (Plate-1). These measured 23 to 25 mm in length and 20 to 22 mm in width and weighed 8 to 10 g. Body contour was elliptical and widest in the region of spiracular plate. Posterior border of the engorged tick showed evidence of festoons (Plate-2). Microscopic findings were long mouth parts with palpal article 2 longest and more than twice the length of other articles (Plate-3); hypostomal dentition 4/4 with dental articles on the inner two files smaller than the outer files (Plate-4); basis capitulum rectangular dorsally with two oval shaped large porose areas (Plate-5); scutum triangular with narrow posterior angle, presence of distinct golden brown ornamentation in the form of cervical stripes fused posteriorly (Plate-6) and dark brown numerous punctuations of variable size; eyes distinct, large, pale and flat and slightly bulging beyond the contour of the scutum (Plate-7); legs long and reddish brown with pale areas at the distal joints; tarsi abruptly attenuated, coxa I with two subequal spurs (Plate-8), the external being larger and Coxa-IV with single broad rounded spur; spiracle plate triangular in shape (Plate-9) and the anal groove posterior to the anus (Plate-10). Based upon these morphological findings the specimens were found indistinguishable from female adult *Amblyomma testudinarium* .

A. testudinarium is one of the largest among hard ticks which are usually 1-9 mm long before engorgement and reaches upto 23 mm in length after feeding . Conforming to these reports, the engorged females in the present case measured upto 25 mm length and weighed 1-5 gm as the highest.The present report of *A. testudinarium* in Mizoram is in conformity with earlier records of this species from neighbouring states like Assam ,Meghalaya , Arunachal Pradesh and Sikkim of the North East India infesting cattle, mithun, tiger, wild

boar, barking deer and elephant. Considering the host relationship and distribution in the previous records and also in the present one, it may be suggested that this tick usually parasitizes the wild animals and may infest domesticated large animal near about the forested area. Highest record prevalence in the North Eastern border of India adjacent to Myanmar might be due to favourable ecological and climatological factors as evidenced by the dense forested hilly environment with average temperature ranging from 10°- 20° C in winter and 15°-30° C during summer months. The present record includes Mizoram also in the *A. testudinarium* distribution map of India.

Plate-1 Dorsal and ventral view of live tick

Plate-2

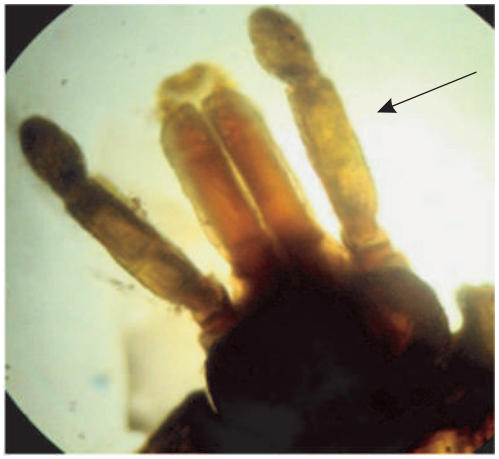


Plate-3

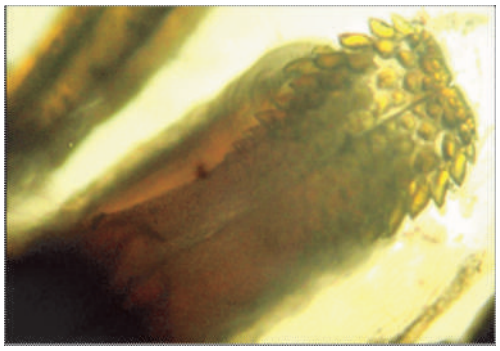


Plate-4

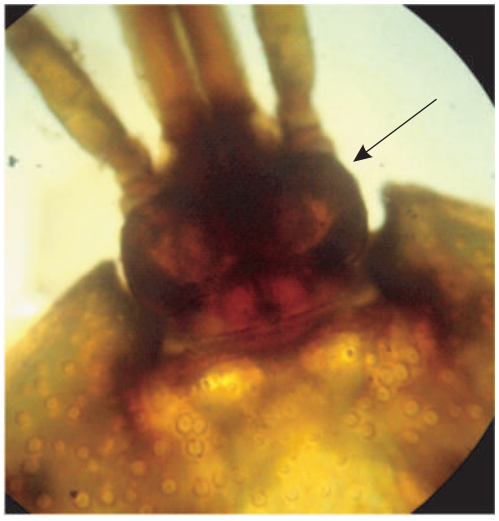


Plate-5

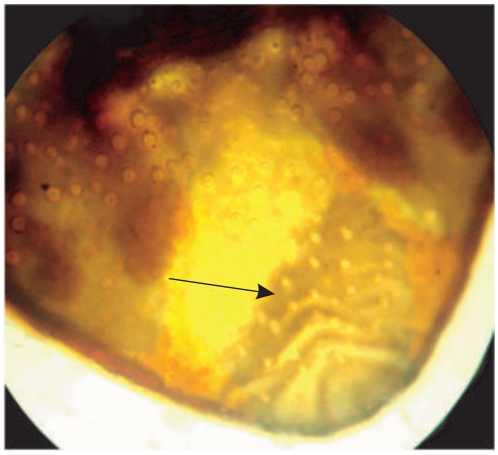


Plate-6

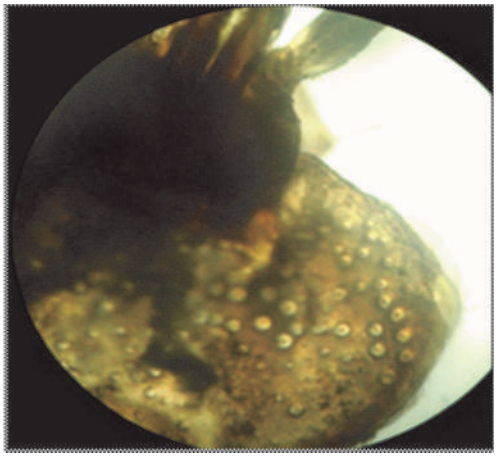


Plate-7 Right eye

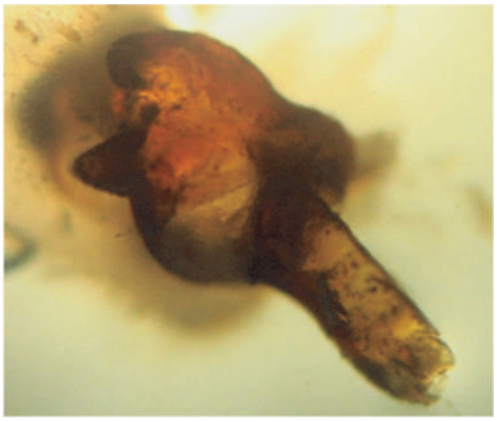


Plate-8

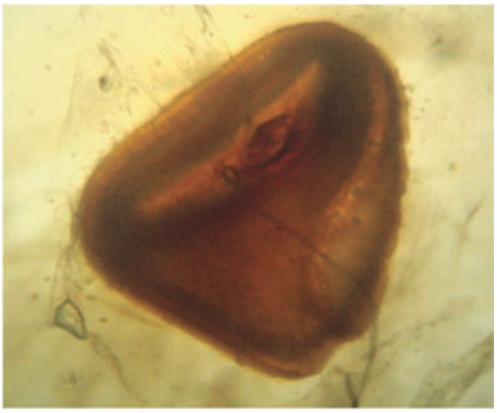


Plate-9

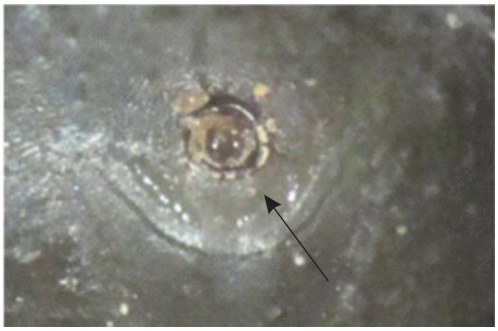


Plate-10

Plate-1: Dorsal and ventral view of live tick

Plate-2: Posterior border showing festoons and colour change with development of dark markings in dead tick

Plate-3: Longirostrate with long palpal article 2.

Plate-4: Hypostomal dentition 4/4

Plate-5: Showing rectangular basis capitulum and porose areas.

Plate-6: Triangular scutum showing ornamentation and punctuations.

Plate-7: Right eye

Plate-8: Coxa-I showing subequal spurs

Plaet-9: Showing triangular spiracle plate.

Plate-10: Showing anal groove posterior to the anus.

Efficacy of chemical and herbal Anthelmintic drugs against naturally infested gastrointestinal helminthiasis in Mithun calves (*Bos frontalis*)

An investigation was conducted to study the efficacy of commonly used chemical as well as few new herbal anthelmintic drugs against gastrointestinal parasite infestation in growing mithuns raised under semi domesticated management system at the Institutes livestock farm. The drug, Ivermectin showed 99.59% efficacy against *Toxocara vitulorum*. While the herbal drugs i.e. Vet worm and *Ficus hirta* have shown efficacy of 88% and 84.88% respectively, which was below the recommended standard efficacy of 98%. Nevertheless the Neem (*Azadirachta indica*) was found to be effective (98.52%) to some extent against *Toxocara vitulorum* infection. The efficacy rate of anthelmintics drugs like Albendazole, Fenbendazole and a combination of Fenbendazole and Praziquantel was 100% and for all the treatment was observed to be effective from day 14th of treatment.

Trichobezoars in mithun calves

Trichobezoars is a common phenomenon in ruminant, but the reports were meagre in mithun calves. The present case study reports the occurrence of hairball in unweaned mithun calves.

Case History and Observations

Two unweaned mithun calves (< 4 months of age) with the symptoms of anorexia, frequent diarrhoea, reluctance to move and lethargy were treated with supportive medication and antibiotic. But the calves did not respond to treatment and died few days later. In post-mortem examination, the abomasum was filled with five small and seven large size hairballs and was compacted. The abomasal mucus membrane was inflammed, peeled off easily and small perforating ulcers were noticed.

In one calf, the hair balls were hard, densely packed and were weighing of 45 to 54 gm and length was 3 to 5 cm. In another calf, most of the hairball was 12 to 17 gm and were soft as in cattle calves. This hairball might be formed due to mineral deficiency especially either copper or microbiological agents or stress and the calves' behaviour of licking body coats of other mithun calves The ingested hair was formed gradually into

oval or round hair balls with gastric juice and enzyme and became hard over a period of time.

Thelaziasis in mithun - A case report

The present investigation formed a part of the animal health check-up and treatment at a camp organized in Papumpare village of Arunachal Pradesh, India. During the health check-up, 30 animals were found to have infested with eye worms. These parasites were carefully collected in specimen vial containing 70% alcohol. The adult parasites were placed in Langhans Lactophenol for taxonomic identification of specimens under microscope with the help of keys of Soulsby (1986).

Grossly, the specimens were milky white in colour and 1.2-1.5 cm in length. Microscopic findings were the worms are milky white in appearance having prominent cuticular transverse striation; mouth cavity is short and broad, widest at middle.

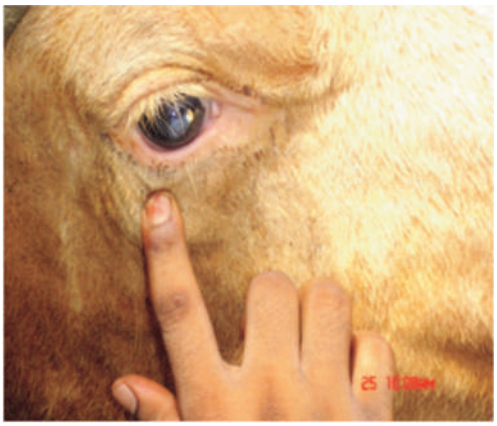


Figure. Infected animal with Thelazia sp

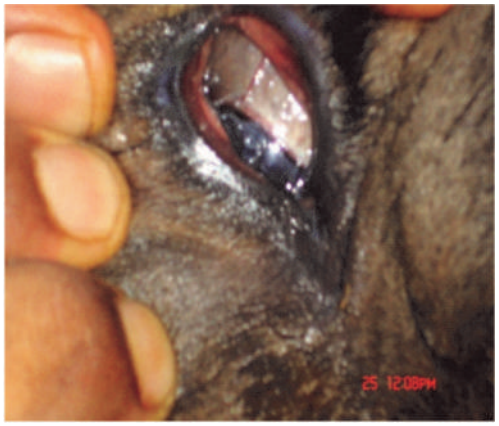


Figure. Infected mithun with Thelazia sp.

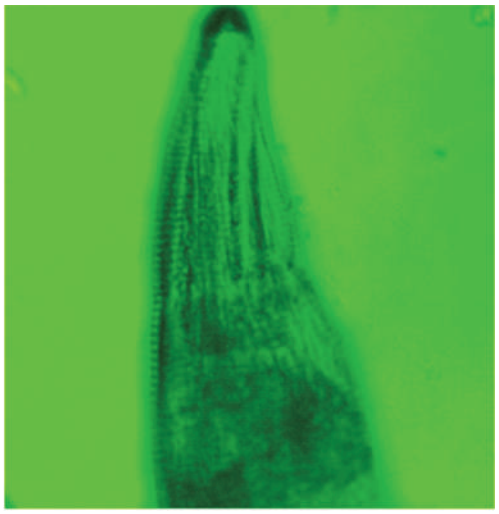


Figure. Anterior end of Thelazia sp.

Figure. Posterior end of Thelazia sp.

Survey for common diseases of mithuns

A total of 750 animals have been surveyed in 16 villages in Nagaland, Arunachal Pradesh and Mizoram and about 300 serum/blood samples were collected form free ranging Mithuns for sero-monitoring of different diseases. 150 reared under intensive system of rearing were surveyed, sampled and screened for common diseases of bovine including brucellosis, IBR, and BVD. In free range condition a prevalence of brucellosis was observed to be 13.33% (40 of 300). Sero-prevalence studies for IBR revealed a prevalence of 9.33% (28 of 300 animals) while sero-prevalence of BVD antibody was found to be 12.66% (38 of 300) in free mithun and that of BVD antigen was observed to be 1.33% (4 of 300) by using commercial ELISA Kits.

Cutaneous warts were found to be prevalent in 2.28 % (16 of 750) of Mithuns and BPV2 has been identified from cases of warts in mithun by PCR. The cutaneous warts have been diagnosed as fibropapilloma by Histopathological studies.

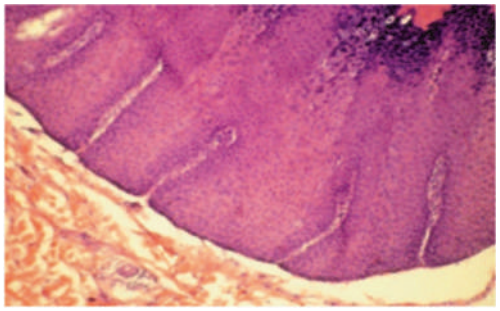


Figure. Endophytic Fibropapilloma

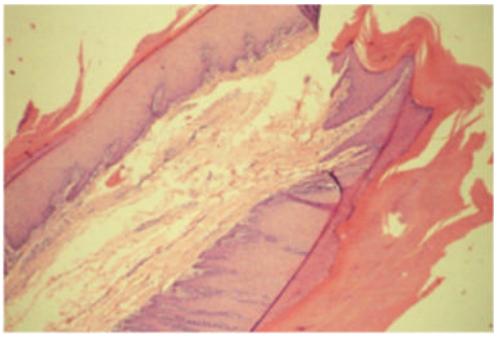


Figure. Rice grain Fibropapilloma

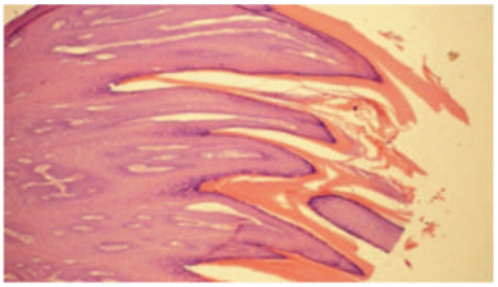


Figure. Exophytic Fibropapilloma

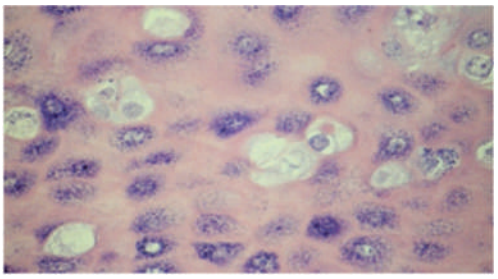


Figure. Keratohyaline granules and inclusion bodies

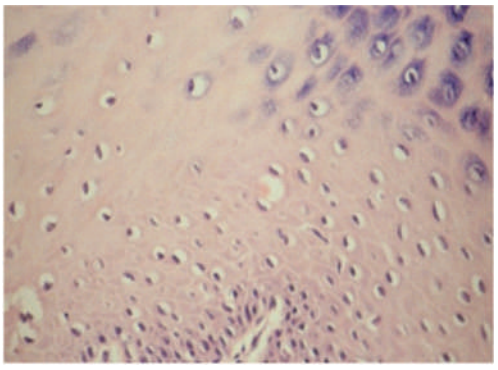
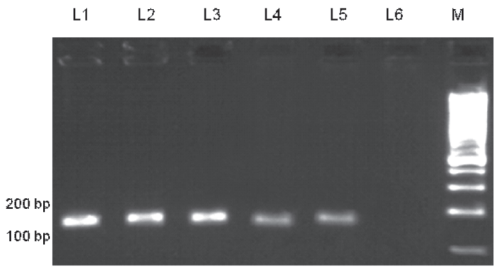


Figure. Koilocytes in papilloma



PCR assay of warts in mithun, M 100 bp marker

FMD outbreaks have been recorded in five different areas in Kohima, Dimapur and Phek districts of Nagaland.

Pulmonary tuberculosis was diagnosed in a mithun by necropsy examination and acid fast mycobacterium tuberculosis bacteria were observed identified and were DNA extracted from the caseated lung tissues were amplified by PCR. Naval ill was diagnosed in three numbers of mithun calves and E. coli were cultured, isolated and identified from all the three cases.

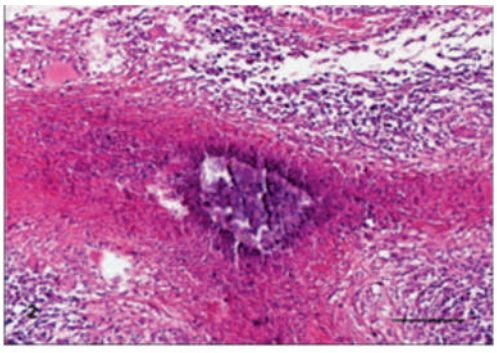
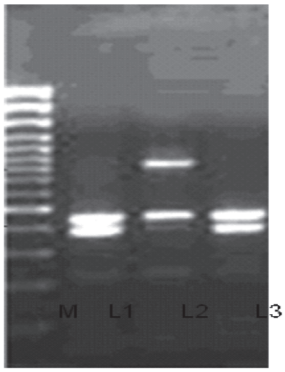


Figure. Bronchial LN: central area of caseous necrosis and mineralization, surrounded by multinucleated giant cells and lymphocytes



PCR assay of Tuberculous lungs. M:100 bp marker, L1-L3 tuberculous modules from lungs

Figure. Multiple drug resistant strains of Enterobacter agglomerans and Pseudomonas aeroginosa were isolated and identified from the eyes of swamp bufflaoes suffering with conjunctivitis. They were found to be sensitive to tetracycline, ciprofloxacin and amoxicillin but were resistant to ampicillin, gentiamicin, nitrofurantoin and cephalexin.