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Rural Industries Research and Development Corporation

Australian Water Buffalo

Genetic and reproduction improvements

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Genetic and Reproduction Improvements

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Foreword

Despite almost 190 years in Australia, a thriving hide shooting industry for 60 years and pet-meat production for many others, the water buffalo only became an established meat industry post 1960. Exports of meat and a thriving local trade which were quite significant for the Northern Territory peaked in 1987. The Brucellosis and Tuberculosis Eradication Campaign (BTEC) ultimately removed the majority of the feral buffalo population by 1992 and meat exports finally ceased with all export abattoirs closing in the Northern Territory.

In 1994 a new light appeared on the horizon in the form of imports into Australia of the River Buffalo from Europe and the US. For meat production, an increased growth rate of 40% over the existing Swamp breed was far too big to ignore, but the dual potential of milk production was also explored in Victoria and later in Queensland. Buffalo cheeses have now collected numerous awards in Cheese Shows in Australia from NT-derived and Victorian buffalo and also now in 2008 in New Zealand from dairy buffalo crosses from the NT.

The aim of this project was to try and help to accelerate industry progress for this very new enterprise direction by incorporating new and improved technologies and processes. This involved four strategies:

i. To test and recommend suitable AI synchronizing protocols over as many States as possible
ii. To set up a register and database to allow for objective-based selection in the meat and milk buffalo industries
iii. To analyse previously collected and current NT data to establish the genetic parameters for future selection within the industry
iv. To communicate and liaise with local and overseas experts in buffalo matters.

The importance of the research is to help bring the industry abreast with other Australian meat and dairy industries that have evolved in southern Australia over a time period of up to 4 times longer since European settlement began, by strengthening the genetic basis of the available gene pool currently present in the national buffalo herd. This could only be achieved by researching the available data in Australia, setting up a means of tracking animals, being able to identify animals of superior merit for meat and milk traits and providing the tools to create linkages in the buffalo population, to be able to multiply the best genes as quickly as possible. This necessitated being able to use the genetics of well established buffalo industries overseas, such as Italy for dairy buffalo via frozen semen from superior bulls.

Producers and potential producers should now eagerly embrace the technologies that have been available to the cattle industry for 20-30 years and use them to greatest advantage for the advancement of the buffalo industry.

This project was jointly funded by the RIRDC from industry revenue which is matched by funds provided by the Australian Government and the NT Department of Primary Industry, Fisheries and Mines. It is an addition to RIRDC's diverse range of over 1800 research publications and is part of our New Animal Products Program which aims to accelerate the development of viable new animal industries. Most of our publications are available for viewing, downloading or purchasing online through our website:

- downloads at www.rirdc.gov.au/fullreports/index.html
- purchases at www.rirdc.gov.au/eshop

Peter O'Brien
Managing Director
Rural Industries Research and Development Corporation

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He would also like to make known his appreciation of the steadfast, solid support and encouragement offered throughout the project by Dr Peter McInnes, the RIRDC Co-ordinator for the Buffalo Industry Program.

The Staff of the Beatrice Hill Farm are congratulated on their management of the Buffalo Herd and their ability to present the stock in good condition throughout the year and in all weathers, their persistence and co-operation with all aspects of the project.

The Author is thankful for the co-operation of 2 private producers in their permission to use their herds for the Artificial Insemination trials that were carried out on their properties and subsequent losses in production from the herd due to Bulls being excluded for 2 months prior to the program. The Haldane (Yambuk, Vic.) and Humphries (Millaa Millaa, N Qld) families are warmly thanked for their participation.

Abbreviations

ABIC	Australian Buffalo Industry Council
ABRI	Agricultural Business Research Institute (University of New England, NSW)
BCS	Body condition score
BHF	Beatrice Hill Farm (Humpty Doo NT 0836)
BTEC	Brucellosis and Tuberculosis Eradication Campaign
CIDR	Controlled Internal Drug Releasing Device
CL	Corpus luteum
CR	Conception rate
E_2	Oestradiol
ET	Embryo Transfer
eCG	Equine Chorionic Gonadotrophin
FSH	Follicle Stimulating Hormone
FTAI	Fixed Time Artificial Insemination
GnRH	Gonadotrophin Releasing Hormone
hCG	Human Chorionic Gonadotrophin
IBC	International Buffalo Congress
i/m	intra-muscular
LH	Luteinizing Hormone
MOET	Multiple Ovulation Embryo Transfer
NT	Northern Territory
NT DPIFM	Northern Territory (Government); Department of Primary Industry, Fisheries and Mines.
ODB	Oestradiol Benzoate
ODV	Oestradiol Valerate
P_4	Progesterone
PAI	Progesterone Auricular Implant
PD	Pregnancy diagnosis
PG	Prostoglandin
PGF2α	Prostoglandin F2alpha
PMSG	Pregnant Mare Serum Gonadotrophin
PRID	Progesterone Releasing Intra-uterine Device
WBC	World Buffalo Congress

Contents

Executive Summary

What the report is about

This report is the culmination of 5 years work carried out in 3 states of Australia for the Australian Buffalo Industry. It reports on the work done to try and bring the Buffalo Industry into line with other mainstream industries such as cattle. This is about being able to use the latest technologies available in order to bring about rapid genetic progress in an animal that has had mostly only feral slaughter or capture value in Australia since its introduction in the 1820s into the Northern Territory. As a farmed animal in Australia it has really only been 40 years in gestation, compared with other animal industries that have been well established over 120 years. Rapid expansion over all states of Australia has occurred over less than 20 years, hampered by and despite the latest long running drought. However a critical mass for long term industry survival has not yet been safely achieved.

Who is the report targeted at?

This report is targeted at mainly producers and potential producers of water buffalo in Australia. It attempts to show that the required background equipment has now been put in place that will allow the industry to become a lot more competitive - the ability now to be able to identify those superior animals objectively and make use of them for the good of both the meat and dairy sectors of the industry.

Background

The water buffalo is a hardy animal with great adaptive features for many locations around Australia for both meat and milk products. In recent years it has also shown live export potential with orders and supplies to New Zealand, South Africa, Chile and Japan for dairy heifers and bulls. Traditional meat and live breeders to SE Asian markets are still being supplied from the NT. After 150 years in Australia, domestication was finally commenced with an animal that had survived for so long under heavy shooting and climatic pressure in the north. In southern states the novelty value was probably high to start with, but then people realised the healthy value of the meat and after the Riverine buffalo importation in the 1990s, the dairy value of this new animal. Its ability to milk well off grass will stand it in good stead in the future as prices for concentrate feeds will inevitably soar. The popularity of buffalo cheeses has been acknowledged by Cheese judges at premier cheese shows in Australia and New Zealand, made by cheese-makers, often at the start, with only limited supplies and experience in both countries. All four new buffalo cheese-makers in Australia and NZ have won significant awards in their country, mostly as first time entrants. Whilst its popularity may never approach that of Mozzarella in its native country of Italy, it is certainly worth trying to emulate the role of the Italian dairy buffalo industry.

As buffalo have a very low base number in Australia, the industry needs to be vigilant for inbreeding. It needs to source animals that are most efficient in their business and make use of the best overseas genetics that are available. However until they know well what they already have, it is difficult for producers to make good investment decisions.

Hence the need for a comprehensive register and database that can track all buffalo in Australia, while the industry is still in its infancy, was paramount in importance. To be able to identify animals with superior traits for meat and dairy is essential to make good progress towards efficiency in the future.

Aims/Objectives

- Develop and test protocols for Artificial Insemination (AI) of buffalo cows that will give acceptable outcomes for the Industry. This needs to at least approach the 50% mark for a single insemination opportunity.
- Set up a suitable Register/Genetic Database for the Australian Buffalo Industry that will cater for its present and potential meat and dairy needs for the long term.
- Analyse the past and present genetic data generated from the NT DPIFM Beatrice Hill Farm recordings since 1983, when the decision was first made to bolster the research effort being made by the NT DPIFM on behalf of the industry.
- Maintain local and overseas industry contact through attendance at ABIC meetings and at international buffalo congresses

Methods used

- 12 rounds of AI were carried out in 3 States using 3 different protocols, with Ovsynch the most tested of the 3 protocols. There were many variations of timing of the inseminations, using both one and two at 0,and 12 or 24 hours. Finer Embryo Transfer pistolettes were used to endeavour to navigate the heifer cervix in particular. A DNA sample taken from the calf after birth, was used to determine timing of the successful conception by using different semen on consecutive inseminations. Fat measurements were taken at the start to make sure that the rump injection site was going to allow the needle to reach the muscle.
- ABRI was commissioned to create the register and database for the industry. The database has been populated so far with Beatrice Hill stock plus 2 herds from Queensland and 3 from Victoria.
- The data from the Beatrice Hill herd were tested against various models but was found not to have sufficient data with good progeny framework due to untimely interference of Tuberculosis causing total destruction of the herd and rebuilding from scratch with new animals of unknown parentage.

Results/Key findings

- The magic 50% success rate has remained elusive over the 4 years, however our knowledge of the processes is becoming more confident with time. A total of 67 successful inseminations occurred from a total of 248 which equates to a success rate of 27% overall. The best single achievement was 41.7%
- The register is now open for business and the database is ready and capable of calculating Estimated Breeding Values for the industry. Accuracy will improve as entries increase over time.
- The total database does not have enough total progeny yet with known parents to be able to estimate genetic heritabilities of the various traits with any accuracy. Producers need to be encouraged to supply data for their stock to speed up the rate of increase in the database population. Within the short term a lot more production information will become available from this dataset
- Both the 7th and 8th World Buffalo Congress were attended, including a pre-Congress cheesemaking course and post Congress tour of the Italian dairy buffalo industry. Updates and regular Buffalo News articles were contributed.

Implications for relevant stakeholders for:

Industry:

Whilst AI procedures are still not perfect, a lot of useful knowledge has been gleaned from experts in the field and from the trial work carried out. There is sufficient confidence to believe that there is now enough built-up knowledge to have good control over outcomes to be able to achieve benchmark conception rates within the short term. This will allow breeders to employ AI now to upgrade their herds with reasonable confidence of success.

The Buffalo Database also has the potential to make Australia a world leader in genetic expertise particularly in meat production if producers run with the now available technology.

Communities

There is a possible future for buffalo in dairy communities where deregulation has bitten hard. Buffalo may be a more efficient producer of protein and fat than dairy cattle under cost-price squeeze situations where concentrates will become increasingly expensive and more difficult to afford. Buffalo would be able to be substituted into an existing dairy enterprise with very little modification required to facilities or infrastructure. Present experience indicates prices for buffalo milk to be 3-6 times that available to whole cow milk producers.

There is no reason that other countries cannot make use of this resource that has been created to pool information and create a more powerful database for overseas buffalo producers. There is potential to bring in countries such as ones to which Australia has recently exported and to include the US, Canada, and any countries that may like to pool their data in the common interest of genetic improvement for beef and dairy. We obviously have a long way to go to get to the current Italian milk recording and genetic database that they have developed for the buffalo dairy industry. The widespread use of Italian dairy semen by many countries would provide good genetic linkages.

Policy makers and others

In the NT there needs to be further support for the farmed industry to target better markets that reflect the potential value of the farmed product into the future. There needs to be clear distinction between the farmed and feral product for overseas meat markets that reflect the costs of production for both sectors

Recommendations

i. That producers take full advantage of the systems that are now on offer to genetically improve their industry for the better. This Breedplan technology is a powerful tool in helping them improve their herds and make them more marketable around the world. Our position as a very low disease risk exporter is recognised world-wide

ii. That potential producers think seriously of the water buffalo as a suitable animal for a livestock industry for many parts of Australia, but particularly in current dairy districts, where the infrastucture in dairies and cheese factories is already present.

iii. That for dairying in the tropics, the water buffalo has already proved its potential, and should be encouraged.

1. Introduction

Buffalo were introduced into Australia in the early 1800s from Indonesia as supplies for various early English settlements that came and went in the Top End of the Northern Territory. Animals escaped captivity or were left behind when settlements were abandoned. Swamp buffalo were well suited to the environment of the Top End floodplains and over 150 years built up their population to around 350 000 head at their peak in the 1980s. Subsequently the national Brucellosis and Tuberculosis Eradication Campaign (BTEC) eliminated the bulk of the feral population, leaving 15 000 on farms and 30-50 000 in monitored-free areas of the Top End, mainly in Arnhem Land.

Early utilisation of the species was for hides especially for export, and used for industrial belting from the 1880s to the Second World War. They were then superseded by synthetic materials for this purpose.

Some overseas live exports began in 1958, followed by a lucrative European Community market for frozen meat to be used in the smallgoods trade, as well as harvesting for the domestic meat trade and pet food industry. Domestication commenced around 1970 in the NT, and built up slowly on pastoral properties mainly run as feral harvest operations. The BTEC program required all buffalo to be tested fully for these diseases. Numbers dropped dramatically as mentioned previously. The best market became live export for slaughter to Brunei where buffalo commanded a 10% premium over beef. Between 1994 -1997, importations of Riverine Buffalo (dairy breeds) were made, consisting of 8 head from the United States to the NT, 38 head from Italy and 28 head from Bulgaria to Victoria. The latter 2 were for a private dairy operation, whilst the former was mainly for meat production by crossbreeding with the local Swamp buffalo.

Crossbreeding gave better meat carcases, grew 40% faster, dressed out and yielded at higher percentages than the local swamp animal. Interest also became stronger for dairy production and 2 more dairies were established, both in Queensland where buffalo had previously (officially) been prohibited entry into the State. Buffalo are now in all states of Australia, and have been exported in the last 3 years to New Zealand, South Africa, Chile and Japan, as well as traditional south-east Asian markets. Previously export swamp heifers have gone as far as Nigeria, Cuba and SE Asia in large numbers.

Peak live exports of 7748 head were achieved in 2006 from the NT. Drought in southern Australia had been the main dampener for the expansion of the industry, but also the number of abattoirs willing to slaughter buffalo is diminishing. TenderBuff has been popular in Darwin over a period of 15 years, but the closure of the local abattoir has curtailed production since April 2007. The local market consumed 100-200 head per year at a price that was suitable to encourage local development of pasture-fed animals for local meat consumption. If that rate of consumption was replicated in the main population centres over the rest of Australia, there would be no problem selling 100% of the product that the industry can produce for the foreseeable future.

Buffalo cheese from the 3 dairies and recently also New Zealand, has won many awards at important cheese shows, usually as first time entrants, indicating the popularity of buffalo cheese products amongst judges and the public. The popularity of Mozzarella in Italy is also an example of the strength of a possible dairy industry in the future, from an animal that will however milk successfully off grass pasture without the need for concentrate-based rations. The appeal of buffalo milk to cheese manufacturers is the recovery rate of 5:1 instead of the 10:1 rate for cow milk, the additional product range and the opportunity to blend milks to make different cheese varieties and flavours.

The objectives for this project arose from the need to develop a reliable Artificial Insemination (AI) protocol, to be able to use overseas semen as a means of expanding the genetic base of the industry in Australia. Because only 4 bulls were brought from the United States, and the increasing interest in dairying, there was a need to successfully introduce new genetics from overseas.

Thus testing was specifically required to ensure that experience was obtained at consistently getting the best results possible, from high cost semen from the best available sources. Coupled with this was the need, while the industry was small, to be able to effectively track all the buffalo available in Australia, to be able to estimate their current genetic value for different products in the long term and quickly identify those genes that contributed most to the profitability. A ‘Buffalo Breedplan’ was essential to be put in place as early as possible into the buffalo industry, so that objective measurement of traits of significance to the producer and customer could be identified and used. Because of the small population of buffalo in Australia, historical data from swamp buffalo reared under good management was vital, so Beatrice Hill data from the NT DPIFM herd was needed to be entered, verified and submitted to ABRI for analysis of genetic parameters and relationships. This was a mammoth undertaking that yielded a total of 1821 head with 22 136 individual field records containing variable numbers of condition scores, live-weights, pregnancy recordings, fat measurements, lactation status, and carcase measurements for 14 years of TenderBuff, plus some cull cow and bull turnoff.

Previous RIRDC funded collaborative work by the Victorian Dept of Agriculture with NT DPIFM did a limited amount of AI trial work plus some trial work on MOET, which involved AI. They achieved a 2 out of 6 cows (33%) result in southern Australia using CIDR’s for 8 days followed by prostaglandin at CIDR removal, and oestradiol 24 hours later, with a blanket AI on day 11 in one trial, while in the MOET studies, similar protocols were used, except that the best embryo yield was using a Deslorelin implant instead of a CIDR. As there were only 3 animals per group, there needed to be further trial work to demonstrate the effectiveness of various protocols, using larger numbers of animals in a larger range of localities or regions.

Extensive Brazilian reproduction research indicated that the ‘Ovsynch’ protocol was successfully used in that country to get around a 50% pregnancy rate or better, in one round of insemination in the breeding season and the technology has been adopted by many other countries as well.

2. Objectives

There were multiple objectives for this project with the aim of increasing the understanding of the species and thereby the profitability of the Australian Buffalo Industry. Because industry expansion has been relatively recent, there was an opportunity to develop platforms that will speed its expansion using new, adapted and improved technologies:

- Develop and test protocols for Artificial Insemination of buffalo cows that will give acceptable outcomes for the Industry. This needs to at least approach the 50% mark for a single insemination opportunity.
- Set up a suitable Register/Genetic Database for the Australian Buffalo Industry that will cater for its present and potential meat and dairy needs for the long term.
- Analyse the past and present genetic data generated from the DPIFM Beatrice Hill Farm recordings since 1983, when the decision was first made to bolster the research effort being made by the NT Government on behalf of the industry.
- Maintain local and overseas industry contact through attendance at ABIC meetings and at international buffalo congresses

3. Methodology

3.1. Develop and Test Artificial Insemination protocols.

- Commencing with a Literature Review, the options for best AI outcomes were investigated world-wide to see which protocols were the most used and likely scenarios that could be adopted here in Australia.

- A series of Rounds of AI were commenced that covered the following dates;

Round No.	Date	Total Stock	No of Inseminations	Location
1	5-15/3/04	23 Ovsynch	AM and PM	BHF
2	20-30/7/04	25 Ovsynch	12-AM and PM 13-AM only	BHF
3	27/7-6/8/04	16 Ovsynch	9 -AM and PM 7-AM only	BHF
4	8-18/2/05	17 Ovsynch	AM and PM day 10	BHF
5	19-29/4/05	12 Ovsynch	AM and PM day 10	BHF
6	12-22/11/05	17 Ovsynch	AM and PM day 10	BHF
7	22/3-4/3/06	20 Ovsynch	AM day 10 only	Yambuk, Vic
8	4-14&15&16/4/06	19 Ovsynch 6 Cue-Mate	AM day 10 and AM day 11	BHF
9	28/6-8/7/06	25 Ovsynch	AM day 10 and AM day 11	BHF
10	12-22/2/07	5 Ovsynch	AM day 10 and AM day 11	BHF
11	4/6/07	26 Ovsynch 10 CIDR	AM day 10 only	Millaa Millaa, N Qld.
12	11-21/1/08	26 Ovsynch	AM day 10 only	BHF
Total		248		

Of the synchronising protocols used, ‘Ovsynch’ was the most used. This procedure is;

1 Inject 5ml i/m GnRH at 4 pm on day 0
2. Inject 2ml i/m Prostoglandin at 4 pm on day 7
3. Inject 2.5ml i/m GnRH at 4 pm on day 9
4. Inseminate 8am day 10.

3.2 Develop and Implement a Register / Database system for the Buffalo Industry

A consultant (Dr Brian McGuirk) was engaged to recommend a suitable Registry system that could be implemented to take the industry forward. The consultant contacted many cattle breed societies around the world to enquire as to the best systems available currently. His report is in Appendix 1.

ABRI was chosen to fulfil this role because of their worldwide experience in registration of animal breeds, animal genetics and the development of Breedplan, which is being adopted throughout the world for cattle. Buffalo have been accepted into the system by the Cattle Industry, for which the buffalo industry is very grateful; to be able to pick up on the experience and knowledge accumulated over 40 years without having to re-invent the 'wheel'.

ABRI agreed to carry out the following tasks;

- Estimate a set of genetic parameters for the historical Swamp data set (1983-1998)
- Set up a breed register/database to incorporate both meat and dairy attributes. This was originally to be set up using NT BIC facilities as a focal point but circumstances have changed in the meantime and so DPIFM will provide the 1st point of entry service from the NT at this stage to all Australian producers. A Secure direct access via the Web to the Buffalo Database at ABRI from a desktop PC in Darwin has been initiated. Training in its usage has been carried out.
- Internet registration facility
- Parameter estimation for recent data (Riverine and crossbreeds)
- Provide Breedplan facilities within the Register/Buffalo Database system.

The Register is one feature of the Buffalo Database that has been created by ABRI and populated from NT, Victorian (2) and Queensland (2) herds so far. A linkage via the 'Web' has been set up from the author's office, to the database at ABRI for entry of new animals onto the system and to be able to view or amend all records. This data entry can be achieved via 3 methods

- Single animal entry from small herds manually via the Secure Link to the ABRI database, from a hand written pro-forma printed off from an Excel spreadsheet and posted to the Buffalo Register
- A computer based Excel Spreadsheet saved as a text file and emailed to the 'Buffalo Register' for checking and if large enough, then submitted to ABRI for direct entry
- Files sent from the ABRI established Herdmaster® software program owned by the buffalo breeder or other compatible software, via email, direct to ABRI for uploading.

There will be a per calf charge for entry into the register from 2008 progeny onwards payable to ABRI for the maintenance of the Database. Breeders will be able to avail themselves of Breedplan services on a per trait charge for those buffalo that are registered on the database

A web-based copy of the database will be available for scrutiny by breeders wishing to look at what animals exist in the Buffalo Database. This copy is updated from the Buffalo Database weekly and is similar to other breed society on-line registers.

3.3 Analyse existing data generated by various herds on Beatrice Hill Farm since 1983

The data has been subject to genetic analysis and is reported in Appendix 2.
The herd is in two distinct phases with some overlap due to the arrival in 1994 of the first 2 riverine bulls.

1. Swamp herd with selection for growth rate and culling for reproduction from 1983 to the closure of the herd in 1998. Mating ceased in 1996 due to a detection of an outbreak of TB.
2. Riverine and crossbreeding herd which produced the first F1s in 1995 and has been continued until the present. In 2008, the first calves were born via the crossbreeding and backcrossing pathway. The first River bulls were imported from USA and arrived in 1994. Purebred heifers arrived over the next 3 years and have bred up to currently number around 90 head, with some dispersed to other states, (Qld and Vic). The 4 original imported heifers are all still productive dams and 2 of the 4 bulls are still being used today. (1 died and the other slaughtered due to exposure to TB infected cows).

3.4 Maintain communication and contact with the buffalo industries in Australia and overseas

Each ABIC meeting in Australia was attended by the author with progress reports for this project.
Regular articles were submitted to the ABIC "Buffalo News".
Both the 7th and 8th World Buffalo Congresses were attended in the Philippines and Italy respectively.
The author delivered a paper to the 7th WBC titled "Production of Specialised Quality Meat Products from Water Buffalo: TenderBuff."(Lemcke 2004) See Appendix 3 for Report of visit.
In Italy a Pre-Congress Cheesemaking Course (Salerno) was attended, the 8th World Buffalo Congress proper (Caserta), and a comprehensive post-Congress tour of the Italian Buffalo Industry was enjoyed. (Salerno to Milan). See Appendix 4 for Report on all 3 aspects of the event.

4. Buffalo artificial insemination – Literature Review

4.1 Literature Review; Buffalo Artificial Insemination Project.

There are numerous world wide studies on artificial insemination in buffalo but results vary widely and buffalo have proven more difficult than cattle, to achieve good rates of conception. The factors in achieving high conception rates to AI are multiple and include the need for;

- qood quality frozen semen
- good cow condition to reduce the likelihood of anoestrus
- good handling facilities
- rising plane of nutrition
- favourable weather conditions
- stock with quiet temperament, and stock people with good handling skills
- good heat detection capability
- appropriate synchronizing protocols
- skilled and experienced (quick) inseminators to reduce stress.
- timing of AI in relation to ovulation.

The literature tends not to mention, or monitor some of these factors very closely, but in reality they can be very important, and are possibly why results differ very widely.

4.2 Buffalo Reproduction

4.2.1 Buffalo Oestrus Cycle and Phases

The 4 phases of the cycle are similar to cattle

- Pro-oestrus – lasting 2-3 days, the ovaries are quite active with FSH stimulating the growth of follicles and the rising levels of oestrogen, swelling of the vulva and maybe the start of mucus secretion
- Oestrus – this is the heat period where the animal is restless, urinates frequently and plenty of clear mucus is produced, but it remains generally trapped in the vagina until palpated. Internal and external genitalia swell (giving the uterus its palpable ‘tone’ and ovulation occurs usually 5-24 hours after the cessation of standing heat. In the later stages increasing LH and decreasing FSH causes ovulation and the formation of a corpus luteum (CL) in the ovary
- Metoestrus – lasting 3-4 days with sharply declining levels of oestrogen, swelling of vulva and uterus declining, mucus production becoming cloudy and thinner and eventually ceasing.
- Dioestrus – during this phase the CL produces high levels of progesterone (P_4) which remains if implantation occurs and continues throughout pregnancy. With no fertilization of the egg, the dioestrus period will last for 10-20 days, the CL will regress and P_4 levels will decline markedly and the cycle will be repeated.

The 4 phases can be broadly grouped into 2, the follicular phase (oestrogen influenced) and the luteal phase (progesterone influenced)

4.2.2 Many studies have been done on both Swamp and Riverine buffalo reproduction.

McCool et al (1989) in the NT studied swamp buffalo and found an oestrus cycle length of 18-24 days, the duration of oestrus 11-23 hours and ovulation occurring 12-24 hours after the onset of oestrus.

Jainudeen in Malaysia recorded a mean cycle length of 21 days, 18-21 hours in oestrus and ovulation 15-18 hours after the end of oestrus.

Perhaps the most rigorous reproductive studies have occurred in Brazil over the last 18 years, where many studies on large numbers of buffalo have been reviewed on the website of the University of San Paulo. (available on www2.fmvz.usp.br/bufalos/ . They have used radiotelemetry to closely study oestrus cycles and endocrinology/ultrasonography to study follicular dynamics. Below is a listing of significant findings in oestrus detection induced by prostaglandin (Porto-Filho, 2000);

- Oestrus duration in heifers – 11.8 ± 5.6 hours (Range 1-25 hours)
- Start of oestrus to ovulation – 30.0 ± 4.9 hours
- End of oestrus to ovulation – 17.9 ±4.1 hours
- Av no. of mountings per oestrus – 24.6 ± 18.2
- Duration of the mounting – 3.6 ± 0.7secs
- Total mounting duration – 94.2 ± 80.7secs
- PGF to LH surge – 45.6 ± 10.8 hours
- Oestrus onset to LH surge – 3.6 ± 3.4 hours
- LH surge to ovulation – 25.6 ± 2.6 hours

Other Brazilian research provides additional data.

- Oestrus duration in cows – 14.7 ± 7.3 hours Range 6-48 hours
- Ovulation – 16.9 ± 6.5 hours (Baruselli 1994)

Follicular waves occur during the cycle as synchronized development of a follicle group in a certain period. The first starts 3-4 days after start of the cycle until day 11. The second wave starts on day 12 till the end of the cycle. There may be 2-3 waves during a cycle. 2 wave cycles are the most common. Cows that exhibit 3 waves/cycle generally exhibit longer luteal phases; ie 10.4 ±2.11 vs. 12.66 ± 2.91 days ($P<0.05$), inter-ovulatory interval; 22.27 ±0.89 vs. 24.5 ±1.88 days ($P<0.01$) and oestrus cycle 21.84 ± 1.01 vs. 24.00 ± 2.21 days ($P<0.01$) (Baruselli 1997) for 2 and 3 wave cycles respectively.

Buffalo heat detection is more difficult than in cattle due to lesser homosexual mounting activity displayed amongst groups of buffalo (Kanai and Shimizu, 1983; Hill et al, 1992 [6.3% in heifers]) and fewer external signs of heat. Vasectomised or deviated bulls are more reliable indicators for detecting cows in oestrus. Chin-ball harnesses are effective markers if close observations are not practical under current conditions (Hill et al, 1992). Baruselli (1992) however maintains that traditional twice daily observations on herd activity will only miss a very small percentage (3.5%) of heats.

4.2.3 Seasonality of Buffalo Reproduction

Seasonality in buffalo is dependent on location in the world. In the tropics the buffalo is a poly-oestrus continuous breeder. Where buffalo are produced further north or south of the tropics of Cancer and Capricorn, then the more seasonal is the breeding pattern. Baruselli et al, (2007) state that responses to treatments for AI, (when done in the off-breeding season), are dependent upon factors affecting the depth of anoestrus ie; seasonality (distance from the equator), body condition, age and interval from calving. See Figure 1.

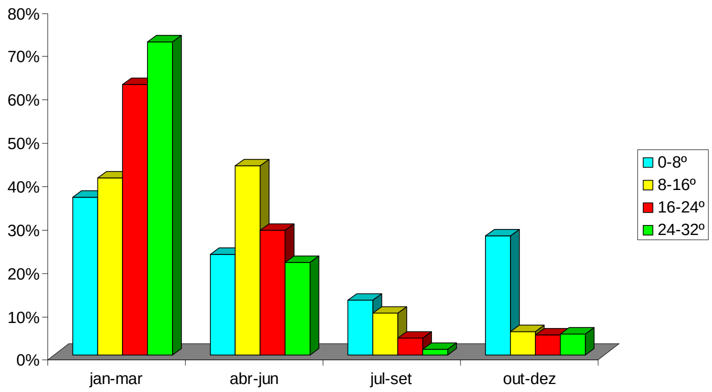


Figure 1: Buffalo calving distribution throughout the year according to the latitude (n=16,487). Brazil, 2001. (Baruselli 2007)

In Pakistan, Chohan et al (1998) injected sub-oestrus Nili-Ravi cows with PG (2ml Cloprostenol) in the peak breeding season Sept-Feb and Mar-Oct (low breeding season) and again after 11 days if no oestrus after the 1st injection. AI was done at 12 and 22 hours after onset of oestrus. After the first injection, 26/30 and 21/35 showed oestrus for peak and low seasons respectively. All showed oestrus after the second injection, and pregnancies resulting were 16/30 (53%) and 8/35 (23%) for the peak and low seasons.

Putu et al (1986) recorded ovarian activity, oestrus and conception in 75 **swamp** buffalo after oestrus synchronization in 2 seasons and at 2 levels of nutrition (ad lib grass plus 1 or 5kg of concentrate per day). They found that 95% of cyclic cows were detected in oestrus, and 91% of oestrus cows conceived during service periods (1 return only after a single synchronised oestrus). They found no significant effect of season or level of nutrition on oestrus or conception in cyclic cows. The big effect was the level of nutrition on the percentage of acyclic cows after treatment (35% vs 3% of cows at low vs high nutrition levels.

4.2.4 Body Condition

Baruselli et al (1995) showed that body condition is a big factor in conception rates to AI as seen in table below;

Condition Score	**<2.5(low)**	**3.0 and 3.5 (Mod)**	**>4.0 (high)**
Number of buffalo	42	253	181
First postpartum oestrus	76.6 ± 24.4 [a]	64.6 ± 27.2 [b]	49.3 ±19.4 [c]
Incidence of anoestrus (%)	42.8 [a]	15.4 [b]	3.7 [c]
AI services per conception	2.72 ±1.12 [a]	1.89 ± 0.68 [b]	1.78 ± 0.89 [b]
Interval calving to pregnancy (days)	112.7 [a]	88.1 ± 28.6 [b]	75.1 ± 25.1 [c]
Conception rate to AI (%)	38.1 [a]	74.3 [b]	82.3 [c]
Conception rate to AI plus natural mating (%)	42.8 [a]	80.2 [b]	87.8 [c]

Table: Effect of body condition score at calving on reproductive efficiency in buffalo

Putu et al (1986) explored the role of nutrition in ovarian activity, oestrus and conception in 75 swamp buffalo over 2 seasons (wet and dry) and at 2 levels of nutrition (grass ad lib plus 1 or 5 kg of concentrates per head per day). Over all treatment groups, 95% of cyclic cows were detected, and 91% of oestrus cows conceived during service periods, which allowed a single return to service after synchronized oestrus. Season of mating had no affect on any aspect of reproduction after feeding regimes were put in place and level of nutrition had no effect on oestrus or conception in cycling cows. The big difference was the acyclic cow rate that was 35% compared with 3% for the low versus high feeding levels.

4.2.5 Age

Baruselli et al (2007) demonstrated that the pregnancy rate for primiparous cows (heifers) compared with pluriparous cows to fixed time insemination was 35.5% vs 51.0%.

4.2.6 Timing of Insemination

Vale et al (1991) suggest for visual detection systems the best time for insemination is the first rejection of a teaser bull from mounting a cow that has previously been in standing heat.

4.2.7 Post Calving Anoestrus

Shah et al (1990) and some other authors have shown that by giving a 14 day post partum cow an injection of GnRH (100µg & 250µg both used) resulted in quicker completion of uterine involution, earlier resumption of ovarian activity, shorter calving to conception intervals and a better 1st service conception rate in lactating dairy buffalo (non-suckled) compared with saline injected controls. There was no significant difference between the 2 dose rates.

4.3 Buffalo Synchronization Protocols

4.3.1 Prostoglandins

Prostoglandins were probably the first protocols that were widely used in Buffalo. It is possible to use a single dosage, but more often 2 doses are injected at 12 day intervals. There are many different brands available on the market and most are used at a dose rate of 2ml i/m to cause luteolysis of a functional corpus luteum and bring on the onset of oestrus within 2-4 days. On a single dose those animals that do not have a functioning CL will not respond to treatment ie those that have recently been on heat. Hence the second dose at 11-14 day intervals ensures all animals are fully synchronized.

Baruselli (1994a) found that there was a great variation in the duration of oestrus manifestation (36-96 hours) after the administration of prostaglandin. This was partly explained by trial work by Porto Filho et al (1999) where observation by radiotelemetry on cows injected before and after day 10 of their oestrus cycle was carried out. Cows treated before day 10 showed significantly shorter oestrus and ovulation intervals. (40.4 ±2.1 vs. 56.3 ±3.3 hours for PG to onset of oestrus and 70.0 ± 11.3 vs. 87.2 ± 12.9 hours from PG to ovulation; both $P<0.01$)

Perera et al 1977 carried out 2 trials with buffalo heifers giving 2 injections, 11 days apart, using natural mating Trial 1 and AI (Trial 2) at 72 and 96 hours after the 2nd injection(fresh semen) and got pregnancy rates of 33% to natural mating and 30% to the AI. Difficulties were encountered in getting semen into the uterus in 7 out of the 12 heifers in the AI trial. 2 of the 3 conceptions to AI were uterine depositions of the semen.

Khattab et al (1996) compared 4 different analogues of prostaglandin on Egyptian buffalo and found significant variation in response and conception rates over a 5 year period. Cows showing no response to the first injection were given a second 11 days later. Enzaprost® gave the lowest results for oestrus exhibition (57% response to the 1st injection compared to 73.2%, 70.7% and 79.5% for Lutalyse®, Prosolvin® and Estrumate®). Estrumate® gave the highest conception rate of 72.9%, 69.0% and 70.7% for Enzaprost® and Prosolvin® with Lutalyse® the lowest at 41.5%. Between seasons there was no difference in percentages showing oestrus, but conception rate was much lower in summer (39.1%) than winter (64.0%).

Honnappagal et al (1991) tested 3 levels of PGF2α at 25, 50 or 100µg delivered on day 10 of the oestrus cycle. The interval to oestrus from the injection was respectively 67.25% ± 3.8, 65.8% ±3.3 and 57.5% ± 4.2 hours, and the conception rates were 14.2, 12.5 and 25% respectively following 2 inseminations.

Jindal et al (1988) found that after palpation of a mature CL, 90% of lactating cows and 100% of heifers showed oestrus after a single injection of PG, while 80% randomly cycling, lactating cows and 67.7% of heifers showed oestrus after 2 injections at 11 day intervals. Oestrus occurred at 72-96 hrs after injection time.

Ibrahim (1987) compared 5 PG analogues administered during the luteal phase of the oestrus cycle to 300 cows. Animals which showed oestrus within 2-5 days were AI'ed once; those treated a second time 11days later were AI'ed twice at 48-72 hours after the 2nd treatment. See table below

Brand	Dose rate	% cows showing oestrus after 1 injection	Conception Rate %
Enzoprost®	2.5 ml	44	53
Estrumate®	2 ml	37	65
Lutalyse®	5 ml	25	56
Prosolvin®	2 ml	53	70
Prolan®	8 ml	95	68

Table: Oestrus and Conception rates after PG injection of different products

El-Menoufy et al (1986) split 50 lactating Egyptian buffalo into 2 groups and gave group 1, two injections of PGF 11 days apart and the control group no treatment. Group 1 (92% exhibited oestrus) were joined with bulls for 4 days after the 2nd injection, while the group 2 cows were joined for 22 days. There was no significant difference in pregnancy rate after 60 days from the joining period of 52% versus 48% for the controls.

Bachlaus et al (1979) synchronized 9 heifers with 2 doses of PG, 12 days apart. The average time from the second injection to oestrus onset was 3.00 ± 0.26 days. 8 of the heifers had their cycles synchronized, 6 ovulated and 4 conceived. 6 of the heifers appeared to synchronize on the first injection according to their hormone profiles.

Several references allude to cost savings in prostaglandin by using an alternative route for injection. At this site, 75µg is injected into the intra-vulval submucous membrane. Work by del Rei et al (2002) showed that this method halves the effective dose needed and found that 25% (n=59) of lactating cows responded to the 1st dose and 42.4% to the 2nd dose 12 days later. The best response was cows in the 120-149 days (83.3%) post calving period of the 30-239 days used. Chohan (1998) reported similar work earlier using Estrumate® at 25% of the i/m dose in the intra-vulval submucosal membrane.

Situmorang (1997-Indonesia) improved the efficiency of PGF2α by giving an extra injection of 500 IU of hCG 48 hrs after the 2nd PGF2α injection in grazing swamp buffalo (n=34). The results are in the following table;

	Oestrus by 48 hr after PG2nd	Oestrus by 72 hr after PG2nd	Preg Rate of good condition buffalo
- hCG n=16	68.8%	81.3%	50.0%
+hCG n=18	83.3%	100%	86.6%

Table: Effects of augmentation of PG with hCG at 48 hrs.

Synchronization of oestrus was significantly better in buffalo in good condition than those in poor condition.

Chohan in Pakistan (1993) with sub-oestrus Nili-Ravi were treated with 500µg of PGF2α in peak and low breeding seasons. If the cow did not come on heat, it was treated again 11 days later. AI was done at 12 and 22 hrs post onset of oestrus. See table below,

	Showed oestrus after 1 injection	Oestrus after 2nd injection	Pregnancies
Peak Breeding season	26/30	30/30	16
Low Breeding season	21/35	35/35	8

Table: Seasonal effects on reaction to PG administration

4.3.2 PRIDs

The most commonly used Prids are CIDR's in many countries. A new variant is available in Australia using the same hormone but a different design and is sold as Cue Mate® (Bioniche P/L)
Hill et al (1992) using imported Australian swamp buffalo heifers used CIDR's to synchronise oestrus and found that from PRID removal to onset of mating with bulls was a mean of 50.3 hrs, but the range was quite variable from 31-70 hours. Standing mounts for the bull occurred for 19.3 ± 5.1 hrs and resulted in 62% of heifers mated. Tailpaint was not useful because of premature removal and CIDR's were lost from 38% of heifers (and replaced) until tails were removed from the device. The protocol used was 12 days to CIDR removal with a 150 IU dose of PMSG at removal. 10mg of ODB was injected at CIDR insertion. Pregnancy rate was not reported.

In Brazil, (Vale, 2007) PRIDs are the basis of recommended protocols for out-of-season AI where anoestrus is common particularly when buffalo are raised further from the equator. The most recent recommendation is the following protocol;

- Day 0 at 6pm - PRID insertion plus 2mg ODB
- Day 9.at 6pm – PRID removal plus2 ml PGF2α, 400IU eCG
- Day 11 at 6 pm - 10µg GnRH
- Day 12 at 8am – FTAI

Rajamahendran et al (1979) in Sri Lanka treated 18 head of buffalo in the luteal phase of their cycle with a single injection of PGF2α after which 3 exhibited oestrus and 2 became pregnant to natural mating. The second trial had 16 head randomly chosen for ovarian activity, all injected with oestradiol 17β at PRID insertion. After 12 days the PRID was removed and 13 cows retained the device for the full period. Of the 13, 10 displayed oestrus between the 4th and 5th day after removal (mean of 102 ± 10 hours), of which 8 became pregnant to the bull joining when observed in oestrus. This gave a 50% pregnancy response to PRID protocol and only 11% for the single PG injection.
De Santis et al (2004) compared Ovsynch and Prids plus PMSG in Italy in the non-breeding season (Feb-May) on cycling and non-cycling heifers and mixed parity cows. Cycling groups CHE (heifers) and CMP (mixed parity) were given Ovsynch and non-cycling group NCMP given PRID + PMSG. Conception rates were CHE, 11/30 (36.6%), CMP, 6/14 (42.8%) and NCMP, 12/17 (70.5%). The PRID protocol was a 9 day implant with a day 7 injection of 1000IU of PMSG and AI at 72 and 96 hours after PRID removal.

R Züge et al (2004) used PRID protocol in mid-summer (out of season) on 6 heifers and 3 cows using

- Day 0 - PRID 1 g of progesterone plus 10 mg ODB
- Day 9 – Remove PRID, 150µg Cloprostenol and 2500IU of eCG
- Day 11 – 1500 IU hCG
- Day 12 – AI once only

The results were 4/6 heifers and 3/3 cows pregnant.
Vale (2004) suggests a slightly different recipe to that above with all treatments carried out at 1800 on day 0, day 9. day 11 and FTAI on day 12 at 0800 using only 500 IU of eCG and 1500 IU of hCG
Bartolomeu et al (Brazil 2002) reported poor efficiency with both Crestar (with ODV) and PRID protocols out of the breeding season.

Neglia et al (Italy 2003) compared PRIDs (Group A, n=111) (+ 10 mg ODB capsule for 10 days then PG +PMSG 1000IU at removal, AI at 60 and 84 hours post PG injection), and Ovsynch (Group B, n=117) (100µg GnRH day 0, 375µg PG day 7, 100µg GnRH day 9, with AI at 18 and 42 hrs after 2nd GnRH injection.) Pregnancy rates were not significantly different between A&B at 36% vs.28.2%. Ovsynch was more efficient in the cycling buffalo, where PRIDs were more useful in non-cycling buffalo.
Rensis (Italy 2001) achieved oestrus in about 50% of buffalo cows with PGF2α, with an interval of 2-5 days. Ovsynch + FTAI gave a pregnancy rate of 30-40% during the breeding season (not effective out of breeding season) and PRID protocol (P4 +ODB +PMSG.+ PGF2α) gave 40% in season and less than 30% in non-breeding season.

PRID implants were applied for 10days by Barile et al (1997) to 62 cows during the low breeding season. 39 were naturally mated and 23 were FTAI at 48, 72 and 96 hours from PRID removal. Conception rate to the induced oestrus was 21% and up to 56.5% up to the 3rd oestrus. To AI the induced conception rate was 34.8%.

Bhosrekar et al 1994 compared 3 different protocols on a large number of female buffalo (n=403) given 2 inseminations; See table below

	Group A	**Group B**	**Group C**
Protocol	2 x PGF2α 11 days apart	PRID	Synchromate B + PMSG ear implant.
Showed visual oestrus signs	72.4%	82.6%	82.5%
Rectal Palpation of oestrus	94.4%	98.1%	97.6%
CR non-cycling (summer)	29.3%	34.0%	31.1%
CR cycling summer	25.8%	32.5%	38.1%
CR non-cycling rainy season	50.5%	57.0%	55.0%
CR cycling rainy season	52.1%	68.7%	61.3%
CR non-cycling winter	42.3%	54.3%	50.5%
CR cycling winter	57.6%	39.7%	42.9%

Table: Comparison of cow and seasonal effects on conception rates for 3 protocols

Conception rate was significantly affected by season of insemination and body condition of the cow. The mean interval from insemination to ovulation was 34.86 hours.

Feng JC et al in China compared PRIDS with progesterone implants plus 1000IU PMSG after removal at 12 days. See table below,

	PRID (post removal)	**P4 Implant + 1000 IU PMSG**
Number of Cows	61	59
Crystalline cervical mucus	68 ± 9.1 h	60 ± 11.2 h
Accept mounting by male	75.5 ± 12.6 h	80.1 ± 13.3 h
Follicular growth	93 ± 17.9 h	90 ± 18.1 h
Ovulated	114.3 ± 29.2 h	115.9 ± 18.6 h
Inseminated at	105 ± 19.7 h	109 ± 20.1 h
Inseminated No.	53	50
Calves from single insemination	15	10
Calves from two inseminations	7	11

Table: Comparison of 2 protocols on buffalo cows in China

Subramaniam et al 1991 in India compared the newly available CIDR with + or – PGF2α (5mg deposited at the cervix) or PGF2α alone. The quickest and greatest response was from PRID + PGF2α and the best pregnancy rate (50% vs. 37.5%) as well. Insemination was 12 hours after onset of oestrus.

4.3.3 Ear Implants

The commonly used ear implant contains norgestomet and is sold as Crestar®. It has been used in buffalo. It is basically used as a direct substitute for a PRID.

The typical protocol for cattle is an ear implant on day 0, accompanied by an injection of Oestradiol Valerate, removal of the implant on day 9 with a 400IU PMSG and 56 hours after removal FTAI for cows and 48 hours for heifers. A day 7 PG injection is recommended for dry dairy cows, 2 days before the removal of the implant.

The latest recommendation from Brazil is (Baruselli 2007) is different to the cattle protocol;

- Day 0 at 6pm – PAI insertion plus 2mg ODB
- Day 9 at 6pm – PAI removal, 2ml PGF2α and 400IU eCG
- Day 11 at 6pm – GnRH injection
- Day 12 at 8am – FTAI

Martins et al (2002) tested Crestar plus Folligon (PMSG) for oestrus induction in female Murrah buffalo subdivided into groups according to the stage of the oestrus cycle. They concluded that the protocol was efficient at inducing oestrus synchronization.

Patel et al (2003) in India used Crestar +ODV on 10 cows and 10 heifers in the breeding and non-breeding seasons. 100% of cows and 70% of heifers responded with oestrus. The response was 90% in the breeding season and 80% in the non-breeding season.

Innocente et al (1996) in Italy used 14 buffalo cows 4-6 yrs of age with Crestar implant + 5mg norgestomet + 5 mg oestradiol on day 0. After 8 days they were injected with PGF2α and implant removed on day 10. This was accompanied by 1000 IU of PMSG (groupA) or 1000 IU FSH/LH (group B).

- Group A 71% oestrus within 40 hrs, 70 hrs duration of oestrus
- Group B 86% oestrus within 40 hrs, 48 hrs duration of oestrus

Ultrasounds showed abnormal responses in group A, and embryo recovery was poor in both groups, with only one cow per group responding.
Diaz et al (1994) tried FTAI on 15 cows lacking a palpable CL with implants of norgestomet for 12 days with injections of 3mg norgestomet and 5mg oestradiol (Group 1), and 19 cows with CL 2

injections of PGF2α 12 days apart (Group 2). Fixed time inseminations were carried out 68 h post implant withdrawal, or at 68 hrs (A) or 65&74 hrs (B) after 2nd PG. Treatment had no significant effect on interval from treatment to signs of oestrus (53-57 hrs) or % of females showing oestrus (92-100%) Conception rate (CR) for group 1 was 43%, and for 2A, 67% and 2B, 83%.

Luthra et al (1995) used Crestar implants on 7 cattle and 7 buffalo cows (9 anoestrus and 5 prepubertal) to synchronise oestrus and mated to bulls. At implantation 5mg of ODV was injected and implant removed on day 9. On day 8, an i/m injection of PMSG (400IU) was given. Al cows and 6/7 buffalo showed oestrus within 24-36 h after implant removal. 5 cows and 3 buffalo became pregnant on the first oestrus and 1 of each on the second. The conception failures were all in poor body condition.

In Thailand, Virakal et al (1992) got 3 twin calf outcomes from 24 swamp cows where oestrus was synchronized with norgestomet or norgestomet plus PMSG. 3 of the twins died within 5 weeks and birth weights were 19.0 ± 2.0 kg compared with single births at 28.5 ± 1.9 kg.

Yadav et al (1994) in India, tested, on 100 anoestrous Murrah buffalo cows in village situations, 5 different synchronizing drugs plus a control group. The groups received either, Lugol's iodine solution, Oestradiol valerate, PMSG, GnRH or Synchromate B (same as Crestar) and the control group was not treated. The percentage of animals which showed oestrus was 42.3, 50.0, 66.7, 53.8, 75.0 respectively and 30.8 for the controls. Average time intervals in days from last treatment to onset of oestrus for each treatment were 22.4, 4.2, 4.6, 19.3, 24.7 hrs, and 28.4 days in the control group.

4.3.4 Ovsynch

This is probably the most widely used protocol in recent years in most countries except for out-of-season breeding. (Cows that are anoestrus)

Zain et al (2001) studied the effect of using the Ovsynch protocol on 3 groups of pluriparous cows where the GnRH was substituted with saline injections on day 0 and day 9. GnRh on day 0 reduced the numbers coming into oestrus between day 0 and day 6 (10.8% vs. 25.8% in control group. Mating with bulls gave pregnancy rates to the first insemination of 31.3% (no GnRH), 58.8% for no second GnRH dose and 68.8% for full Ovsynch.

Berber et al(Brazil, 2001) substituted LH for the second dose of GnRH in the Ovsynch protocol and then AI (timed) was used on 305 non-pregnant buffaloes greater than 60 days post calving. A second insemination occurred between 16 and 25 days using oestrus detection methods (twice daily inspection and teaser bull). The pregnancy rates achieved on first and second inseminations were for GnRH 56.5% and 51.6% and for LH were 64.2% and 54.8% respectively. Overall for the double insemination the pregnancy rates were 66.9% vs. 75.5% for GnRH vs. LH which was significant at the 0.05 level. The intervals between timed AI and oestrus detection were the same for both treatments.

Singh and Madan (1999) studied the ovulation response to a combined PGF2α plus eCG given to pre-pubertal buffalo heifers. At oestrus, half were injected with GnRH (200 mg Fertagyl). Ovulation was synchronised within 14.67±1.77 hours post-GnRH injection whereas it was delayed in the control heifers to within 61.60±13.74 hours.

Camelo et al (Brazil, 2002), compared 12 (G1)and 24 hour fixed time AI (G2)after Ovsynch with natural oestrus detection and AI at 24 hour (G3)after onset of oestrus in 89 lactating buffalo cows. The results for PD after 45-60 days were 46.7%, 27.6% and 36.7% for G1, G2, & G3 respectively. They found significant differences in conception rates between body condition score (BCS) and order of the 1st oestrus on pregnancy rate. Primiparous cows also were reproductively less efficient than multiparous cows. Ovsynch was regarded as a practical tool for oestrus induction and more efficient at 12 hours post GnRH injection.

Recent work in Italy by Barile et al (2004) compared the efficiency of 2 protocols on 9 farms, PRID/ODB/PG/PMSG vs Ovsynch in the low breeding season (spring) on 186 lactating cows. Group

A were treated with PRID containing 1.55g P_4, a capsule with 10mg ODB, (both for 10 days), injection of 0.15mg PGF2α and 1000IU PMSG on day 7, group B were given the standard Ovsynch protocol except that the initial dose of GnRH was150μg , same amount as dose 2 on day 9. Fixed time AI was for A, 72 and 96 hours from PRID removal and B, 60 and 84hr after PGF2α. Milk sampling for P_4 was monitored to determine cyclicity. Over the 9 farms the conception rates varied widely from 16-64% with a mean of 45.2%. For Group A the mean pregnancy percentage over all farms was 47.8% while for group B was 42.6% (not significant). The numbers of cows cycling at the start of the trial was high 77.6% vs 80.3% for A vs B. The conception rates for each group are tabled below. Farm 7 had the highest number of non-cycling cows (46.9%), total cows =32 See summary table below

	Conception rate cycling cows	**Conception rate non-cycling cows**
Group A PRID	51.11% - 23/45	38.46%-5/13
Group B Ovsynch	44.9% - 22/49	16.66% - 2/12
Farm 7 A		43.0%
Farm 7 B		12.5%

Table: Comparison of 2 protocols on 9 Italian Farms

The authors suggest that PRID is a much more efficient protocol in non-cycling animals

4.3.5 Determination of Early Pregnancy

Milk sampling at 19-27 days post-breeding was measured for P4. (Arora et al, 1980). A level of P4 greater than 10ng/ml was taken as predictive of positive pregnancy. Milk progesterone levels in pregnant cows are significantly higher than non pregnant cows (P<0.001) at 19, 21, 23, 25 and 27 days post insemination. It is easier to measure in the milk than in blood plasma because the concentration is 4-5 times higher. See table below;

Days from AI	**19**	**21**	**23**	**25**	**27**
% Accuracy of +ve pregnancy	76.9	76.9	86.9	83.3	71.4
% Accuracy of -ve pregnancy	75.0	75.0	87.5	83.3	66.6

Table. **Accuracy of prediction on various days after AI.**

The most accurate time for sampling was then said to be from 23-25 days.
This is in close alignment with Roxas et al (1989) in Philippine Carabao with highest accuracy of 83% at 22-24 days for positive diagnosis and 100% at 24 days for negative diagnosis.

4.3.6 Ultrasonics

Baruselli (2007) recommends testing at 26 days post insemination to enable a second insemination to be carried out, to gain a 12.1 month mean calving interval, after a post partum interval of 50 days so that the second insemination would be at 86 days post calving. Ultrasound detection and diagnosis requires a fair degree of practice to become competent.
In dairy cattle at 28-35 days the accuracy of transrectal ultrasonography (Nation et al, 2003) showed that 100% accuracy was not obtained when compared with a 13 week rectal palpation due to a 9% loss of pregnancy up to 13 weeks. Sensitivity was 97% and specificity was 86% with positive and negative predictive values of 92% and 93% respectively.

4.3.7 Improving post-partum reproduction outcomes

Several authors (Metwelly et al (1999), Nasir Hussain Shah et al(1990)) report that GnRH is effective in increasing reproductive efficiency post calving by injecting a 5ml dose of GnRH on day 14 after birth of the calf.± PG at 9 or 45 days later. This causes cows to return to regular oestrus more quickly than normally expected and reduce the inter-calving interval.
Rastegarnia et al (2002) compared doses of GnRH and found that 50μg was not effective in causing ovulation in River buffalo where 100μg was an effective dose while Nasir Hussain Shah et al (1990) found no differences between 100μg and 250μg doses

5. Buffalo Artificial Insemination Trials Results.

There were 12 separate rounds involving 248 head on 3 properties with 67 positive outcomes. (Victoria, Queensland and the Northern Territory). The results are as follows;

5.1 Round 1.

5.1.1 Ovsynch protocol with double insemination day 10, am and pm.

A total of 23 head was inseminated, consisting of 4 cows and 19 heifers. All cows and 4 heifers conceived and calved.

• Breeds	1 x Swamp, 19 R x SW, 3 River
• Time of year	March 2004
• Pregnancy rate whole group	34.8%
• Pregnancy rate cows group	100%
• Pregnancy rate heifer group	21%
• Mean body condition score	6.0 (1-8 system)
• Mean wt of all pregnant females at start	490.3 kg
• Mean wt of pregnant cows at start	484.5 kg
• Mean wt of pregnant heifers at start	496.0 kg
• Mean wt of non-pregnant heifers at start	446.7 kg
• Site of semen deposition in cows	All uterine am and uterine pm
• Site of semen deposition in heifers	3 u/u and 1 uterine/cervical
• Time taken am session	10.2 mins/head
• Time taken pm session	6.7 mins/head
• Sex ratio of calves	5M:3F
• Semen used	19 Turkey Creek Bill (locally collected)/4 Imp. Italian
• Semen successful	8 /23 100% TC Bill
• Mean and (range) days to calving	322 (312-332) days
• Wt change during protocol (preg)	-589 gms/head/day
• Wt change during protocol (non-preg)	-419 gms/head/day

5.2 Round 2.

5.2.1 Ovsynch protocol with double insemination day 10, am and pm.

A total of 25 head (all heifers) was inseminated. Difficult heifers (14 head) were tried with ¼ ml ET pistolettes (thinner) after 1/2ml normal pistolettes failed to achieve uterine deposition. Semen was first decanted to ¼ ml straws, then loaded singly into ET pistolette. Only 10 of 25 head were inseminated during the pm session.

• Breeds	1 x Swamp, 14 R x SW, 10 x River
• Time of year	July 2004
• Pregnancy rate whole group	24.0% (All River) (6 from 25)
• Pregnancy rate single inseminations	3/14 (21.4%)
• Pregnancy rate double inseminations	3/11 (27.3%)
• Mean body condition score	6.44 (1-8 system)
• Mean p8 fat thickness	22.1 mm
• Mean wt of all heifers at start	447.9 kg
• Mean wt of pregnant heifers at start	422.3 kg
• Mean wt of non-pregnant heifers at start	476.9 kg

- Site of semen deposition in pregnant heifers — All uterine, but 3 with ¼ ml pistolette
- Time taken am session — 13.5 mins/head
- Time taken pm session — 7.5 mins/head
- Sex ratio of calves — 5M:1F
- Semen used — All Turkey Creek Bill (locally collected)
- Semen successful — 100% TC Bill
- Mean and (range) days to calving — 316.5 (310-325) days

5.3 Round 3.

5.3.1 Ovsynch protocol with double insemination day 10, am and pm

.A total of 16 head (all cows) was inseminated. Only 9 of 16 head were inseminated during the pm session. 7 head were treated with Estumate® and 9 with Juramate® on day 7. 1 of each group became pregnant. Both pregnancies were from the am insemination only.

- Breeds — 15 x purebred River, 1 x 7/8 RX
- Time of year — July-August 2004
- Pregnancy rate whole group — 12.5% (All River) (2 from 16)
- Pregnancy rate single inseminations — 1/7 (14.3%)
- Pregnancy rate double inseminations — 1/9 (11.1%)
- Mean body condition score — 6.63 (1-8 system)
- Mean p8 fat thickness — 29.3 mm
- Mean wt of all cows at start — 626.4 kg
- Mean wt of pregnant cows at start — 610.0 kg
- Mean wt of non-pregnant cows at start — 476.9 kg
- Site of semen deposition in pregnant cows — Both uterine
- Time taken am session — 6.9 mins/head
- Time taken pm session — 8.4 mins/head
- Sex ratio of calves — 1M:1F
- Semen used — 15 x Turkey Creek Bill (locally collected)/ 1 only Imported Italian "Bellissimo"
- Semen successful — 100% TC Bill
- Mean and (range) days to calving — 316.5 (304-329) days

5.4 Round 4.

5.4.1 Ovsynch protocol with double insemination day 10, am and pm

A total of 17 head (13 cows and 4 heifers) was inseminated. 5 of the cow group became pregnant. There were 2 pregnancies were from the am inseminations and 3 from the pm inseminations. Different semen was used for the am and pm sessions, and DNA used to identify the sire when the calf was born.

- Breeds — 17 x purebred River
- Time of year — Feb 2005
- Pregnancy rate whole group — 29.4% (All River) (5 from 17)
- Pregnancy rate double inseminations — 5/17 (29.4%)
- Pregnancy rate for heifers — 0% (0/4)
- Pregnancy rate for cows — 38.5% (5/13)
- Mean body condition score start — 6.76 (1-8 System)
- Mean p8 fat thickness start — 22.6 mm
- Mean wt of total group at start — 608.9 kg
- Mean wt of all cows at start — 640.7 kg
- Mean wt of pregnant cows at start — 611.2 kg
- Mean wt of non-pregnant cows at start — 659.3 kg
- Mean wt of non-pregnant heifers at start — 505.3 kg

- Site of semen deposition in pregnant cows All uterine
- Time taken am session 8.9 mins/head
- Time taken pm session 5.4 mins/head
- Sex ratio of calves 1M:4F
- Semen used 16 x Turkey Creek Bill (locally collected) Imported Italian 13 x "Bellissimo", 5 x "Bestiale"
- Semen successful 2/16 TC Bill, 1/13 Bellissimo, 2/5 Bestiale
- Mean and (range) days to calving 309.6 (305-313) days

5.5 Round 5.

5.5.1 Ovsynch protocol with double insemination day 10, am and pm

A total of 12 head (8 cows and 4 heifers) was inseminated. 5 of the cow group became pregnant. There were 5 pregnancies were from the am inseminations. Different semen was used for the am and pm sessions, and DNA used to identify the sire when the calf was born.

- Breeds 12 x purebred River
- Time of year April 2005
- Pregnancy rate whole group 41.7% (All River) (5 from 12)
- Pregnancy rate double inseminations 5/12 (41.7%)
- Pregnancy rate for heifers 0% (0/4)
- Pregnancy rate for cows 62.5% (5/8)
- Mean body condition score start 6.75 (1-8 System)
- Mean p8 fat thickness start 34.6 mm
- Mean wt of total group at start 655.5 kg
- Mean wt of all cows at start 708.8 kg
- Mean wt of pregnant cows at start 712.4 kg
- Mean wt of non-pregnant cows at start 702.7 kg
- Mean wt of non-pregnant heifers at start 549.0 kg
- Site of semen deposition in pregnant cows All uterine
- Time taken am session 7.9 mins/head
- Time taken pm session 4.2 mins/head
- Sex ratio of calves 5M:0F
- Semen used 12 x Turkey Creek Bill (locally collected) Imported Italian 1 x "Bellissimo", 6 x "Bestiale", and 5 x "Barbanera"
- Semen successful 1/1 Bellissimo, 1/6 Bestiale, 3/5 x Barbanera
- Mean and (range) days to calving 313.0 (304-318) days

5.6 Round 6

5.6.1 Ovsynch protocol with double insemination day 10, am and pm

A total of 17 head (6 lactating cows and 11 heifers) was inseminated. 2 of the cow group became pregnant and 1 heifer. There were 2 pregnancies were from the am inseminations, and the third can't be determined as the same semen was used am and pm. Where different semen was used for the am and pm sessions, DNA used to identify the sire when the calf was born. "Bill" semen was mainly used on the 2nd insemination.

- Breeds 6 x purebred River cows, 3 x 1/2, 1 x ¾, 6 x 7/8, and 1 x 15/16 river crosses
- Time of year November 2005
- Pregnancy rate whole group 17.6% (3/17)
- Pregnancy rate double inseminations 17.6% (3/17)
- Pregnancy rate for heifers 9.1% (1/11)

- Pregnancy rate for cows | 33.3% (2/6)
- Mean body condition score start | 4.9 (1-8 System)
- Mean p8 fat thickness start | 8.0 mm
- Mean wt of total group at start | 433.6 kg
- Mean wt of cows at start | 510.5 kg
- Mean wt of heifers at start | 391.7 kg
- Mean wt of pregnant cows at start | 493.0 kg
- Mean wt of non-pregnant cows at start | 519.3 kg
- Wt of pregnant heifer at start | 371.0 kg
- Mean wt of non-pregnant heifers at start | 393.8 kg
- Site of semen deposition in pregnant cows | All uterine
- Time taken am session | 7.4 mins/head
- Time taken pm session | 5.4 mins/head
- Sex ratio of calves | 1M:2F
- Semen used | 21 x Turkey Creek Bill (locally collected) Imported Italian 5 x “Cabirio”, 4 x “Millennio”, and 4 x “Appulo”
- Semen successful | 1/5 Cabirio, 1/4 Millennio, 1/21 x Bill
- Mean and (range) days to calving | 315.3 (314-318) days

5.7 Round 7

5.7.1 Ovsynch protocol with single insemination day 10, in the am

A total of 20 head (all heifers) was inseminated in Victoria. 10 of the group were tested pregnant to ultrasound at 78 days post-insemination. Subsequently only 6 calves were born.

- Breeds | 19 x purebred Italian heifers and 1 x Bulgarian Murrah
- Time of year | March 2006
- Pregnancy rate whole group | 10/20 (50%) to ultrasound at 78 days.
- Calving rate | 31.6% (6/19) 1 pregnant heifer died well before calf due date
- Mean body condition score start | 6.5 (1-8 System)
- Mean wt of total group at start | Not measured
- Site of semen deposition in pregnant cows | All uterine
- Time taken am session | 3.5 mins/head
- Sex ratio of calves | 2M:4F
- Semen used | 3 x Turkey Creek Bill (locally collected) Imported Italian 7 x “Cabirio”, 7 x “Millennio”, and 3 x “Appulo”
- Semen successful pregnancy | 2/3 Appulo, 2/7 Cabirio, 5/7 Millennio, 1/3 x Bill
- Semen successful calves | 1/3 Appulo, 2/7 Cabirio, 3/7 Millennio, 0/3 x Bill 1
- Mean and (range) days to calving | 310.8 (308-314) days

5.8 Round 8

5.8.1 19 head (4 cows, 15 heifers) Ovsynch protocol with double insemination day 10, am and day 11 am (24 hours apart) and 6 head (cows)on Cue-Mate® protocol with double inseminations at 24 hour intervals.

A total of 25 head (5 lactating, 5 dry cows and 15 heifers) was inseminated. 2 Cue-mates were missing, one on the 11/4 and the other on the 13/4. The latter did conceive successfully. Where different semen was used for the second day, DNA used to identify the sire after the calf was born. "Bill" semen was mainly used on the 2nd insemination.

- Breeds — 6 x purebred River cows, 3 x 1/2, 1 x ¾, 6 x 7/8, and 1 x 15/16 river crosses
- Time of year — April 2006
- Pregnancy rate whole group 72 days — 36% (9/25)
- Calving rate of whole group — 5 from 25
- Pregnancy rate for heifers — 26.7% (4/15)
- Calving rate for heifers — 0% (0/15)
- Pregnancy rate for cows to Ovsynch — 75% (3/4)
- Pregnancy rate for cows to Cue-Mate — 33% (2/6)
- Mean body condition score start — 7.0 (1-8 System)
- Mean p8 fat thickness start — 16.4 mm
- Mean wt of total group at start — 552.3 kg
- Mean wt of total group at insemination — 542.9 kg
- Mean liveweight loss over protocol — 940g/hd/day
- Mean wt of cows at start — 633.4 kg
- Mean wt of heifers at start — 498.2 kg
- Mean wt of pregnant cows at start — 632.0 kg
- Mean wt of non-pregnant cows at start — 634.8 kg
- Site of semen deposition in pregnant cows — All uterine
- Time taken 14/4 — 4.8 mins/head
- Time taken 15/4 — 5.2 mins/head
- Sex ratio of calves — 3M:2F
- Semen used — 31 x Turkey Creek Bill (locally collected) Imported Italian 5 x "Cabirio", 5 x "Millennio", 4 x "Appulo", "3 x Barbanera", "4 x Bestiale"
- Semen successful — 1/5 Cabirio, 2/4 Appulo, 2/31 x Bill
- Mean and (range) days to calving — 314.0 (305-321) days

5.9 Round 9

5.9.1 Ovsynch protocol with 2 inseminations on am day 10, and am, day 11

A total of 25 head (10 lactating, 4 dry cows and 11 heifers) was inseminated. TC Hillary was joined with the group on the day of the second insemination from 9th July to 13th September (66 days). Hillary got a further 13 pregnant in this period. This would indicate animals that came on heat later than the scheduled AI times or on subsequent heats. One heifer calved during the AI 'window', 11 of the others in a 3 week grouping 3 weeks later and one heifer another month later. 7 of the cow group became pregnant to AI and no heifers. All conceptions came from the 1st inseminations on the 8th July. Where different semen was used for the following day, DNA used to identify the sire after the calf was born. "Bill" semen was mainly used on the 2nd insemination or on heifers that were difficult to get a uterine deposition.

- Breeds — 14 x purebred River cows, and 4 heifers, 1 x ¾, 5 x 7/8, and 1 x 15/16 river crosses
- Time of year — June-July 2006
- Pregnancy rate whole group — 28% (7/25)
- Pregnancy rate for heifers — 0% (0/11)
- Pregnancy rate for cows — 50% (7/14)
- Mean body condition score start — 6.5 (1-8 System)
- Mean wt of total group at start 27/6 — 612.8 kg
- Mean wt of cows at start — 510.5 kg
- Mean wt of heifers at start — 535.3 kg
- Mean wt of pregnant cows at start — 694.0 kg
- Mean wt of non-pregnant cows at start — 653.4 kg
- Site of semen deposition in pregnant cows All uterine
- Time taken am session day 1 — 4.2 mins/head
- Time taken am session day 2 — 4.3 mins/head
- Sex ratio of calves — 4M:3F
- Semen used — 19 x Turkey Creek Bill (locally collected) Imported Italian 4 x “Cabirio”, 4 x “Millennio”, 6 x “Appulo”, “8 x Barbanera”, “9 x Bestiale”
- Semen successful — 2/4 Cabirio, 3/6 Appulo, 2/9 x Bestiale
- Mean and (range) days to calving — 310.0 (302-314) days

5.10 Round 10

5.10.1 Ovsynch protocol with 2 inseminations on am day 10, and am, day 11

A total of 5 head (2 lactating, 2 dry cows and 1 heifer) was inseminated. Three head were ones not pregnant to the bull in previous Aug-Sept, and the other 2 were lactating cows that were in lower condition, but more than 120 days from calving. (Oct 06)

- Breeds — 4 x cows and 1 heifer all purebred River
- Time of year — Feb 2007
- Pregnancy rate whole group — 0% (0/5)
- Pregnancy rate for heifers — 0% (0/1
- Pregnancy rate for cows — 0% (0/4)
- Mean body condition score start — 6.0 (1-8 System)
- Mean Fat at P8 site at start — 20.6 mm (3-55 mm)
- Mean wt of total group at start 12/2 — 603.0 kg
- Mean wt of cows at start — 639.5 kg
- Wt of heifer at start — 457 kg
- Site of semen deposition in cows — All uterine
- Site of semen deposition in heifer — Vaginal/Cervical (good signs of heat day 1)
- Time taken am session day 1 — 7.6 mins/head
- Time taken am session day 2 — 7.6 mins/head
- Semen used — 4 x Turkey Creek Bill, Imported Italian 2 x “Cabirio”, 2 x “Millennio”, 1 x “Appulo”, “1 x Barbanera”

5.11 Round 11

5.11.1 In North Queensland, 26 head (all lactating cows) were treated with the Ovsynch protocol with single insemination on day 10, am and 10 head (cows) on CIDR® protocol with single insemination on observed heats. All were observed twice daily, during milking and also during the day whilst in the paddock grazing. Four early pregnancies were not detected at the start of the protocol, so that these cows aborted due to the prostaglandin and were not suitable for AI

- Breeds — 6 x purebred River cows, 3 x 1/2, 1 x ¾, 6 x 7/8, and 1 x 15/16 river crosses
- Time of year — June 2007
- Pregnancy rate whole group 72+days — 21.9% (7/32)
- Calving rate of whole group — 31.3% (10 from 32)
- Pregnancy rate for cows to CIDR — 37.5% (3/8)
- Pregnancy rate for cows to Ovsynch — 29.2% (7/24)
- Mean body condition score start — Not recorded but all in very good condition
- Mean p8 fat thickness start — Not measured
- Mean wt of total group at start — Not recorded, no facility
- Weather conditions during AI — Cool and some drizzle at times.
- Site of semen deposition in pregnant cows — All uterine
- Sex ratio of calves — 7M:3F
- Semen used — 1 x Turkey Creek Bill, Imported Italian 10 x “Cabirio”, 9 x “Millennio”, 9 x “Appulo”, “2 x Barbanera”, “1 x Bestiale”, 1 x “Bellissimo
- Semen successful — 4 (3M/1F)Cabirio, 5 (3M/1F) Appulo, 1 (1M) x “Bestiale”
- Mean and (range) days to calving — 312.1 (305-319) days

5.12 Round 12

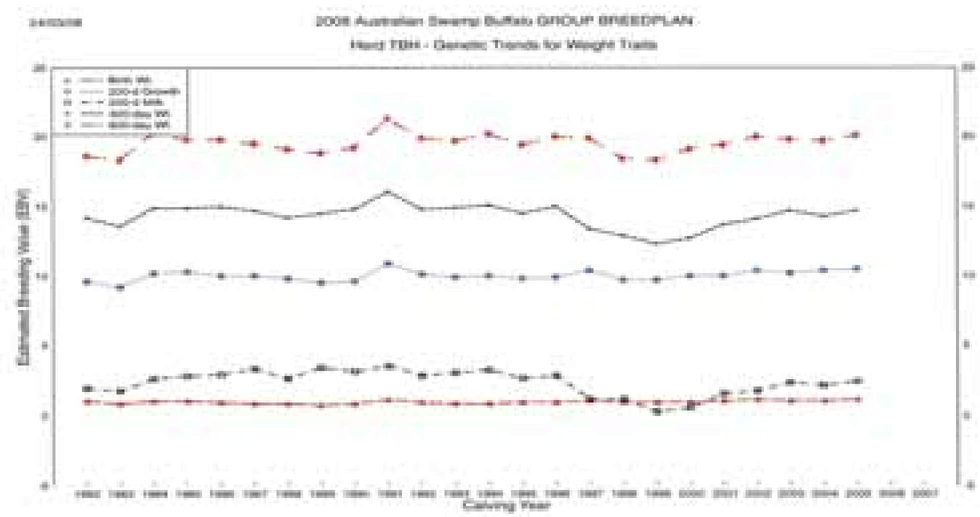
5.12.1 Ovsynch protocol with single AM insemination day 10

A total of 26 head (all dry cows) was inseminated. 10 were diagnosed pregnant at 43 days and again confirmed at 93 days.

- Breeds — 26 x purebred River cows
- Time of year — January 2008
- Pregnancy rate whole group — 38.5% (10/26)
- Mean body condition score start — 5.96 (1-8 System)
- Mean p8 fat thickness start — 16.8 mm
- Mean wt of total group at start — 615.9 kg
- Mean wt of pregnant cows at start — 545.4.0 kg
- Mean wt of non-pregnant cows at start — 660.0 kg
- Site of semen deposition in pregnant cows — 9 uterine/1deep cervix
- Time taken am session — 7.4 mins/head
- Time taken pm session — 5.4 mins/head
- Sex ratio of calves — Not yet born.
- Semen used — 2 x Turkey Creek Bill (locally collected) Imported Italian; 8 x “Cabirio”, 9 x “Millennio”, and 7 x “Appulo”
- Semen successful — 2/2 x Bill, 2/8 Cabirio, 3/9 Millennio, 3/7 Appulo
- Mean and (range) days to calving — Not yet born

6. Buffalo Data Genetic Analysis

Some preliminary results have been made available only recently for the Beatrice Hill herd. See graph below.



These results show that genetic trends for the herd are not showing much change over the 1983-96 period, apart from one noticeable year (1991) where there was a substantial rise, apparently due to the influence of a single mating bull that was used during that time. Its 600 day EBV was almost double the herd average at that time. This bull was coloured pink, and had a birth weight of 42 kg, but was killed after only one season of mating by other bulls during the non-mating season. His EBVs stand out from all others in the whole herd, but the influence was short lived. Pink colouration is generally not favoured by the industry. This is why we need Breedplan to flag these animals, before they die!
Reproduction EBVs for the same period showed a flat trendline, similarly for the current herd.
There is a rising trend in growth EBVs for the current herd, a period during which there has been no selection for growth whatsoever! The same bulls were used throughout.
Separation of the two herds appears warranted from the results that have been obtained from the preliminary analysis

7. Discussion of Results

7.1. Artificial Insemination Trials

.Whilst success rates of these trials have not reached overseas benchmarks (Bhosrekar et al, 1994, Situmorang et al, 1997, Berber, 2001, DeSantis et al, 2004, and Züge et al, 2004) there is still some optimism that within the short term, with increasing knowledge, comprehension and understanding, 50-60% conception rates will be reached. Part of the reason could be the tropical location of most of the trials in the NT, with the feeling that in the southern areas, that good conception rates may be easier to attain (best conception rate of all trials was on the Vic herd that was all heifers!), however more heed will need to be taken of seasonality than in the NT, where nutrition seems to take precedence over seasonality. Overseas literature suggested that the Ovsynch protocol is the most efficient for animals that are ovulating and most of our trials have used this protocol. There were a number of rounds that gave good rates, but consistency was elusive. This may be due to a number of factors, seasonality, body condition of stock, lactation stress, semen quality or bad synchronization. However most of the conceptions occurred to the first insemination. That is why we used different semen to isolate the best timing by DNA testing the calf for its sire. There is still however a need to evaluate other protocols so that we can dispense advice for all Australian conditions where buffalo may be found. Many of the original trials were lacking in success because of the large number of heifers involved and the reputed low success rates of Ovsynch with heifers. (Baruselli et al, 2003). These trials observed variable calving results with heifers from 0% in Rounds 4, 5, 8 and 9, 9.1% in Round 6, 21% and 24% in Rounds 1 and 2, and 31.6% in Round 7. Rounds 7 and 8 suffered large losses from pregnancy testing to calving; of 50% down to 31.6% and 36.0% down to 20.0% respectively.

The particular feature of heifer pregnancy difficulties appears to be the inability of inseminators to be able to easily penetrate through the cervix into the uterus to deposit the semen. Often there is penetration for ½ to ¾ of the cervix, but failure to progress any further. In order to overcome this problem, the inseminator opted to split the ½ ml straw between 2 ¼ ml straws and use an ET pistolette which is narrower than the standard ½ ml AI pistolette. The majority of successful heifer pregnancies were the result of uterine semen deposition. Generally most cows are quite easy to manipulate the pistolette through the cervix in a short timeframe. Cows that were difficult as heifers to AI, usually are no problem once the first calf has successfully been born.

Delegates with good results to AI at the 8th WBC emphasised the need for good condition in cows to reduce the possibility of anoestrus, maintaining feed quality during the protocol, and using good quality semen.

7.1.1 Semen Quality

The results appear to indicate wide differences in semen quality that we have received from overseas as allocation of semen has been made quite randomly to animals, except to avoid Sire over daughter inbreeding. It is meaningless to enter the local semen ('Bill") in the table as Bill was used for the second insemination **many** times, where there have been few conceptions, and also used for difficult animals eg heifers where a calf has not been through the cervix as yet. While only 2 Italian bull semen batches are **statistically** significantly different in conception rate, this has a lot to do with the small numbers used so far. The results (see table below) are probably a timely warning that some of our problems have a great deal to do with semen quality and producers should be aware that quality is highly variable. There is a wide range of conception outcomes from the 6 imported bulls ranging from 10% to 41.2%, so it would probably be very advisable for all producers using AI to record all inseminations and add to our recorded information. See table below;

Semen Bull Name	Mean success rate achieved	Total straws used	Confidence Interval (5%)	Significance at 5% probability
Bellissimo	0.100	20	±0.131	a
Bestiale	0.158	19	±0.164	a,b
Barbanera	0.240	25	±0.167	a,b
Appulo	0.412	34	±0.165	b
Cabirio	0.293	41	±0.139	a,b
Millennio	0.195	41	±0.121	a,b

Table: Comparison of Italian bull semen pregnancy outcomes from AI trials.

7.1.2 Sex ratio of calves born.

Over all the trials there have been produced 34 males to 23 females from AI. This equates to the percentage of males being 59.6%.

7.1.3 Where to in the future with AI?

The next RIRDC project for this organization has allowed for the continuation of AI, so the number one priority will be to obtain semen of excellent quality as well as genetic merit. Up to now we have had little choice in what we could obtain. Since visiting Italy we now realise what is available on the market. We know our own local semen has good quality, but expert testing will be carried out in future to assess viability and motility in comparison with our own.
Other considerations will be;

- Seasonality; further comparisons between protocols in various seasons, particularly in southern Australia
- Body condition and weight will be monitored more closely to provide possible reasons for poor conception
- We need to find a better method of determining the actual onset of oestrus, although all indications from previous work are that the synchronization period is pretty tight with PG + GnRH. With buffalo the heat mount detector is not a serious contender for this, because of lack of strong female mounting behaviour. Many also get removed in wet weather, and the 'scratchie' type self-adhesive detectors are not at all useful; they do not adhere well enough to buffalo hair and hide. The cotton/breakable coloured capsule type products also lose a substantial number in a short time period. The problem is interpreting whether the loss is due to mounting or some other way.
- The effect of nutrition during the protocol needs to be investigated.

7.2 Register/Database

The Buffalo Register/Database will be run by ABRI and per head charges will depend on the level of usage; a basic fee for animal registration, then additional charges according to the number of Breedplan traits to be measured and analysed. Certificates of registration will be available and reports sent to owners of their current EBV calculations. As with cattle, accuracy will increase as numbers of animals entering the system with production records, increases.

Initially in the project it was envisaged that the Northern Territory Buffalo Industry Council would act as the monitoring/vetting agent for the industry of animals being registered. This situation is no longer a viable arrangement, so the author will act as the Registrar on behalf of the Industry for the medium term from the NTDPIFM Office in Darwin.

There is no doubt that the more the system is used by the buffalo industry, as has been the case with most of the cattle breeds, the quicker benefits will accrue and the sooner the better genetic animals will be identified. This will help the industry to decide where new genes should come from and other issues, such as the effect of Italian genes on meat production and quality, the milk producing ability of the US sires compared with Italian semen imports, what happens to quality of milk if volume is chased heavily, and is there enough individual variation between bulls to be able to fix that dilemma. We also need to be able to isolate those critical factors that are important in the manufacture of buffalo dairy products, their genetic origins and whether selection is possible. We need to avoid if possible the Friesian/Holstein experience where yield was paramount, and quality, reproduction, animal size and udder health deteriorated.

This Buffalo Database has the potential to be a powerful tool for the industry for performance improvement and to boost sales in the long term as customers realise its potential.

7.3 Genetic Data Analysis of Beatrice Hill Herds.

Spreadsheet and Database records of the current and former herd were converted into a customised ILR1 database at ABRI. The data presented to ABRI posed some problems, not the least of which was the culling of the swamp herd for Tuberculosis exposure by the end of 2008. This restricted that herd to a 13 year reproductive period. The current herd had to build up from scratch again and there was a subsequent space of 3 years where offspring numbers were very low and no linkage with the previous herd. There were also complications of the Riverine breed introduction; no linkage with new swamp herd with the original swamp group and the restricted mating practices with the crossbred herd due to the shortage of usable bulls and their restriction to a single crossbred level for an extended period of 12 years for management simplicity. Not enough milk production records were available for analysis at this stage but they are being built up in the meantime. With time, parameter estimation will be possible as enough data become available. There is another 3 years of latest current herd data to enter onto the database; that will help to pick up some the numbers required and extends the pedigree lines a few years.

7.4 Communication and Liaison

All World Buffalo Congresses are very well conducted events and are a vital arena for meeting the experts in the buffalo field.

The Australian Buffalo Industry Council (ABIC) has performed very well for the most of it’s existence, and is a good forum for contact with all states of Australia.

The “Buffalo News” is an excellent communication tool and has a large Australian and overseas clientele base and is sent electronically as well.

8. Implications

8.1. Artificial Insemination Trials

Whilst satisfactory rates have still to be achieved, we have learnt a great deal from what has been a very interesting number of trials carried out so far. A large number of variables need to be taken into account apart from the protocol that is adopted.

8.2. Buffalo Register / Database

The benefit of the new database is that there is the potential for all Australian buffalo to be listed while the industry is relatively small. Because most expansion has been within the last 15 years the public memory in being able to trace animal origins is still available, particularly for the Riverine introductions. The advantages of this are that in the future, both buyers and sellers will be readily able to see what is available anywhere in Australia, and be able to choose from the widest selection, those animals which suit their breeding objectives. This will help those entering the dairy buffalo market to objectively select animals that meet their needs and there is less chance of being sold a crossbred animal that does not have the stated percentage Riverine blood. What will make the database even more valuable in the longer term is if all dairies carry out regular milk testing and these records are entered into the database. It is suspected that most dairy animals would be fed off grass for the foreseeable future with only a minimal amount of concentrate going into the diet. Being able to milk from grass is one advantage that dairy buffalo, compared with dairy cattle, will have as the country begins to wrestle with carbon trading, climate change, reducing water supplies, higher temperatures, higher food prices, higher fuel prices and competition for arable ground for food production vs bio-fuels and energy production. Because buffalo produce a protein and energy rich product without the need for large concentrate input, it will be a distinct player in the market.

Visual and scanning access to the Database on the Net will be a great boon for both Australian and international buyers and should help increase demand for the product in the long term. Already there have been buyers that have specified particular milking attributes for their purchases which are not presently possible.

The Buffalo Database will provide the tools necessary for a vibrant competitive stud industry.

8.3. Beatrice Hill Herd Data Analysis

As can be seen from the genetic analysis, there is a need for more data collection to provide useful and precise genetic parameters, and linkages with other countries may be useful in achieving this at a faster rate. ABRI already has data from Thailand, and as exports from Australia for dairy are increasing we should be encouraging the recipients to become dedicated recorders. The more people to contribute to the recording, the greater will be the precision of the estimates of genetic heritability, hence EBVs and in the long term, recognition of superior genes for meat and milk will be correctly made. The problem for the current milk industry is the very shallow genetic base of the Australian herd (74 head imported), so it is important to be able to identify suitable genetics overseas, in countries where semen or embryos can be sourced, to be able to assess its possible contribution to increasing the genetic base and production potential of the herd. Because it originated in 3 countries, that has some future advantages in determining the best source of future genes. Also because Italian semen has been used world-wide in the dairy buffalo industry, there will be some vital genetic linkages being recorded for the whole world buffalo industry.

8.4. Maintain communication and contact with industry

This contact has been crucial for the project, particularly in sorting out the difficulties with the AI process. This is evident in many ways, as so many practical aspects of carrying out an AI program are not mentioned in a scientific paper. One example was that in being able to measure fat in Beatrice Hill breeders, it was found that when trying to give a rump injection in a very fat buffalo cow (some have 60 mm of fat on the P8 site) that a 40mm needle is not going to be physically able to reach a muscle, so the neck is a much better site as there is a lot less fat over that site and the needle easily accesses muscle there. A Brazilian expert relayed that absorption from fat takes up to 5 days longer than if injected into the muscle, which totally obliterates any chance of synchronization.

Further use will be made of the Buffalo News as a vehicle for dissemination of results as they come to hand, along with NT DPIFM publications avenues such as Agnotes, News magazines and technical bulletins.

9. Recommendations

9.1. Promote the uptake and usage of the database

Having now been created, the buffalo industry needs to use the resource of the Buffalo Database to its fullest extent to make it work to progress the industry to the next stages. Equipped with 'Buffalo Breedplan' the industry can now make rational decisions based on objective measurement, once a significant body of recordings have been made. This applies to both meat and dairy sectors.

This needs the cooperation of all stakeholders by contributing objective data to make the database more effective and the internal genetic relationships more precise. The value of gaining overseas customers into the database should not be forgotten.

The use of AI will help build these linkages even more widely. The NTDPIFM could make this happen quicker by making available much cheaper semen than is currently purchased from overseas, from its current supplies from "Bill". We could also collect and produce semen from a number of new trained bulls, if the demand was there.

These aspects would need to be discussed at industry forums and promoted in the "Buffalo News"

9.2. Make wider use of AI where practical.

Using AI semen or bulls on the database already will help speed up production improvements by providing the necessary genetic linkages. Although the author is not satisfied that enough progress has been made to make hard and fast recommendations to industry on which protocols are the most effective, we have learnt a massive amount on the factors involved in getting good AI outcomes and can advise accordingly.

9.3. Maintain linkages with overseas experts and institutions

This is a necessity for our competitiveness with the world buffalo industry that we maintain linkage with people and institutions that are better resourced than ourselves. We now though have a cutting edge database and records system that can be useful to them as well.

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Appendices

Appendix 1.

Consultants Report on Registry / Database needs for Buffalo Industry in Australia

Database Requirements for the Australian Buffalo Industry
Dr. Brian Mc Guirk
Sept 2004

Conclusions and Suggested Course of Action

While cattle Breed Societies in the UK have adopted varying strategies to meet their registration needs, I suggest that the pedigree registration service offered by ABRI is the most likely to meet the needs of the Australian Buffalo Industry. The good experiences that the Angus Society in the UK has had with the ABRI system would confirm this assessment. The ABRI system already meets the needs of the Australian Buffalo industry, they have staff who can help implement what they already have to offer, and it is clear that new versions, presumably enabling telephone registrations, are already in the pipeline.

For the Australian Buffalo Industry, specific advantages of the ABRI system would include:

- That it was developed in Australia, and is widely used there by Australian Breed Societies.
- It already has a proven track record with many Australian and overseas cattle breed societies.
- ABRI is already included in the RIRDC Buffalo Project, albeit in a different capacity.
- The ABRI pedigree registration software can be linked with accounting packages, so that members can be charged for services, and enables a smooth interface with genetic evaluation software, which might be required for buffalo at some point in the future.

Given that assessment, I suggest that a formal approach be made to ABRI, to ask ABRI to design and implement a pedigree registration service for the Australian Buffalo Industry. However, before that approach is made, and certainly before any commitment with ABRI is made, then a number of decisions need to be made.

As regards scope of the system, I suggest it should be seen as consisting of a number of modules, including herd details, animal identity, birth and pedigree details, growth (weight) information, and an ability to accept electronic or other forms of input, such as from milk parlour software.

As regards size of the system, I suggest that it should be restricted, at least initially, to animals in a grading up or crossbreeding programme, such that they are either purebred or crossbred river animals. In other words, at least initially, feral swamp animals should be excluded, other than perhaps including a swamp dam identity for a crossbred calf. If this approach is agreed with, then the number of animals to be included will be quite small.

As regards mode of operation, then I suggest we should look at one point of data entry for all herds, including Beatrice Hill and Millaa Millaa, and that this might be based at the office of the Northern Territory BIC. However all “registered” herds would be able to view information. The option for herds, such as those at Beatrice Hill or Millaa Millaa to modify data, using some form of PIN identifier, needs to be discussed. I would imagine that initially we would be looking primarily at paper-based or internet (email) systems for passing data, with the entry point acting as a bureau type service, but from which data amendments can be made. But while the database might initially be of an ACCESS-type arrangement, there must be longer-term plans for telephone data entry.

ABRI is already mentioned in the RIRDC Project proposal, but to calculate Estimated Breeding Values (EBVs). While this might be seen as a long-term aim, to do so within this Project would, to my mind, be a mistake.

- The numbers of animals will be small, and mostly of various crossbred generations.
- An analysis of those data within the Project, on a lap top or similar PC, using more appropriate software, would produce estimates of genetic parameters on which later EBV analyses might be based, provide information on the performance of different crossbred generations, and allow for the incorporation of "foreign" information, such as milk EBVs from Italy.

For such analyses to be possible, the ABRI system must be able to produce suitable output files (say .csv files which are EXCEL or ACCESS readable) which can then be analysed.

As regards the cost of the system, this needs to be agreed with ABRI, taking on board decisions about the size, scope and mode of operation.

I suggest that funds need to be found for an agreed initial period of use of the ABRI system, say for five years.

Funds for such a venture should fall in part on members, but some at least can be found within the RIRDC project and budget, including moneys allocated otherwise to ABRI and other cost savings.

Database Requirements for the Australian Buffalo Industry

Details of discussions with UK Cattle Breed Societies and other interested parties.

Discussions with Suzanne Harding, Holstein United Kingdom (HUK), Rickmansworth, 11th August 2004.

HUK operates a registration scheme for animals registered with its own Breed Society, plus Jerseys, Guernseys, Ayrshires and Belgian Blues.

The scheme is geared towards telephone registrations and is based on SQL server. This is needed to cope with the volume of records and to provide web-based access. However, it can also incorporate information written on ACCESS databases. The scheme has grown out of earlier ACCESS databases, such as the one still operated by the Charolais Breed Society, which was developed by HUK.

In its present form, the HUK database can incorporate proof information, from Signet (beef), MDC/ADC (dairy production) or HUK (dairy type). Equally it can deal with milk records from milk recording organisations such as CIS (wholly owned by HUK) or NMR.

The database is linked to membership information, is interfaced with the UK national tag numbering system, and can produce all registration papers and milk certificates.

The service offers options for AI, natural service, or ET. For the Belgian Blues, the breed society provides a list of likely bulls. Where ET is used, the recipient does not have to be a registered animal.

The system is "cow-based", as are registrations. When a new cow enters, the system can look at calves born in that herd, or other registered herds, to pick up relevant information.

While CIS offer disease recording options, these are little used by farmers.

Bruce Fairlie at Paisley can look at milk records etc, and can "manually" alter "flagged" errors. In other words, outside the central system, some degree of monitoring can be applied at the bureau level.

The HUK system could be made generally available, although negotiations would be needed with Dave Hewitt, CEO of HUK, who can decide on purchase or licensing arrangements and charges. In all probability, the Breed Society would have the task of charging members for the services.

All costs associated with data transfer and any modifications or developments (eg growth information) would need to be paid for by the Breed Society. On an on-going basis, data storage charges would be on a per animal basis. If HUK was asked to edit or manipulate data, this would also generate a charge.

Suzanne Harding indicated that she would be generally happy with the wider use of the HUK system, but that they would not want to be involved in "trouble shooting". The task of operating the system would rest with the Breed Society that had licensed/purchased the system.

<u>Discussions with Dr Duncan Pullar, MLC/Signet, 11th August 2004-08-12</u>

MLC/Signet operates a pedigree recording and genetic evaluation service for beef cattle and sheep. Pedigree information is imported on a monthly basis from the larger breed societies. For smaller breeds, MLC constructs a file of the necessary information for recorded animals.

Most growth information is provided to MLC directly by farmers, although I assume that for the larger Breed Societies the point of contact for farmers is with the Breed Society, and not MLC.

Signet provides a weighing service, and also a scanning service, although these activities are often contracted out. Use of a scanning service is not a pre-requisite for membership to the scheme.

A file of pedigree and production information is then extracted for BLUP evaluation to produce EBVs.

All programs for these activities were written in the MLC. The beef evaluation system is a legacy of Crump and Amer, while the sheep system is due originally to Derrick Guy, which has subsequently been upgraded "in-house". However it cannot readily accommodate new traits, such as CT scan or egg count information for sheep, so needs to be updated.

Members are charged a fee, which entitles them to have records taken/processed on a small number of animals. Total charges are capped.

MLC/Signet is now moving to an agreement to use the BASCO system, which is jointly owned by a number of sheep and beef breed societies. Development work is the responsibility of Graham Technologies (Willie Fisher, 0141 533 4049).

MLC will pay some development costs for this system. The plan is that pedigree and performance data will be passed to SAC for evaluation.

It is proposed that data entry will be possible by farmers, or through a bureau service, such as might be operated by Breed Societies. With special software, errors can be detected and corrected in real time, on a PC.

Duncan Pullar suggested that ABRI is the obvious candidate organisation to operate a breed registration service for the Australian buffalo industry, with data entered by a breed society office in the first instance, with pedigree, growth and milk information all being accepted.

<u>Discussions with David Benson, Secretary, Charolais Society, 12th August 2004</u>

The Charolais Society uses a "stand-alone" system for registration purposes. The system was developed by HUK, and is in ACCESS. It was done before the current SQL-based system now being used and extended by HUK.

At regular intervals, the Charolais Society passes pedigree/registration information to MLC/Signet, who combine it with the performance data they receive from Charolais breeders (or service providers, as in the case of scanning information).

The present system works perfectly well as a bureau service. However it does not accept telephone or internet registrations, which some (few) members might want, and on-screen cannot enable breeders to check the herdbook, or to search out bulls that meet certain requirements, rank bulls etc. However these latter tasks can be undertaken by the Society using the present system, using criteria identified by breeders etc.

The planned demise of the Signet/MLC system will cause problems. HUK's new SQL system is one option for pedigree registrations, with the possibility that further options could include growth and scanning data.

The MLC is proposing the BASCO system be used to combine pedigree and performance data, prior to despatch to SAC for genetic evaluations. ABRI is the other option for providing both pedigree registration and evaluation. This might be done using the Angus Society as the link organisation in the UK. The Charolais Society is still to decide finally which of these two routes to follow.

Phone Discussions (10768 88775) with John Fleming, Secretary, Belgian Blue Society, 13th August 2004.

A phone conversation was deemed sufficient, as the basic ingredients of the Belgian Blue approach were already known.

The Breed Society transmits performance data to the Angus Society offices in Perth on a regular basis, and there is also a regular transfer of pedigree information.

The Belgian Blue Society uses the HUK registration service, and will then use ABRI for their BLUPs, courtesy of the Angus Society facility in Perth.

The Belgian Blues like the HUK software, both because of its flexibility, but also because of the ability of the HUK system to communicate with BCMS in Workington, to obtain passports. For this Breed Society, Caesarian deliveries could be classified as standard or optional.

The Belgian Blue Society has not yet tested Angus/ABRI system.

Discussions with Ron McHattie, Chief Executive Officer, Aberdeen Angus Society, Perth, 23rd September, following a phone conversation (01738 622 477) on 3rd September 2004.

I will complete a full report on the Angus/ABRI system as soon as possible, as it seems to offer a suitable model for the Australian buffalo industry.

The Angus Society has been operating the Australian ABRI system since 1st August, is quickly overcoming teething problems, and the Angus/ABRI system would seems to offer a suitable model for the Australian buffalo industry.

Their previous Angus registration system was based on an old DOS-based system, and was essentially obsolete. In addition, the SIGNET/MLC procedure for genetic evaluations was also felt to be less than satisfactory.

The ABRI system offered a pedigree registration system that seamlessly interfaced with a genetic evaluation system. It can also be integrated directly with the SAGE accounting package used by the Society. Registration details are extracted from the form breeders complete for UK animal tagging requirements. Registration details on the system covers the last 24 herd books. The registration data are emailed to Australia once a week, and are returned within 24 hours. Breeders and other interested parties can interrogate the system on-line, to find out animal or breeder details.

Performance data can be submitted by breeders either on paper or via a secure internet system. On receipt of performance data, an interim EBV is calculated, although ABRI will also conduct formal breed-wide evaluations every six months. Performance data from the past 20 years have been obtained from MLC/Signet. EBVs will be returned from the ABRI system on 13 traits, and various indexes will be calculated. The interrogation capability of the ABRI means that interested parties can search the system for bulls that meet EBV criteria. For example, interrogating sale catalogues is one option open to buyers.

From now on, the breeder retains ownership of all performance data, but any EBVs are now the IP of the Breed Society.

The introduction of the ABRI system has been greatly helped by the involvement of Murray Scholz (programming) and Jack Allen (evaluations). ABRI can provide an accounting package to sit beside the registration system, and can provide as little or as much of the data entry needs as are requested. They clearly have further versions of their software in the pipeline, so that telephone registrations are envisaged. That move will presumably require an upgrade of the current database to an SQL system.

Appendix 2.

Australian Water Buffalo Performance Data – Preliminary Results

Australian Water Buffalo Performance Data

Preliminary Results

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2.1 Preliminary Report on Australian Water Buffalo Performance Data

The data was supplied by NTDPIF&M from various PC databases and converted into a customised ILR1 (International Livestock Register) database at ABRI. The performance data used in this analysis was collected at Beatrice Hill. No other performance data was available at the time.

Basic Statistics

For this report, animals were extracted for analysis if they had a performance record. The parents and grandparents of these animals were also extracted. Table 1 outlines the information available from the extracted data. The sex ratio of animals in the extract was even and virtually all animals were from single births.

Table 1. Basic statistics of the extracted data

Total animals in extract	3245
- **those with a known sire**	**1302**
- **those with a known dam**	**1715**
- **those with a birth date**	**3110**
Total weights in extract	22034
- **animals with at least one weight**	**2911**
Total scrotal records in extract	526
- **animals with scrotal record**	**346**
Total fat depth records in extract	1780
- **animals with fat record**	**771**
Total condition score records	24816
- **animals with condition score**	**2875**

Weights Statistics

The aim of the initial analysis is to look at what data is available to estimate genetic parameters for Swamp Buffalo. As such, we need known sires, dams and birth date. Twins and ET calves are also excluded from the parameter estimation analysis as well. The weight data was pruned to exclude various categories of animals and then divided into traits based on standard BREEDPLAN age ranges (Table 2).

Table 2. Pruning of the Weights File

Total weight records	22,034
No sire	**- 10,518**
No dam or sire	**- 462**
No DOB or 01/01/yy	**- 2**
Twins/Triplets	**- 65**
Useful weight records Remaining	10,989

Table 3. Weight records by trait

Trait	Age Range	Records	Wt Range	Animals
BW	**age = 0**	**1,177**	**15kg to 69kg**	**1,177**
W2	**80 – 300**	**2,825**	**30kg to 493kg**	**1,105**
W4	**301 – 500**	**2,016**	**93kg to 632kg**	**1,032**
W6	**501 – 900**	**3,194**	**122kg to 668kg**	**1,002**
Young/Old	**1 - 79 and > 900**	**1,777**	**excluded**	

To account for variation in environment over time, weight records were placed into contemporary groups based on birth year / sex / management-group /slice for birth weights and birth year / sex / weight-date / management-group / slice for post-birth weights. In all cases, age slicing was set to 60 days (ie only animals born within a 60 day period or slice are included in a contemporary group).

Further edits were imposed to the data based on requirements for Variance Component Estimation. Records were excluded if the birth date of the dam was unknown; and contemporary groups excluded where only a single sire or a single animal was represented. Table 4 outlines the number of records used in the Variance Component Estimation for each weight trait. From a parameter estimation perspective, this is a very small data set. At best, only ballpark estimates can be obtained from such a data set as the standard errors around the estimates will be high.

Table 4. Number of weight records available for variance components estimates.

Trait	**Records**	**Animals**	**Sires**	**Dams**
BW	761	761	51	382
W2	1,315	821	53	401
W4	1,522	792	52	390
W6	2,436	766	52	383

Repeat records were available for W2, W4 and W6 traits.

Figures 1 to 4 show the distribution of the weight records by year of weighing. This clearly shows the dislocation of the data from the "old" and "new" groups of animals as a result of culling the entire "old" herd due to Tuberculosis. The gap in the recording of data shown around the year 2000, compromises the data structure and is not ideal for estimating reliable genetic parameters. However, due to the small numbers of total records available, we used the entire database ignoring this data structure issue. This may also present problems when a BLUP analysis is run.

Figure 1. Distribution of birth weight records across year of weighing.

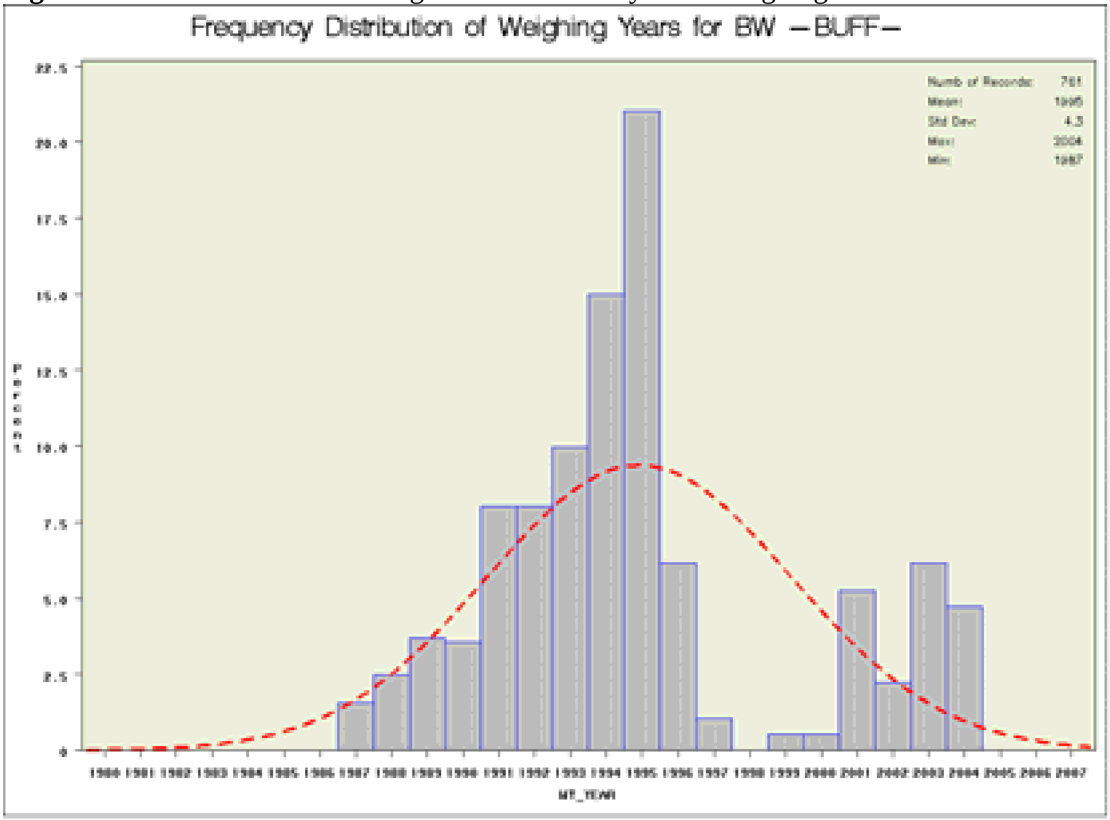


Figure 2. Distribution of W2 weight records across year of weighing.

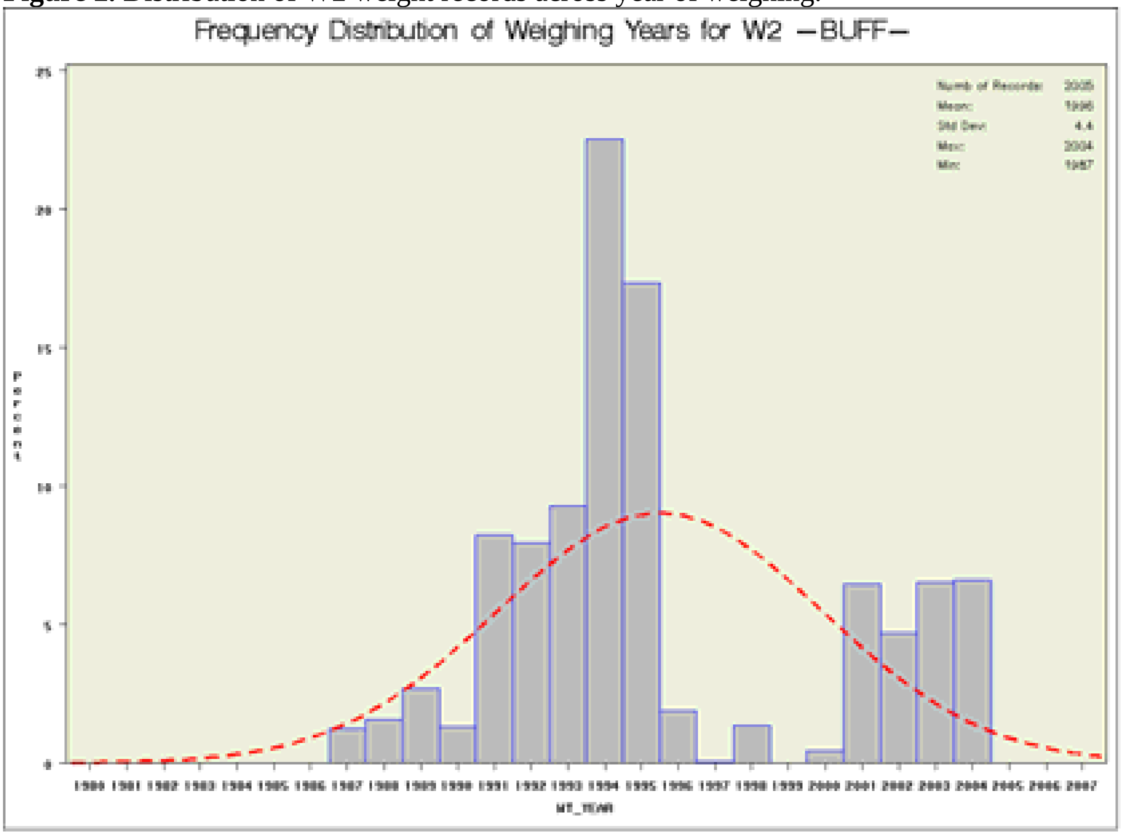


Figure 3. Distribution of W4 weight records across year of weighing.

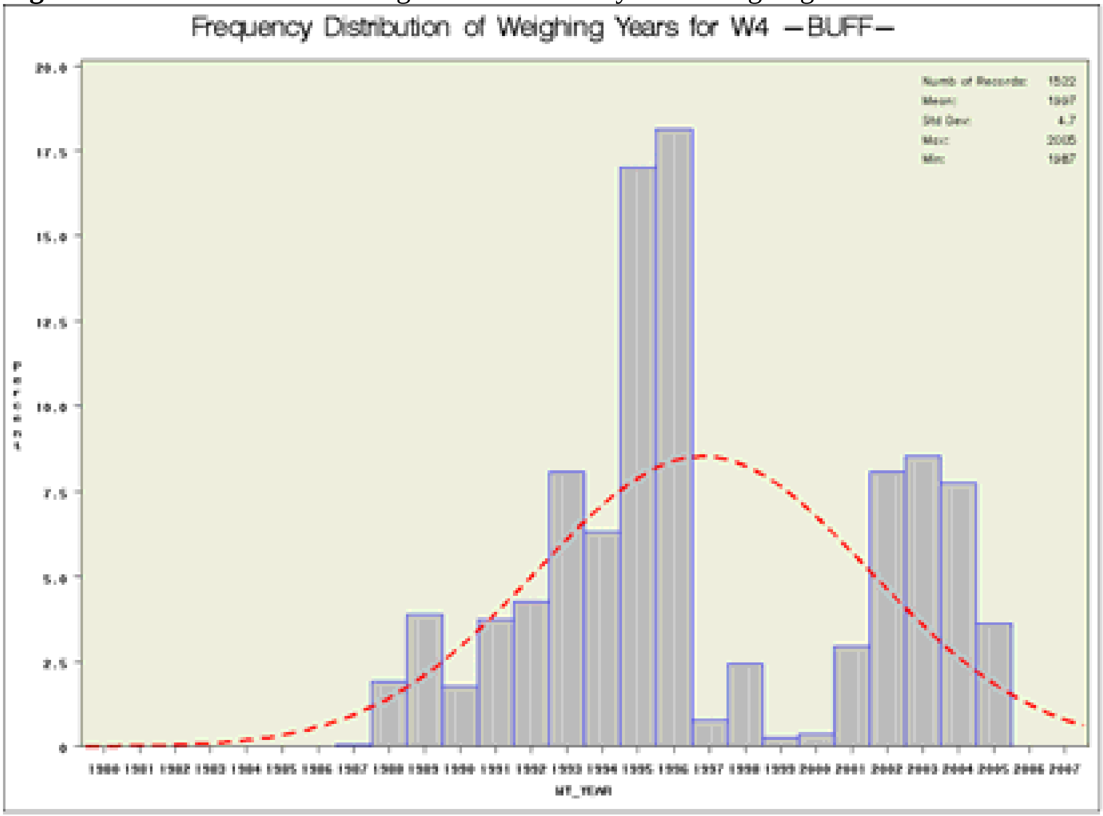
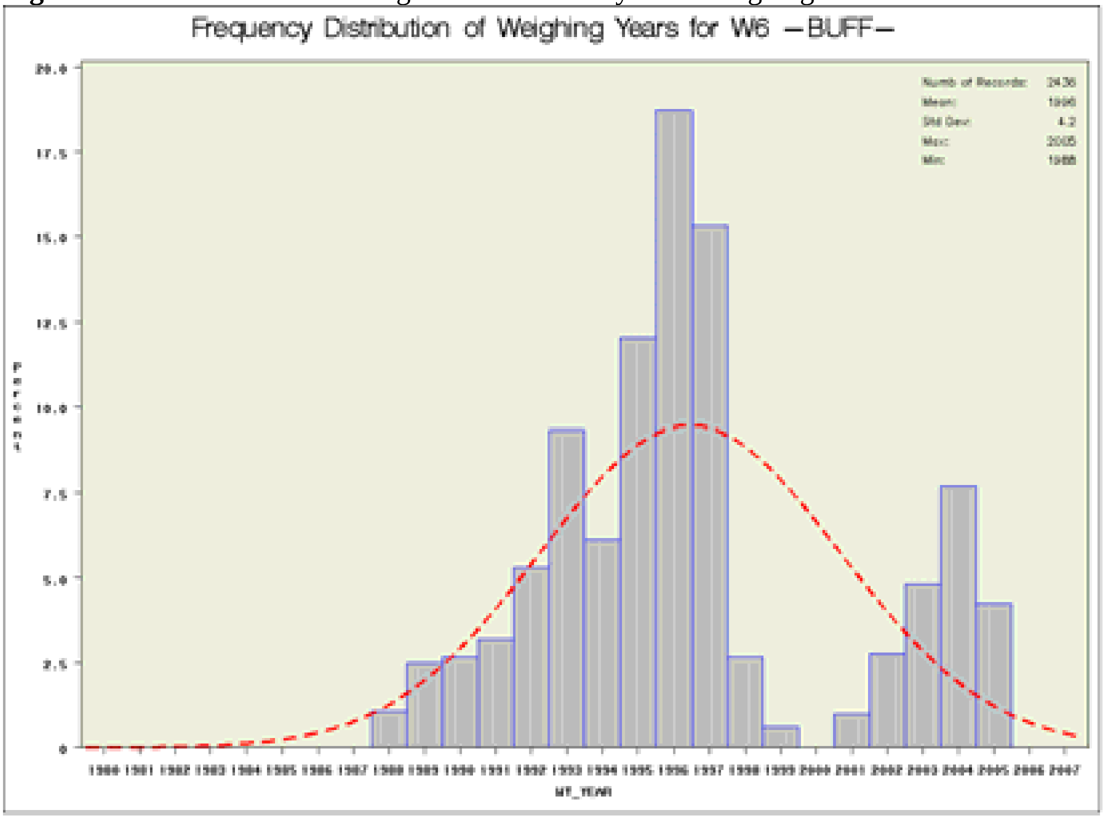


Figure 4. Distribution of W6 weight records across year of weighing.



As part of the analysis to estimate variance components for this dataset, a repeated records model (fitting the permanent environment of the animal effect) was fitted to account for the repeated records for the same trait. In addition, the W4 and W6 records were checked for month of weighing distribution (Figures 5 to 8). The W4 weights tend to be scattered throughout November to June with only small numbers weighed in the July to October period. The majority of the first W6 weights were taken around September, but month of weighing was less clear as multiple W6 records were included.

Figures 9 to 12 outline the distribution of the raw weights for each trait.

Distribution of birth weight and W2 weights tend to follow normal distribution, while W4 and W6 weights tend to be bi-modal and probably reflect different management over time and types and sex of animals. However, given the small number of records available, this was not investigated further.

Figure 5. Distribution of first W4 records based on month of weighing

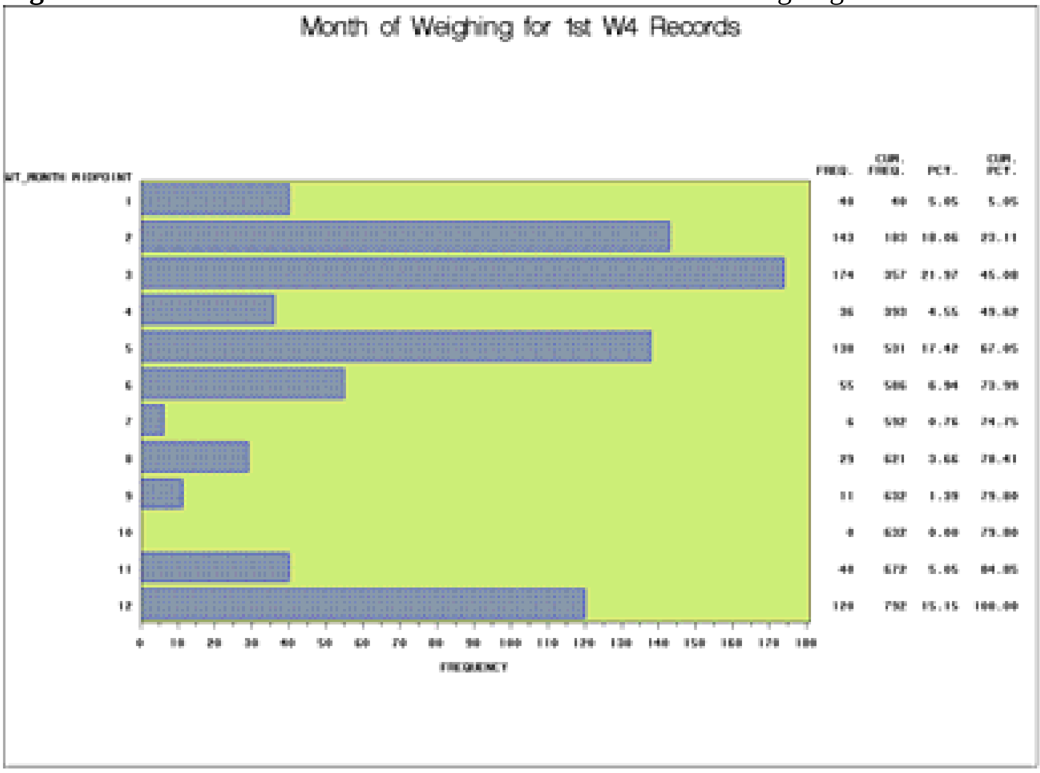


Figure 6. Distribution of all W4 records based on month of weighing

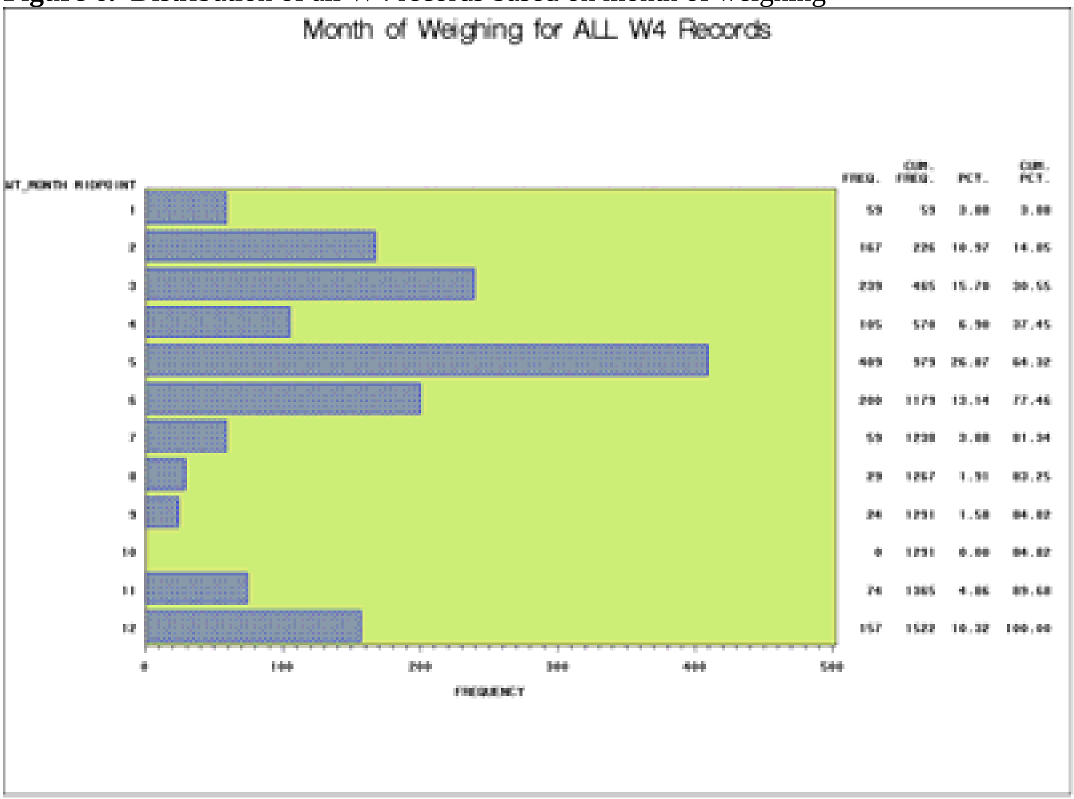


Figure 7. Distribution of first W6 records based on month of weighing

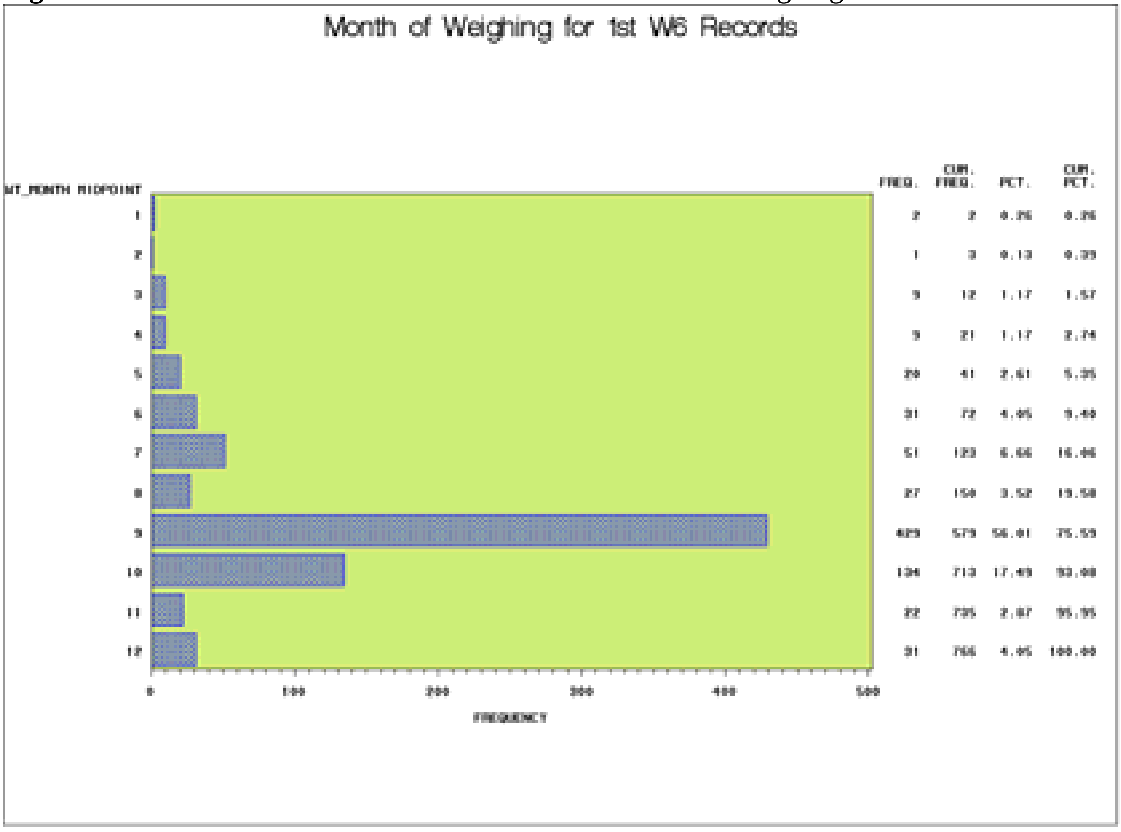


Figure 8. Distribution of all W6 records based on month of weighing

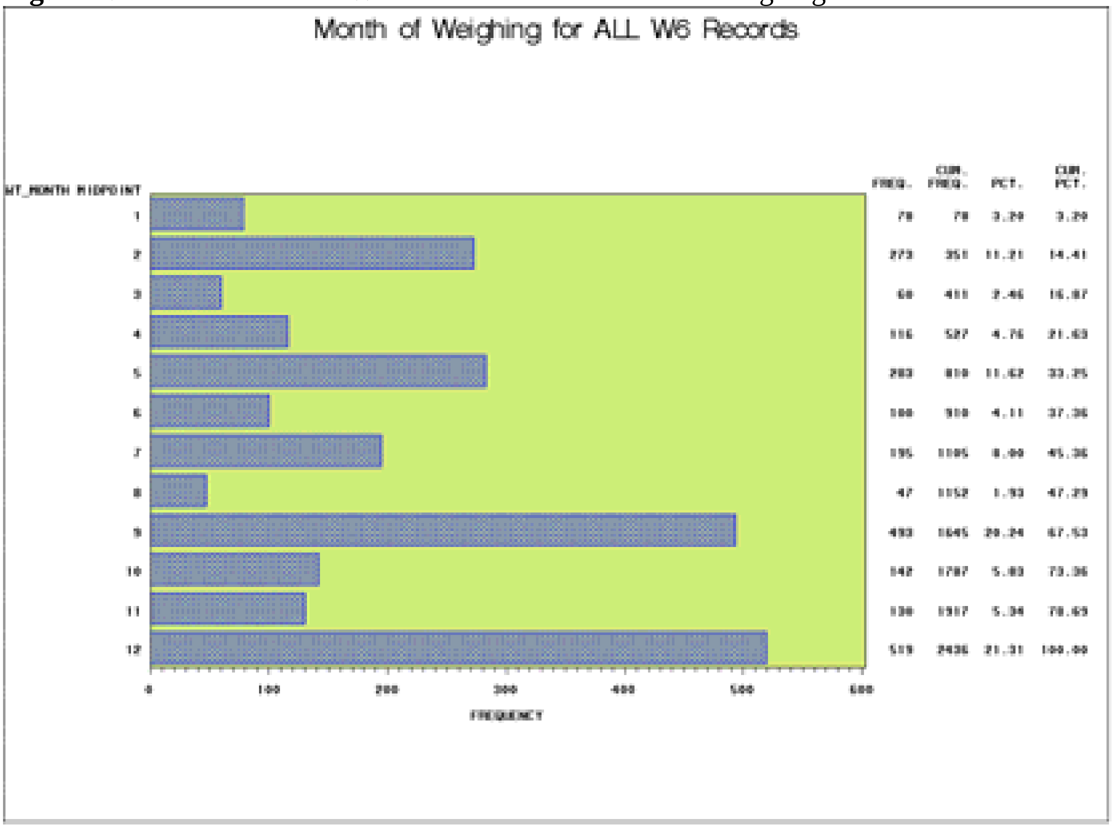


Figure 9. Frequency distribution of birth weights

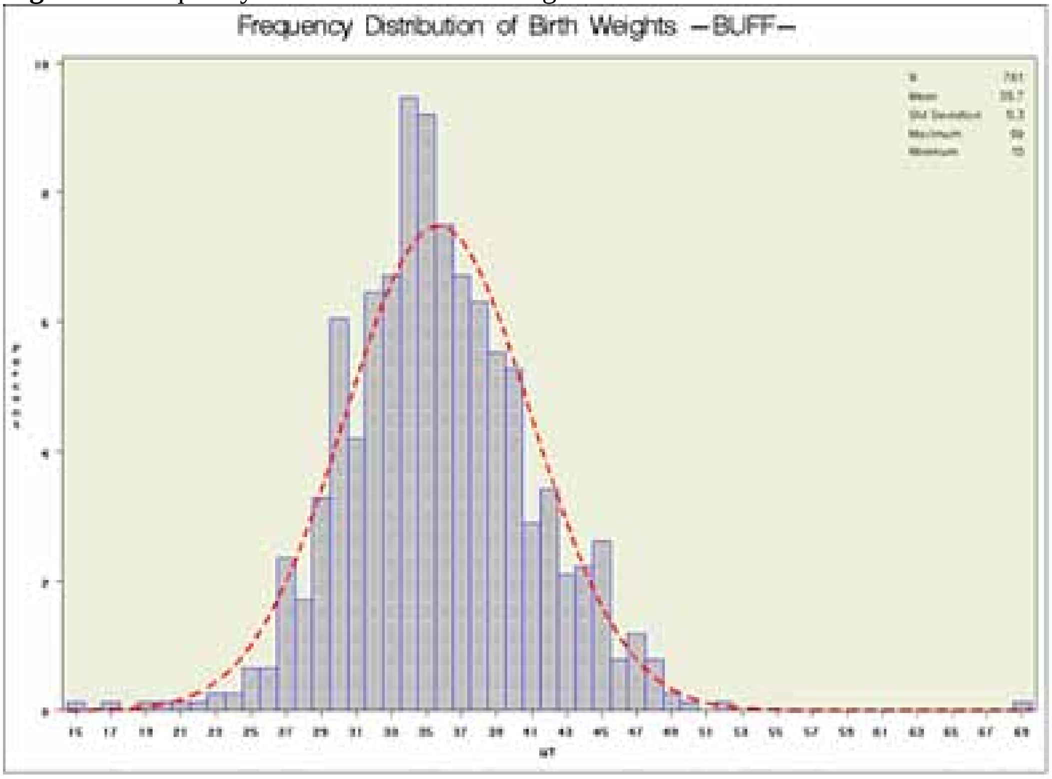


Figure 10. Frequency distribution of W2 weights

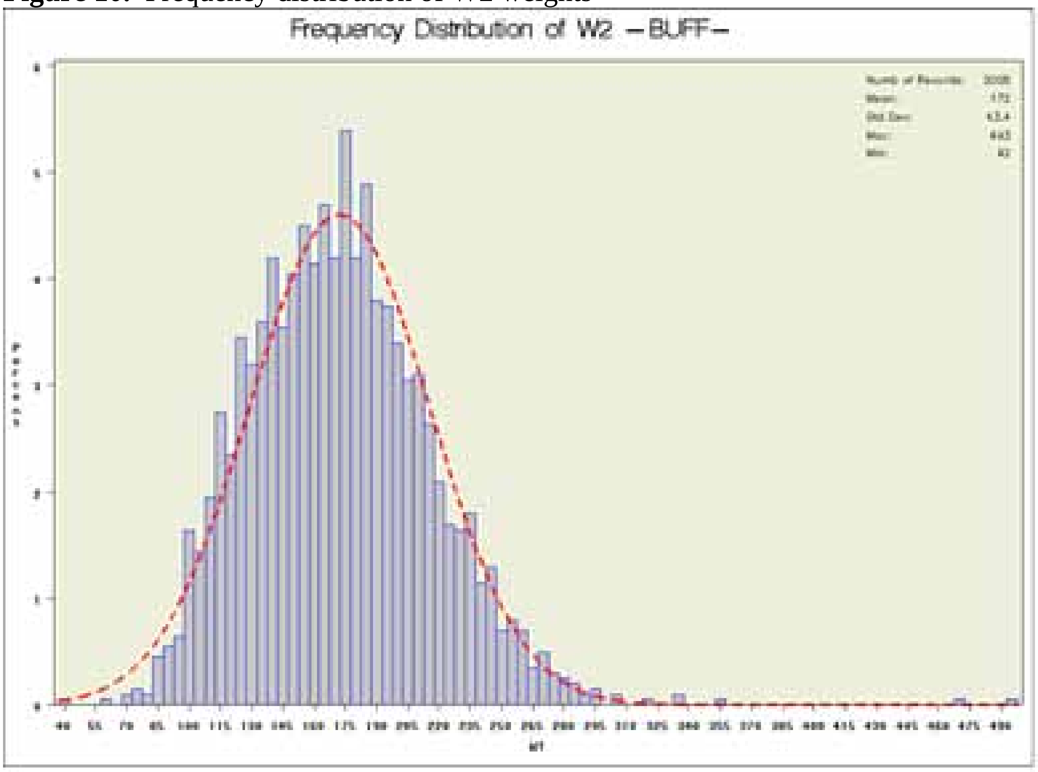


Figure 11. Frequency distribution of W4 weights

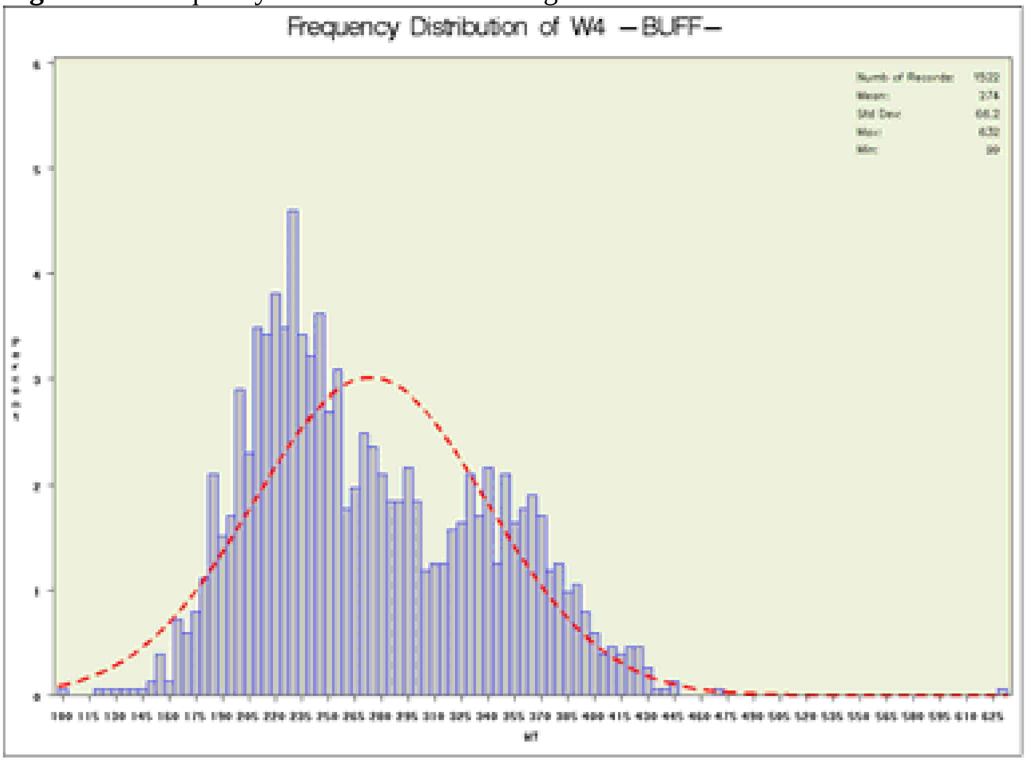
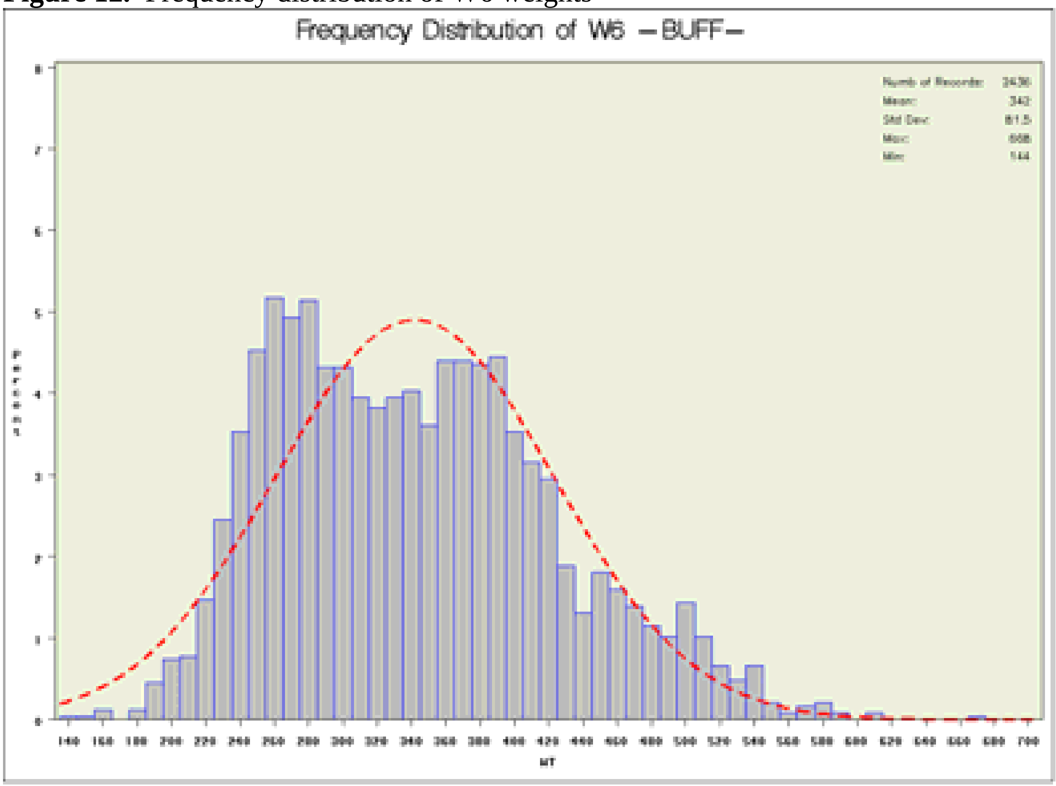


Figure 12. Frequency distribution of W6 weights



Data Analysis Models

Different models were fitted to the weight traits. The models used for BW (Table 5) are slightly different to the models used for W2/W4/W6 (Table 6).

Due to the small amount of data available for parameter estimation, sire breed effects (Swamp, Riverine) were not considered. While it is expected that there will be performance differences between the two, the structure of the data isolates the performance into separate contemporary groups so head to head comparisons were not available.

Table 5. Description of models used for estimating BW parameters

BW	Fixed Effects				Random Effects			Corr
	CG	AOD	AOD^2	HFD	Anim	Dam	PE(Dam)	A-M
Model 1	x	x	x	x	x			
Model 2	x	x	x	x	x	x		
Model 3	x	x	x	x	x	x	x	
Model 4	x	x	x	x	x	x		x
Model 5	x	x	x	x	x	x	x	x

CG = Contemporary Group effect fitted as a class (absorbed before the analysis)
AOD = Age of the Dam defined as deviation from 5 year old, fitted as a co-variable
AOD^2 = AOD quadratic effect, fitted as a co-variable
HFD = Age of the Dam (Heifers) nested within the Heifer class
Anim = random additive genetic effect for animal
Dam = random additive genetic effect for dam
PE(Dam) = random permanent environment effect of dam
A-M = additive maternal correlation

CG for BW is defined as:
Original Owner + Sex + Calving_Year + Mgmt_Group + Heifer

Original Owner = Owner/breeder of animal
Sex = bull or heifer
Calving_Year = year when the calf was born
Mgmt_Group = Management Group defined by the breeder
Heifer = being (Y/N) if dam was less than 3.5 years old at calf birth

Table 6. Description of models used for estimating W2/W4/W6 parameters

WEA	Fixed Effects					Random Effects				Corr
	CG	AOC	AOD	AOD^2	HFD	Anim	PE(Anim)	Dam	PE(Dam)	R_{AM}
Model 1	x	x	x	x	x	x				
Model 2	x	x	x	x	x	x	x			
Model 3	x	x	x	x	x	x	x	x		
Model 4	x	x	x	x	x	x	x	x	x	
Model 5	x	x	x	x	x	x	x	x		x
Model 6	x	x	x	x	x	x	x	x	x	x

CG = Contemporary Group effect fitted as a class (absorbed before the analysis)
AOC = Age of the Calf at measurement nested within sex, fitted as a co-variable
AOD = Age of the Dam deviated from 5 yo is fitted as a co-variable
AOD^2 = AOD_DEV quadratic effect, fitted as a co-variable
HFD = Age of the Dam (Heifers) nested within the Heifer class (Y/N).
Anim = random additive genetic effect for animal
PE(Anim) = random permanent environment effect of animal
Dam = random additive genetic effect for dam
PE(Dam) = random permanent environment effect of dam
R_{AM} = additive genetic and maternal correlation

CG for W2/W4/W6 is defined as:

Original Owner + Sex + Calving_Year + Mgmt_Group + Wt_Date

Original Owner = Owners unique ABRI's key
Sex = bull or heifer
Calving_Year = year when the calf was born
Mgmt_Group = Management Group defined by the breeder
Wt_Date = Date at weighing.

Results

The results for each trait from the various models that were run are summarised in Tables 7 to 10. As expected, the results are constrained by the limited amount of useful data available for estimating genetic parameters. The standard errors on the variance components of all traits analysed are significantly large, hence these influenced our discussion of results and recommendations.

Explanation of headings for Tables 7 – 10:

- V_P – total phenotypic variance
- V_A – additive genetic variance
- V_D – additive variance due to the dam (maternal)
- COV_{AD} – covariance of additive and dam (maternal) effects
- PEA – permanent environmental effect of animal
- PED – direct permanent environment variance
- RES – residual variance
- h^2d – heritability of the direct component (standard error in brackets)
- ICC – intra class correlation for repeat records (standard error in brackets)
- h^2m – heritability of the maternal component (standard error in brackets)
- R_{AM} – correlation between direct and maternal effects (std error in brackets)
- Shaded row indicates possible preferred model for trait.

Table 7. Parameter estimates for BW trait (standard errors in brackets)

BW	V_P	V_A	V_D	COV_{AD}	PED	RES	h^2d	h^2m	R_{AM}
Model 1	24.8	16.8	-	-	-	8.0	0.68 (0.11)	-	-
Model 2	23.0	6.0	4.8	-	-	12.2	0.26 (0.13)	0.21 (0.7)	-
Model 3	23.0	6.0	4.8	-	0	12.2	0.26 (0.13)	0.21 (0.7)	-
Model 4	22.8	5.2	3.7	1.4	-	12.6	0.23 (0.13)	0.16 (0.12)	0.32 (0.75)
Model 5	22.8	5.2	3.7	1.4	0	12.6	0.23 (0.13)	0.16 (0.12)	0.32 (0.75)

Table 8. Parameter estimates for W2 trait (standard errors in brackets)

W2	V_P	V_A	V_D	COV_{AD}	PEA	PED	RES	h^2d	ICC	h^2m	R_{AM}
Model 1	627	411	-	-	-	-	216	0.66 (0.02)		-	-
Model 2	565	199	-	-	155	-	211	0.35 (0.09)	0.63 (0.02)	-	-
Model 3	546	30	126	-	178	-	211	0.06 (0.06)	0.38 (0.04)	0.23 (0.04)	-
Model 4	543	32	50	-	166	84	211	0.06 (0.05)	0.37 (0.04)	0.09 (0.09)	-
Model 5	599	298	255	-189	21	-	213	0.04 (0)	0.54 (0)	0.50 (0)	-.69
Model 6	599	298	253	-188	21	1.4	213	0.50 (0)	0.53 (0)	0.42 (0)	-.69

Table 9. Parameter estimates for W4 trait (standard errors in brackets)

W4	V_P	V_A	V_D	COV_{AD}	PEA	PED	RES	h^2d	ICC	h^2m	R_{AM}
Model 1	1291	1060	-	-	-	-	230	0.82 (0.01)	-	-	-

Model 2	1033	244	-	-	566	-	224	0.24 (0.09)	0.78 (0.02)	-	-
Model 3	1007	54	154	-	574	-	225	0.05 (0.06)	0.62 (0.05)	0.15 (0.05)	-
Model 4	1007	53	115	-	567	47	225	0.05 (0.06)	0.62 (0.05)	0.11 (0.09)	-
Model 5	1008	1075	589	-655	-	-	227	0.06 (0.07)	0.63 (0.05)	0.19 (0.10)	-.33 (0.7)
Model 6	1007	62	146	-30	560	45	225	0.06 (0.07)	0.62 (0.05)	0.15 (0.13)	-.32 (0.8)

Table 10. Parameter estimates for W6 trait (standard errors in brackets)

W6	V_P	V_A	V_D	COV_{AD}	PEA	PED	RES	h^2d	ICC	h^2m	R_{AM}
Model 1	1222	953	-	-	-	-	268	0.78 (0.01)	-	-	-
Model 2	1070	499	-	-	305	-	266	0.47 (0.09)	0.75 (0.02)	-	-
Model 3	1066	457	36	-	306	-	266	0.43 (0.11)	0.72 (0.05)	0.03 (0.05)	-
Model 4	1064	444	0	-	298	55	266	0.42 (0.10)	0.70 (0.05)	0	-
Model 5	1114	772	276	-336	137	-	266	0.69 (0.19)	0.82 (0.08)	0.25 (0.12)	-0.73 (0.14)
Model 6	1113	776	176	-320	120	95	266	0.70 (0.18)	0.81 (0.08)	0.16 (0.14)	-0.87 (0.21)

Of the 526 scrotal circumference records in the data, 268 records were available for analysis after data edit procedures were applied. The average scrotal circumference was 19.1 cm with a standard deviation of 2.2 cm. There was not enough scrotal data available to do any further analysis.

Of the 1780 fat depth records in the data, 630 records were available for analysis after data edit procedures were applied. The average fat depth was 16.1 mm with a standard deviation of 12.8 mm. There was not enough fat depth data available to do any further analysis.

Condition scores are expected to be highly correlated with weight. As such, the weight traits need to be defined within a model prior to considering the condition score. Given the high standard errors around the weights parameter estimates, it was felt that it was not worth analysing condition score at this point in time.

Mature cow weight records are dependent upon the cow and calf being weighed at about the same time. As it is difficult to define the calf weights at present, it was felt that analysing the mature cow weight data should be held over until the parameters for the W2 trait were better defined.

Discussion

The data set has relatively few records suited to variance component estimation. The maximum number of animals available for variance component estimation for any trait was 821 animals (Table 4). While there are many more performance records available, the lack and structure of the pedigree information is the main limiting factor in using them for variance component estimation. The pedigrees are also shallow - which further constrains variance component estimation.

It is expected that this lack of pedigree will be gradually overcome as the Beatrice Hill herd stabilises and rebuilds after being decimated by Tuberculosis. By necessity, the rebuilding of the Beatrice Hill herd used females from various sources that did not have birth and pedigree information available.

As well as effecting the reliable estimation of variance components, the disjointed nature of the Beatrice Hill herd over years (original herd pre 2000 and current herd post 2000) may cause problems from a genetic evaluation perspective as well. While this problem was forced upon the NTDPIF&M, we may need to separate the original herd from the current herd when running genetic evaluations due to the lack of genetic linkage between the two. This will be investigated further.

The results obtained from the variance component estimations show big standard errors which are a reflection on the amount and structure of the data available. It is expected that diligent recording of performance data and pedigrees in the current Beatrice Hill herd will overcome the problem. As such, parameter estimation should be considered again in a few years when enough records are collected. As a guide, reasonable parameters can be estimated for most traits when there are at least 2000 (preferably more) animals in the data set with a complete pedigree structure, with genetic linkage of these animals across years, and recording of the traits to be estimated. Some consideration needs to be given to what traits are a priority and ensuring that actions are put in place to collect the information in as short a time frame as possible within the constraints of budgets and personnel. The volume of data may be enhanced from other herds contributing information.

No milk production records were available at the time of this work. However, it is anticipated that milk recording will start to be recorded soon. Therefore, further parameter estimations should consider the milk traits as well.

Any new parameter estimation work should also include estimation of raw data adjustment factors and age of dam effects.

While the current data set does not give parameter estimates that we could confidently run a genetic analysis, it has been a useful exercise in getting a profile of the data. It is anticipated that ABRI and NTDPIF&M can work together to build on the existing data to develop a useful database over time.

Some of the preliminary results indicate that certain variance components are reasonably close to the preliminary genetic parameters estimated for Water Buffalo in Thailand (Table 11) – developed as an outcome of a joint project funded by the Australian Centre for International Agricultural Research.

Table 11. Genetic parameters calculated for Water Buffalo in Thailand.

	BW	W2	W4	W6	BWm	W2m
BW	0.21	**0.40**	**0.2**	**0.2**	--	--
W2		0.14	**0.4**	**0.4**	--	--
W4			0.14	**0.4**	--	--
W6				0.19	--	--
BWm					0.08	--
W2m						0.09

Heritability on diagonal (bold), correlations on off-diagonals

It is suggested that the genetic parameters estimated for Water Buffalo in Thailand be used for Buffalo in Australia until sufficient records are available within the Australian data set to estimate their own genetic parameters.

Appendix 3

Report: 7th World Buffalo Congress, Manila, Philippines

20 – 23 October 2004
Shangri-La Hotel, Makati

Attended by Barry Lemcke (DBIRD)
Bob & Christine Cook (ABIC President)
Dr Ken Philips (ABRI, New England University)

The Congress was held in conjunction with the 41st Philippines Society of Animal Science (PSAS) Scientific Annual Convention.

I was invited by Organising Committee to present a paper entitled *"Production of Specialised Quality Meat Products from Water Buffalo - TenderBuff"*. A powerpoint presentation was made to the Congress taking 40 minutes, plus a question and answer period for a further 10 minutes. The presentation was well received. I was also asked to chair two sessions relating to Meat Production; on Thursday 21st October the Symposium IA on Production Systems/Management and Friday 22nd October on Meat (Symposium 5B).

There were up to 3 simultaneous sessions going at the one time so that not all presentations could be attended, however the Organising Committee made available all the presentations in printed form during the Congress. This included:

1. A volume of all the invited papers
2. A volume of contributed papers
3. A volume of recent developments in animal production (non-buffalo) for PSAS convention
4. A volume on the pre-congress training in the use of reproductive biotechniques in water buffalo
5. A volume on the abstracts of all the papers
6. A CD covering invited papers, contributed papers and abstracts

This was brilliant effort by the committee to have all these available in printed form before the end of the Congress.

I was unable to join the Congress tour on Saturday 23rd October and overnight, of the Philippines Carabao Centre in Neuva Ecija (National Gene Pool) and visit to small holder farmers and buffalo-based village cooperatives because the flight availability out of Manila. I have previously seen the PCC centre in 2001. Mr Bob Cook went on the tour and will report on that via ABIC.

3.1 The Current World Buffalo Situation

Numbers are now estimated at 166 million of which 97% (161 million is in Asia) India is the largest with 103 million head, slaughtering 10% per year and producing 1.43 million tonnes of meat, of which 300,000 tones are exported, particularly to Malaysia 80,000, Philippines 47,000 tonnes and the balance to the Middle East.

Areas with the greatest rate of buffalo growth are in South America, where the big movers are Brazil (now 3,000,000 head), Venezuela (250,000 head), Argentina (1,300 in 1970 to 70,000 now), Columbia (40,000 head) is also increasing rapidly. Interestingly, Columbia has a premium market in Germany for organic meat. They report in a paper in the proceedings extremely good growth rates of buffalo on pastures, well above that described in Venezuela and Brazilian and Australian data. There must be very good climatic conditions for pasture growth and good use of fertiliser and stocking rates to achieve those figures. Cuba has 30,000 head and USA about 20,000 head.

Progress in Venezuela is in the production of new and different cheese varieties.

In Europe, Italy is increasing in numbers – now 300,000 up 143% since 1993. In Turkey, Bulgaria and Romania numbers are decreasing. There are 500,000 in Iran. Increases in Italy are mainly in cheese making. Recording is carried out in about 30% of total herd with a mean 270 day lactation of 2,168 kg (8 kg per day). Some individual cows produce 5,000-6,000 kgs which averages out at 18-22 kg/day.

It is interesting to note the mindset and disparity between production parameters accepted by the different regions of the world. In areas of the world where buffalo have free access to good quality pasture with no restriction, productivity is very high. In many regions, productivity is so abysmal, that it is hardly believable. Typical in most of Asia is the slow growth rate, poor reproductive rate scenario and they don't believe or understand the potential of the animal that they have.

The Philippines itself has goals in self-sufficiency in dairy products in the long-term: ie importation replacement. It is investing heavily in buffalo research and development through the Philippines Carabao Centres that have been set up in each region (13). They have imported Murrah mainly from Bulgaria, but also have arrangements with India for Embryo Transfer material.
They are doing a lot of work on biotechnology, milk and meat. They have reported tests comparing cattle and crossbred buffalo for meat. These include chemical, taste panel, and physical attributes and there appears to be only minor differences between the two species. The crossbred buffalo under feedlot conditions grew faster than the cattle. The standout problem was the average pH of the meat, of both species was way too high at around pH 6.0. There must be big problems at the pre-slaughter handling stage.
Anyone who would like a copy of the proceedings of the congress would be able to copy the CD that I brought back from Manila as the best means of doing it efficiently.

There is so much work being done on buffalo in the world at present, and this forum proved a great way of disseminating the information. I was able to discuss our AI problems with the Brazilian delegation and they confirmed my suspicion that fat in some cows may be too thick to penetrate through to the muscle, and there is a 3 day delay between the drug becoming active if it is injected into the fat tissue. An alternative site where there is minimal fat for injecting needs to be located. They still routinely use the rump site for injecting the drugs for synchronising oestrus in the cows. They have done many thousands by this protocol and get 50% pregnancy rates using the method. We still have a long way to go to get that percentage.

There were two other issues:

1. Holding the 2010 (9th World Congress) in Darwin.
2. Determining the practicalities of importation of Australian buffalo into the Philippines.

The 8th World Congress

The 8th World Congress was supposed to be held in Cuba in 2007, however they did not appear at this Congress and were no longer willing to host. China had put a circular around during the day to the IBF Standing Committee members that they would be prepared to host the 8th Congress in Nanning China in November 2007, in conjunction with the 5th Asian Buffalo Congress. However, they did not turn up to the Standing Committee meeting to press their case. It was eventually awarded to Naples in Italy. During the process, Argentina put up their hand to host the 2010 9th Congress before I had a chance to put in an offer. I had previously been told by several other delegates, that it was about time to hold one in Australia. I then gained a little more knowledge on the costs involved in staging the Congress. The Italians said that between $200,000 and $300,000 US was needed to hold the Congress.

The Congress does get some sponsorship from FAO but it is up to the organising committee, ie: the host country, to arrange sponsors. The Italians mentioned agri-business companies as potential sponsors in Italy. The Philippines had FAO, their own PCC, and JICA (Japan International Cooperation Agency) and ILRI (International Livestock Research Institute) as sponsors. There was a cast of ‘thousands’ on the Congress Committee which is no problem in a country the size of the Philippines, but would be a major struggle in the Australian context.

I mentioned a possible NT Government interest in hosting to the Argentina and Venezuelan delegates and they passed it on to the new Italian President and Honorary Secretary. They agreed to allow us to think over our bid and inform them within a year’s time of our decision on hosting the 2010 Congress in Darwin.

Along with the Congress goes the Presidency of the IBF for the 3 year period. To me the task appears to be quite daunting unless there was professional help in organising the Congress and a separate NT Government grant made available to cover the up-front costs up to 2 years in advance of the event occurring. It would showcase our buffalo industry and relies on its ability to continue past the next 5-6 years without folding up. This also spans two further NT Government elections so the commitment is long-term.

The Philippines pulled in the PSAS (Philippine Society of Animal Science) to make a joint congress to help boost the numbers internally. A much lower number of colleagues would be available in Darwin. The final 12 months before the congress would be an extremely busy period for the organising committee of the host country.

Knowing the resources available to me, I would think that it would be a huge commitment for me and a lot of other people to stage this event, so I would be very hesitant without the promise of significant backup. At this stage I am not sure of the proportion of the total cost that was sponsored.

3.2 Australian Importation into the Philippines

There has been virtually no increase in meat production of buffalo in the Philippines between 1991 and 2000, with an annual average human population growth rate of 0.4%. I did not think that there would be any interest in importing buffalo as they have 3.12 million already. The Carabao rate of slaughter was 333,300 head in 2001 – a little more than 10% of the population. Imported Indian buffalo meat represents 40% of the total consumed (estimated at 316,000 head equivalent). There is an increasing trend in the consumption of buffalo meat from 1996 to 2001. Buffalo meat currently fetches around 200 Pesos/kg in Manila. This is equivalent to around $5.00 Australian. At 50% carcase this is around $2.50/kg liveweight, so there is some scope for live export, particularly if the price continues to be significantly lower than the current price of cattle.

On the Friday of the Congress a representative of AustAsia, Philip Bielefeld (Divisional Manager) was at the meeting to gauge interest in selling Australian buffalo there. They already have suitable infrastructure in place in Indonesia and the Philippines and access to the meat markets.

Our best strategy would be to work closely with AustAsia from this end in Darwin to get a trial shipment in place to see how they perform under Philippine conditions in feedlots, and work with them to formulate suitable rations. Because they are based in Darwin this makes it easy to organise the logistics. The Department has suitable stock of both swamp and river cross to supply as a trial shipment with AustAsia.

I feel this is the best avenue, as once they get animals in to the Philippines, other groups such as Del Monte and Monterey, etc will see what is happening and will get interested.

Appendix 4.

Report: 8th World Buffalo Congress, Caserta, Italy - October 2007

Attended by Barry Lemcke (DPIFM) and Mitch Humphries (Queensland Buffalo Milk Producer

4.1 Pre Congress Cheese Technologies Course

Monday 15th October 2007

1. Processing of Buffalo Milk into High Quality Products - F. Addeo, Prof. L. Chianese (Naples)

This first session was by far the most important in getting high quality technical information on making mozzarella cheese. The basics of the process are to heat the whole buffalo milk to 37°C and starter culture is added. After starter culture is added, rennet is also added and the mixture is then allowed to acidify down to a narrow range of pH. In the meantime the curd is cut to allow the whey to separate. When the specific pH is achieved through the breakdown of lactose to lactic acid, the whey is removed by the use of a large colander type basket. The curd is then minced finely and put in a large tub. Hot water is added to heat the curd and it is folded over and over to incorporate 50-65 per cent moisture. Once stretched by folding with a rod or by machine, the stretching water is removed and the curd is removed and moulded into balls in cold water using two people – one creates a smooth ball, the other pinches it off into the cold water. After cooling in the cold water the mozzarella/boccincini balls are then transferred to a brine solution for preservation and transport. This brine contains from 3-20 per cent salt and can be acidified with citric or lactic acid, and depends on consumer preferences.
The Caserta/Salerno region – Campania has the largest numbers of buffalo in Italy and is believed to make the best quality mozzarella. To protect this traditional method there are special regulations and restrictions on its production called Denomination of Origin Protected (DOP), meaning that the traditional methods are used and protected for that area by legislation.
Mozzarella can be made using direct starter, whey or milk-based starters. The whey starter only is allowed for DOP manufacture and it is produced by collecting whey and holding it in storage for usage the next day.
The starter is a mixture of two specific bacteria Lactobacillus and Streptococci (rods and cocci). The cocci are in the majority at the start of the process and the rods dominate at the end of the process.
There are shops and factory outlets everywhere selling mozzarella di bufala di Campania (or whichever Province it is made in) and it is in greatest demand in the summer months.
Unfortunately this is in the non-breeding season in buffalo – they are short-day breeders and in Italy the natural calving peaks are in autumn and winter. Sales drop during the winter when salad usage wanes and tomatoes and basil are not readily available. These are traditional accompaniments when eating the cheese. It is sold usually in plastic bags, or sealed tubs or buckets for export with the transport brine included. Machines are available to mechanize the whole process once the curd is properly formed. This includes the mincing, stretching and ball formation. In machine based manufacture the ball sizes depend on the size of the indentations in the rotating drum that is attached to the machine. It is just a matter of changing drums on the machine for different batches. Traditional mozzarella cheese-making uses the whey starter and whole non-pasteurized milk. Pasteurization is required for export.

There are 8 steps involved and milk needs to have minimum quality of protein 4.2 % and fat 7.2 %.

1. Pasteurization (optional for DOP) Heat to at least 37°C for unpasteurized.
2. Adding starter
3. Rennetting and setting
4. Cutting the curd –demineralization and acidification
5. Whey draining
6. Milling the ripened curd

7. Cooking/stretching
8. Cooling and brine storage

Ball sizes are usually boccincini (cherry size), golf ball and tennis ball sizes. Larger balls can be made by hand forming a long rope 60 cm or so long and then plaiting into elongated rolls 20-30 cm in length.
The product is also traditionally smoked or allowed to dry into lower moisture products particularly for pizza cheese Scamorza (drier no stretching), Caciocavallo (harder) and others. Other balls are tied using twine or string to form a head and body arrangement and further dried or smoked.
The stretching liquid can further be processed to make butter by cooling and skimming. Ricotta is produced by heating the whey to 88°- 90°C using steam and cheese comes to the top and is removed using a special scoop into little or large plastic moulds and allowed to drain, then refrigerated. The cheese can be made smoother by adding extra whole milk to the whey before heating. A second reheating also gives different textured, more coarsely grained ricotta.
Due entirely to the resourcefulness of the New Zealand contingent on the Thursday instead of another factory visit, we were able to participate and observe the full procedure over a period of four hours in the Imposta cheese facility and also visit a local supplier of cheese equipment to see what was available. The Kiwis bought quite a bit of the gear needed for hand-making mozzarella and carried it all over Italy.
The rest of the day was spent in the following lectures.

4.2. Milk Bacteriological Analysis – R. Rosati

Outlined what organisms may be present in milk – and the more frequent mammary diseases in Buffalo. Explanation of mastitis pathogens and how to test for them. Laboratory analysis systems – plating out of quarter samples onto blood agar or Edwards modified medium and McConkeys agar. Incubation at 37°C for 48-72 hours.
Gram staining separates into rods, cocci and others.
The main cocci are streptococci and staphylococci.
Gram +ve rods are coryneforms.
Gram – ve rods are coliforms, serratia or pseudomonas.

Bulk milk sampling:
1. Agitate for 10 mins
2. Collect 50-80 ml from top of bulk tank
3. Label samples
4. Transport to lab at +4°C

Some bacteria are psycotrophic – (able to survive refrigeration temperatures.)
Plate counts require six step dilution 1 ml to 9 ml x 5 then counts after plated.
There are automated systems available (at a price) such as Flow Cell and Bactocount.
Samples must be analysed with duplicate within 24 hours of collection.

4.3. Professor Maria Contesi (University of Naples)

Professor Contesi made a comprehensive explanation and interpretation of EC rules that apply to dairy products covering food safety requirements, presentation responsibilities and obligations of the food trade. Professor also discussed the foodstuff hygiene responsibilities of primary producers.
There appears to be flexibility to enable the use of traditional methods of food manufacture eg. DOP mozzarella.

There are three objectives

1. Food safety
2. Fair trading between operators
3. Reliability of information provided by producers

HACCP principles generally do not apply to primary production, but do in processing of that food.

4.4. Some Diseases of Buffalo Milk – G. Galero (Veterinary pathologist)

Focussed on the incidence of Enterohaemorrhagic E. coli from buffalo dairy products. This particular strain E coli 0157 is very serious to humans. It attacks and damages the alimentary canal and 5 per cent of young children can develop Haemolytic Uraemic Syndrome (HUS) which causes renal failure. The main routes of transmission were from faeces,to milk and meat.

Tuesday 16 October 2007

Riva Bianca Cheese Factory.

The group was divided into 2 to visit several factories to keep the numbers manageable.
Riva Bianca had a retail shop in front of the factory and appear to have a staff numbering around fifteen. They were doing mozzarella batches when we visited and we watched the curd preparation, stretching with hot water and hand shaping the mozzarella balls. Two people each using both hands are needed with one forming the ball and the other pinching it off into the cold water bath. There is a lot of staff involved when producing hand-made mozzarella. After going through the factory, we tasted a selection of four of their cheeses, Mozzarella, Ricotta and two Caciottas.
Prior to lunch we visited the Comat Cheese Equipment Manufacturing Factory in Bellizzi where we saw a large range of machinery made for various sized cheese-making operations. Stainless steel is the basis of most equipment and a lot is coated with Teflon paint to prevent stickiness of the proteins in cheese, especially when heated.
After lunch at Imposta we used the break in the program to accompany Prof Addeo to have a look at a cheese factory supplies shop, the New Zealanders bought some basic cheese-making gear that they were unable to source in New Zealand – cheese curd cutters, moulds, bowls, sieves etc and also shown the recommended starter culture packets, the details of which were noted (Dalton Brand).
We walked back to the Hotel, misjudged the distance at the start – probably 9 km.

Wednesday 17 October 2007

We opted out of the organised factory tour. We stopped at Cheese Equipment Shop to pick up the New Zealand order from yesterday. Due to some smooth talking with Professor Addeo, by Richard Dorresteyn (NZ), we got a private invitation to a mozzarella production session – from start to finish at the “Azienda Sperimentale Imposta” cheese facility.
Each stage was monitored using pH and SH titrations of the whey and curd during the process of setting and acidifying. This was a most useful session for both of us, for Richard to get some of the finer points as he had already tried making it previously in New Zealand, and for me as a complete novice. It is obvious that the master cheese maker has skills honed by many years of experience and much is done by touch and feel, not measurement. The whole process started at 8am and finished about 1pm.

Approximate timing:
Warming at to 37°C at 0 hrs
Starter added @ 37° C 0.1 hrs
Rennet added
1st cutting of curd + 2 hrs
Fine mincing of curd + 2.2 hrs
Removing whey + 2.3 hrs

Making ricotta + 3.0 hrs
Cutting & mincing curd + 4.5 hrs
Putting on table + 5.05 hrs
Stretching in batches + 5.10 hrs

Some made differing designs from the fresh, hot mozzarella, pulling into long rope and plaiting or knotting etc. Smaller balls – usually machine made are "boccincini".

After lunch at Improsta, we prevailed on a local Guiseppe Morese to have a look at his farm – free time for the Cheese Technology people – however he took us – Kiwis and I to Vannulo Farm which was an organic operation. This was a very palatial set-up with beautiful buildings and retail outlets, cheese and yoghurt factories, milking parlour and sheds housing the herd. Surrounded by paddocks where the feed was made and sheds for storage of hay for winter, silage bunkers also for year-round feed use. Very impressive and obviously no shortage of cash/capital spared here.

The farm had a total of 600 head buffalo – with 200 being milked. They raise the male calves for meat production. Rations are silage/grass hay/ straw based rations at 40-50 kg/head/day as fed. This includes the wet silage hence the high levels of feed. The farm has been running buffalo for 100 years and making mozzarella for 19 years. We met the owner in his office, shared some ice cream, yoghurt and puddings made at his shops and also had a look at his leather shop, where purses, bags, handbags etc are made to their own exclusive designs. A nice handbag as an example was available at €300 each (Au$500).

The milking parlour was fully computerised – double tandem arrangement with 7 per side. This allows cows to be milked individually rather than as a batch – at their own speed and cows are milked twice daily.

Here we saw the first mechanical back rubbers for the cows. The machines have an electric motor, with a large conical bristle brush attached, which switches on automatically when a buffalo touches the brush. They visit the brush when coming out from the milking parlour, but can also access from the feeding barn at any time of the day. It is obvious that the buffalo love to use these brushes. Each cow was electronically tagged and individual daily milk production is recorded on computerised system.

We were dropped off in Paestum and walked back to the hotel in the dark – about 4 km after a meal at a local restaurant. The navigation was spot on – a fair amount of luck involved and had a close encounter with about ten sheep guard dogs close to the hotel.

Thursday 18 October 2007

We checked out of the Hotel on to buses and then went to the "Azienda Agrituristica Seliano" at Paestum owned by Ettore Bellelli. Another stately mansion. The agricultural land in this part of Italy costs around €100,000 per hectare to purchase at present. As with the other farms seen, the buffalo were all under cover in open-sided sheds, concrete floors, central feed lanes and automatic pen cleaning with mechanical scrapers which run the full length of each pen – 100 metres plus.
This farm had 900 head with 300 milking.

The pens were larger with more room to move on bare dirt for some of the male groups in particular. The dairy was less modern than Vannulo yesterday and was a four-a-side tandem arrangement with large glass bowl milk receptacles for each set of cups. Each cow could be then recorded individually after milking if desired (manually). We watched some pregnancy testing of some dry cows – the technician reckoned he could tell down to 30 days of gestation accurately. Mostly bull mating is used here and one of this farms bulls "Ettore" is on the A1 lists of two of the AI semen distributors in Italy, CIPAB and COFA. The homestead was two storeys with a beautifully polished horse carriage in the lounge room – quite a mansion.

Lunch was back at Improsta – followed by a tour of the Imposta buffalo experiment farm, by some participants while the rest headed for Caserta – the Congress venue.

We were showed around by Fabio Zicarelli who was a mine of useful information. Because of the fewer numbers of people we were able to question farm practices more closely and learn a lot more.

Improsta is a Regional Research Farm for Campania covering a number of industries other than buffalo dairy, cropping, horticulture and forestry. On the buffalo side there is 60 ha for forage production for the 300 head herd of which 150 head are milked. The total farm size is 135 ha. 700 mm of rain falls between October and April (winter). Calf management involves leaving the calf on the cow for four days then separating to individual pens.

Calves are fed on milk replacer at 5 litre/head/day for another 28 days. If calves don't drink within 2 days then are fed with a stomach tube. By the end of this 28 day period it is aimed to achieve a total mixed ration of 1kg/day of DM. After 75 days, milk is slowly reduced until calf is on a full ration of dry feed.

**Milk replacers for cattle calves often have supplementary-added copper; over 300 mg/kg of powder can be toxic to buffalo calves. It can cause sudden death.

The average milk yield is 2700 kg per lactation with up to 10 lactations to 15 years of age. Lactating cows are fed 17 kg/head/day of DM of a 15 per cent crude protein ration. This includes 27 kg of corn silage, 2 kg of lucerne hay and 8 kg of 18 per cent crude protein concentrate.

The calving interval is 420 days. Lactation length 270 days, some 30-40 days shorter. All mating is with bulls which are out from October to February during the winter. This is to get them to calve during the peak cheese consumption period during summer. Peak natural mating is during this winter period normally.

When cows get pregnant – after 40 days the milk production begins to drop off.

Prolapse of the vagina or uterus can be a big problem in some dairies. This has been attributed to a high Ca:P ratio so 1:1 Ca:P ratio is now recommended in dry cow rations. Increasing Ca ratio increases the prolapse rate. In cattle 2:1 is usually the recommended ratio and this causes big problems in buffalo as high as 2-3 per cent or more of breeders.

Overgrown toes are a big problem with bulls and it is related to the lack of walking in enclosed environments, and living on concrete, and the Ca:P ratio. They look closely at the angle of the feet in selecting bulls to alleviate this fault.

4.5 The 8th World Buffalo Congress

Four lecture rooms were operating concurrent sessions at the Crown Plaza Hotel in Caserta. My plan was to circle the papers of greatest interest and float between rooms. However this became too difficult as when an author was absent, the papers were brought forward to fill the gaps so timing became impossible.

Papers presented were all compiled into two large volumes of the Italian Journal of Animal Science Vol 6 2007 Supplement 2, Parts 1 and 2.

Part 1 - Consists of the invited lectures, genetics, nutrition and feeding and reproduction.

Part 2 - Consists of papers from the following areas:

1. Biotechnology
2. Infectious diseases
3. Parasitic diseases
4. Clinical science
5. Milk production
6. Food safety and technology
7. Meat production
8. Management
9. Rearing and environmental control
10. Economics

I won't attempt to summarise all the papers presented, but just a few notes of salient or interesting points on some of the ones that I attended.

Day 1 Friday 19th October

Paper 1 - Trends in Buffalo Production in Asia – Dr Libertado Cruz

In Asia there is increasing use of milk, meat and grain with a plateau in human population growth.

There is a decreasing use for animal use for draught but increasing milk and meat production.

The world buffalo population is estimated at 172 million head – with 166 million in Asia.

Countries with declining buffalo populations are Cambodia, Indonesia, Thailand, Vietnam, Bangladesh, Sri Lanka, Laos, and Malaysia in last 10 years. Philippines – 75 per cent still used for draught.

In India – there are 11 million dairy farmers with 100,000 village dairy societies.

Crossbreeding swamp with River gives 3 x more milk, 2 x growth rate and double the income.

The "White Revolution" in India has been increasing milk production. The "Pink Revolution" has been the production of meat by keeping male calves – the mortality rate of male calves has been reduced from 80 per cent down to 7 per cent in one village demonstration.

In 10 years the meat export from India has tripled from 159,000 tonne to 459000 tonne (1995-2005). This accounts for the lack of demand in our traditional Brunei market. Malaysia and Philippines are the biggest importers.

As draft buffalo use declines in China, there is a distinct push to crossbreeding to produce a dairy animal, crossing swamp with Murrah and Nili-Ravi buffalo.

Paper 2 - Pakistan: Buffalo vs. Cattle for milk – TN Pasha

Buffalo have a higher proportion of milk in winter with cattle used to even out summer supplies. 500,000 head around Karachi in their urban area alone. The advantage of buffaloes to cattle is that the feed supplies are less concentrated for efficient production. Other differences between buffalo and cattle milk are larger fat global size, lower cholesterol 0.65 per cent vs. 3.14 per cent, higher calcium, higher (antioxidant) tocopherol peroxidase (2-4 x greater activity) and people with cow milk allergies are not affected by buffalo milk. Mastitis levels in buffalo are also generally lower. The Nili-Ravi is the highest yield of all the breeds with Kundi not far behind. Genetic effects on milk yield show a repeatability of 0.30 and a heritability of 0.26.

72 per cent of dairy animals have only one lactation due to the difficulty of getting the animal pregnant again whilst lactating.

Paper 3 - Buffalo as a Social Animal for Humanity – Dr SK Ranjhan (India)

Largest world producer of milk 96 million tonne a year (2005/06) and is growing at 5 per cent annually. India 14.7% of global meat production. Dairying provides employment to 8.5 million people -71% are women.
India has had

1. Green revolution (crops) – saturated
2. White revolution (milk) – still some potential
3. Pink revolution (meat) – large potential

India has 96 million buffalo and 180 million cattle. Buffalo up 1.6 per cent year cattle down 0.5 per cent year. There are 12 establishments exporting meat from India.

Increasing use of male calves, purchased at 150 kg at 15 months of age.

Paunch material is collected, dried and compressed to make pellets for roughage feeding.

The international price for Indian buffalo meat (frozen boneless) is US$1800 / tonne.
Each animal produces 5 tonnes of manure annually.

Indians prefer buffalo milk – 20 R vs. 15 R a litre for cow milk.

Milking management of dairy buffalo:
In November 2007 the International Dairy Federation will be publishing a Bulletin on milking management of dairy buffaloes and will include chapters on milk composition and quality, anatomy of buffalo udder, physiology of milk ejection, udder health, milking machines for dairy buffalo, milking routines, milking hygiene, storage of milk and milk recording.

This will be an invaluable reference for the buffalo dairy industry.

Some salient points;
Oxytocin dosage for milk let down – 1-2 IU is no problem, 30-40 IU can cause prolapse problems. Even in Italy the use of oxytocin for milk let down is widespread. Buffalo differ from cattle in that 95 per cent is held as alveolar milk and only 5 per cent cisternal.

Some cheese factories in Italy require once/day milking to increase the fat and protein percentages. Manual prestimulation of udder for at least one minute appears very important with buffalo in terms of ejection time, average and peak yield flow, milking time and stripping yield.

Paper 4 - Tradition and Innovation in the Water Buffalo Dairy Products – Professor F Addeo (Italy)

(Same lecturer as pre-conference course.)
Mozzarella cheese production.
Calf rennet is preferred and needs to have high Chymosin content and negligible pepsin content (maximum 5 per cent).
Casein proteolysis is not desirable prior to stretching.
Casein proteolysis is required for mozzarella for pizza usage (lower moisture content).
Starter culture provides the fermentation activity.
Rennet provides the clotting activity.
Starter culture is a mixture of lactobacillus and streptococcus.
Curd stretching optimum pH is 4.85-4.95.
The Ca content drops with decreasing pH.
With machinery stretching, the faster the screw RPM the lower is the moisture content.

Curd stretching in hot water gives a 10-15 per cent increase in moisture content.
Examples of new products from buffalo milk:
Camembert, Gouda, Grana, Gorgonzola, Strachno also Yoghurt, Probiotic Yoghurt, Kefir, Ferment whey and milk products (eg. Ricotta), Fractioned butter from stretching fluid, and whey protein hydrolysates.

Paper 5 – Nutrition of Swamp Buffalo – Metha Wanapat (Thailand) (Khon Kaen University)

In Thailand buffalo numbers have been slowly rising between 2000-2004 to two million total.

As oil prices get higher, greater usage of buffalo for draft integration with rice, sugarcane and cassava.

Comparisons with cattle – buffalo are more nitrogen efficient and differences in microflora composition and numbers.

Bacteria (cellulolytic, proteolytic, amylolytic) numbers are higher.

Feeding technologies:
Urea treated rice straw (5 per cent Urea) or (2 per cent Urea + 2 per cent $Ca(OH)_2$).
Intercropping cassava and cowpea. Cassava harvested at three months and hay has 25 per cent CP plus condensed tannins for rumen protection. Also intercropping with Cassava and a legume *Phaseolus calcaratus* – which has seeds suitable for human consumption.

Day 2 – 20 October 2007

In Vivo Embryo Production in Buffalo: Present and Perspectives - A Misra (India)

Multiple ovulation in buffalo is still unsatisfactory compared with cattle.

Some of the factors are stage of oestrus cycle, nutrition, age of donor, seasonal effects, genetic effects, sire fertility all have impacts.

Still plenty of research needed to get MOET to suitable rates in buffalo.

In Thailand they have produced calves from somatic cells (cloning) using swamp buffalo.

Interrelationships between nutrition, metabolic homeostatis, ovarian function and oocyte quality in buffaloes and cattle. Michael D'Occhio (Australia)

This is an important paper suggesting important links between fertility and nutritional status. The digestive hormones of insulin, leptin and IGF1 may have big effects on reproduction. Improvement in liver condition where nutritional status is higher can lead to increase in cholesterol which leads to an increase in fertility. Animals on higher energy diets tended to show higher numbers and better quality oocytes.

This concept may be critical in maximising outcomes to AI at Beatrice Hill Farm.

Fixed Time Artificial Insemination in Buffalo (FTAI) – Pietro Baruselli (Brazil)

FTAI basic data:
Brazil 306 days gestation.
Oestrus average 12-15 hours.
From Prostaglandin injection – 45.6± 10 hours for buffalo to ovulation.
Oestrus display after prostaglandin – 36-96 hours with peak at 60 hours.
Rates to FTAI in buffalo: (Fixed Time AI)
35.5 % in heifers

51 % in cows
Suckling reduces outcomes
40-60 days to first oestrus after calving.
Breeding season is important the further from the tropics that you get (higher latitudes).
In the breeding season, Ovsynch is the best protocol.
In out-of-breeding season, PRIDS or Crestar perform better.
Best timing to get twelve month cycle using AI.
50 days post calving – do first FTAI.
Ultrasound at 26 days and do second FTAI if not pregnant (at day 86).
Should get 70 per cent plus pregnancy rate after 2 inseminations.
Breeding season is autumn – winter.
Out of breeding season is spring – summer.

Embryonic mortality in Buffalo cows – G. Campanile

Reported on percentages of embryonic losses in Italy, Brazil and the periods following conception – major cause of losses in fertility.
Found there are relationships between seasons (breeding) buffalo in decreasing daylight breeder – higher losses outside the breeding season. Can be as high as 40 per cent in Italy during this time. 7 per cent in Brazil during breeding season appears to be linked to progesterone concentration in the blood – more likely where progesterone levels are lower.

Environmental Affects on Reproduction – William Vale (Brazil)

Factors affecting reproduction include:

1. Climate/seasonal
2. Weather short term
3. Nutrition
4. Parasites/diseases
5. Social factors (stress within the herd)
6. Interaction with man

Thermal Humidity Index as measure of thermal stress:

THI ≤ 74 – no problem
75 – 78 some decrease in rate of weight gain
79 – 83 significant loss of productivity
≤ 84 emergency conditions

Heat stress can have effects on oestrus, pregnancy, bull fertility, nutrition (lack of water).

Heat stress can be ameliorated by providing shade, housing ponds for cooling etc. shelters, showers.

Because of their black skin and fewer sweat glands, they appear out of place in the tropics, but they do adapt despite these things with proper husbandry and management.

Can we Consider Buffalo a Non-precocious and Hypo-Fertile Species – L. Zicarelli

He is implying no, as when buffalo are given good management and nutrition, they are quite fertile.

Calving intervals in Italy are decreasing. Now at 435 days which is down 35-40 days from 30 years ago, despite out-of-season mating practices. If culling rates are increased on animals with long inter calving periods the average will come down.

Out-of-season mating is from February-September (7-8 months) during the summer.

Urea Molasses Multi-nutrient Block improved Milk Production – S Akhanum et al (Pakistan)

Consumption of 300-500 g/head/day – in 78 per cent of dairy cattle – 10 per cent no change – 12 per cent decreased production. 44 per cent molasses, 6 per cent urea, 8 per cent cottonseed meal and 28 per cent wheat bran. Cost benefit 1 : 4 value of UMMB and milk.

Grazing Behaviour of Buffalo Heifers in Salerno – F Napolitano

Area just native pasture 600 m above sea level, 950 mm rainfall September to March (autumn/winter/spring) 16 head on 40 ha (lightly stocked).
Weight gains as good as intensive systems.
Activity pattern:
48% grazing – (spring 60%, summer 41%, autumn 43%)
11% walking
14% resting – (spring 11%, summer 19% autumn -)

Animals ruminated and lay down more in summer and autumn and grazed longer in spring.

Study only covered six hour day so grazing in summer and autumn may have been more also.

Wild Buffalo (Tamarau) in Mindoro (Philippines) – Professor Ishihara
Around 250 head stable over last 5 years. Conservation area 4000 ha 600 m – 1000 m above sea level.

Japanese study – 16 vantage points simultaneous continuous observation points.

Mainly bulls with 1, 2 or 3 cows plus progeny.

234 of 269 in groups – 29 solitary animals (50 per cent males).

Fresh dung samples are checked for coccidia and liver fluke. Coccidia 10/15 Fasciola 3/15.

Calf Feeding Colostrum and Subsequent Survival – Mastellone et al (Italy)

The amount of IgG transfer in colostrum appears to be directly related to growth rate and survival of calf on the dam. There was no correlation between IgG levels and growth performance of calves when fed milk replacer.

Calves allowed access to dam were 5.4 kg heavier at 30 days of age than milk replacer fed calves.

Day 3 - 21 October 2007

Buffalo in Venezuela – J Reggetti

27.2 million hectare of available grazing land
52% extensive ranching
43% semi intensive cattle
4.7% intensive cattle
Official figures 170,000 from Agric Ministry.
Breeders think 300,000 closer to mark.
Most in floodplain areas.

Average 3-6 litres/cow. Mostly to cheese production 26.7 per cent Murrah 50-200 head herds. Buffalo out perform cattle but government is not doing much to help. Demand for animals outstrips supply in the country for supplying needs of new and prospective producers. Priorities – production register to identify superior genetics, increased research and development.

Buffalo Breeding in Brazil – O Bernades – Presented by J Reggetti

Started with 200 head in 1890s now 3-3.5 million.

- 25,000 herds
- 63,000 in 1961
- 495,000 in 1980
- Murrah 69%
- Mediterranean 20%
- Jaffarabadi 8%
- Carabao 2%

Mainly for meat production, but greater interest in milk since the 1980/90s.
One of the main challenges in Brazil is getting profits back to the farmer. Most of the profit share is generated in the distribution sector – not on the farm. The buffalo herd is growing at 3-3.5 per cent increase/annum.
Big difference in performance between milk and meat producers in weights of male weaners eg. male meat calves weaned at 306 kg and male milk calves weaned at 176 kg.
As in Venezuela there is not any distinction between beef and buffalo in the meat market place.
Annual milk production (buffalo) 45,000 tonne.
There is annual slaughter of 744,000 head -155,000 tonnes meat – 185,000 tonnes dairy products.
Buffalo milk is valued at twice that of cow's milk.
Biggest increase is in areas with dairy factories (150).
Seasonal supply of milk.
1 milking/day 1,460 kg/lactation, 2/day 2,080 kg/lactation, best 2,955 kg/lactation.

Argentina and Rest of South America – Marco Zava

In South America buffalo numbers are growing at 12.7 per cent per annum.
Chile – 10 head from Australia in 2006 (9 cows 1 bull).
Uruguay – Started with 100 head in 1987 – now 500 head with dairy about to start.
Bolivia – 1964 start now 5000 head – one producer has 2,000 head and milking 200 head.
Paraguay – 8,000 total mostly in small herds 20-50 head. Used solely for meat production, weaned 8-10 months 180-330 kg.
Argentina 85,000 head. 40 per cent Mediterranean 20 per cent Murrah, rest cross breeds. All are north of 31°S latitude. Slaughter at 500-550 kg at 24-30 months in age.
La Salamandra near Buenos Aires has 80 milking cows and 2159 kg production record. A well run cheese factory producing mozzarella and other cheeses, yoghurts etc.
Again no distinguishing between buffalo and beef in meat market.

The Buffalypso: The Water Buffalo of Trinidad and Tobago – Steve Bennett (not present) presented by Floyd Neckles

Development started in 1949 in sugar cane Company by Steve Bennett.
Around 5,000 left in Trinidad – 50 per cent are Buffalypso developed as breed from many different breeds of river and swamp. The Bennett herd has been sold. The government and University of Trinidad and Tobago is now trying to ensure that the Buffalypso is maintained and developed into the future.
Selection between 1950 and 1973 to produce a better beef animal – redistribution of muscling to the hindquarter and straightening the backline.

Molecular Changes during Chemical Acidification of Buffalo and Cow Milks – S Ahmad et al (France)

Comparison of physico-chemical characteristics of buffalo and cow milk at natural pH and during acidification.
Total nitrogen higher for buffalo than cows. Normal pH similar for both species.
Casein nitrogen higher for buffalo.

Higher Ca, higher Phosphorus, higher Mg, higher Na, Lower K, Cl.
The pH decreased more slowly for buffalo milk, buffering capacity was higher.
Therefore can't extrapolate recipes for cow's milk to buffalo milk without some modification to account for it (extra acid).

Sensory Profile of PDO Mozzarella di Bufala Campana Cheese – Professor F Addeo (Italy)

Some differences between spring and summer (protein higher in summer).
Measures of odour are milk, butter, cream, yoghurt, musk, mushroom and animal/stable.
Measures of texture are elasticity, hardness, cohesiveness, juiciness, oiliness, chewiness, stridency.
Measures of taste are bitter, sweet, acid and salt.

Sensory Evaluation of Butter – SA Fernandes et al (Brazil)

Taste testing using non-trained panellists to assess colour, flavour and firmness.
Buffalo butter was lower for all three attributes, but greatest difference was colour, the buffalo being white and the cow's one yellow. The scale was 1-9 (1 liked extremely- 9 disliked extremely) and flavour and firmness for buffalo milk rated less than 1 unit lower on the scale. The authors commented that colour could be corrected by using a dye in manufacture. There appears to be the public expectation that butter should be yellow.

Effects of Feeding Systems on Aromatic Characteristics of Buffalo mozzarella – G Cifuri (Italy)

They were looking for the effect of different diets on aromatic compounds. Gas chromatography was used to identify these compounds.
Aldehydes, ketones*, esters, terpenes* and alcohols* were all affected by diet, some* significant statistically.
Hydrocarbons were not affected.
Free fatty acid content also not significant.
Differences in cheese flavours were put down to concentration differences of a common set of flavour compounds, rather than unique compounds associated with a particular feed.

Molecular Characterization of Buffalo Meat using Proteomic Techniques – Pasquali Ferranti et al (Italy)

Buffalo meat has benefits over other meats healthy-eating wise such as lower cholesterol, lower fat, higher unsaturated/saturated fat ratios, greater iron. This group is looking for the molecular markers of quality in buffalo meat.
Three different muscles were used before and after seven days of tenderization (hanging) using electrophoresis techniques. They found differences between the three muscles in the extent of tenderization.

Lipid Oxidation in Buffalo Meats from Vitamin E Supplemented Animals – A Cascone et al (Italy)

They looked at Vitamin E supplementation at high, medium and low levels to see if meat oxidation (of fats) could be inhibited in storage, by feeding Vitamin E supplements before slaughter. It was concluded that supplementation was a promising strategy to prolong shelf life of buffalo meat.

Evaluation of Muscle Tissue Growth in Young Mediterranean Buffaloes Slaughtered at Different Weights – TAB dos Santos et al (Italy)

Evaluated carcases at 450, 480, 510 and 540 kg live weight after feedlotting from 330 kg at 14 months. Concluded after measuring muscle, bone and fat percentages that early growth is mainly muscle when compared to bone and fat.

Comparison and Improvement of Chemical and Physical Characteristics of Low Fat Ground Beef and Buffalo Meat Patties in Frozen Storage –
J Uriyapongson. (Thailand)

Eight different treatments were compared. No difference between beef and buffalo in cooking yield. Addition of modified starches and methylcellulose – reduced the shrinkage of patties when cooked and provided greater stability during storage. Methylcellulose improved the texture of frozen buffalo meat patties (Buffalo in Thailand usually old when slaughtered, so tough and chewy).

DNA Certification of Buffalo Meat – LV Tesciera et al (Brazil)

This group is developing methods to allow the screening of buffalo meat products to ensure there is no substitution with other meat products using DNA techniques.

Relation of Post-Mortem Protease Activity to Tenderness in Buffalo and Brahman meat – K Neath et al (Japan)

This group has demonstrated in buffalo a greater tenderness in the meat compared with Brahman at the same age, gender and diet. There are many factors which control tenderness, particularly the post-mortem degradation of key myofibrilla proteins by calpain enzymes during aging. The weakening of the protein structure causes increased tenderness. Protease activity is higher in buffalo than beef at all pHs tested.

The stock was grown out at PCC in Philippines.

Day 4 Monday 22nd October

Safety and Quality along the Buffalo Milk and Cheese Chain – M Contesi et al (Italy)

Food poisoning outbreaks are reduced if pasteurized milk is used in the processing of cheese. Slow acidification of the curd can be caused by bacteriophage infection of the starter culture. Lactic Acid Bacteria (LAB) and some other factors. Slow acid development leads to poor quality cheese. Bacteriophages are viruses that can only replicate within a bacterial cell. They can also attack the Streptococcus thermophilus that is used in the starter culture for mozzarella cheese.

4.6 Post Congress Tour

Tuesday 23rd October 2007 from Caserta

After a late start we bussed to Salerno for the 3rd National Show of the Mediterranean Italian Buffalo in Eboli, missing the scheduled Temple at Paestum.
Judging of all the classes proceeded for the whole day with parades of each class in an area with good spectator seating available. It was also possible to observe all individual stock in stalls outside the arena.
Lunch was had near the arena and consisted of BBQ buffalo meat and ample cheese and salad. Red wine flowed copiously, but the Italians are used to it.

The various categories included:

Bulls	16 – 24 months
	24 – 36 months
	> 36 months
Heifers	16 – 22 months
	22 – 28 months
	> 28 months
Cows in milk	> 38 months
	38 – 52 months
	52 months – 10 years
Older cows in milk	> 10 years
Cows with	> 600 kg protein (as 200 kg appears to be maximum for a single lactation the 600kg must be a cumulative total for their lifetime production)

After the show we went to the official dinner where the champion trophies and prize winners in all sections were awarded. There was a red very new Ferrari parked conspicuously in the driveway to this palatial villa.

During the day in the stalls after judging some of the lactating cows were milked with portable 2 place milking machines. Some of the cows had been waiting some considerable time for this relief once the judging was finished.

Day 2: Wednesday 24th October 2007

From overnight in Salerno we drove back to Caserta and visited a very large farm that had feedlot pens that housed 1000 head of breeders milking 700 at a time and with 300 dry cows. The farm area was 215 ha and the dairy was a 12 side herringbone. The farm belonged to Guiseppe Morese.

The fodder mix for lactating cows was:
1 kg Oats
24 kg Corn silage
11 kg Ryegrass hay
6 kg Concentrate

For dry cows and bulls:
7 kg Corn silage
1.5 kg Concentrate
7 kg oaten straw
Plus Minerals/Vitamins
The farm has 15 staff and we watched one of the staff teaching a new calf to drink from a bottle. The feedlot was open but had huge sliding doors to move across the ends of each shed for wind breaks in the winter. These doors had a heavy duty plasticised fabric cover (tarpaulin type material).

Males are grown out to 15 months or 450 kg at slaughter. Calves are started on buffalo milk, but once sucking strongly they are transferred to milk replacement powdered preparations. Calves are individually penned until weaned. This farm also had mechanized calf milk feeders. Some are computer controlled and record the calf number from an electronic tag and record also its consumption and intake. There were huge storage bunkers for silage and sheds for hay for full feeding all year round. A lot of the farm was new construction, and no expense spared eg. Galvanised and stainless steel fittings. The very interesting part of this property was the cleaning of yards and recycling of nutrients. The pens were long and narrow and divided into three sections lengthwise. Each bay slopes downhill on a constant grade and daily a huge amount of water is let go at the start of the bay and rushes in a 150 m depth along the whole length of the bay, flushing the manure with it. It is then pumped to a huge settling pond where the solids are removed by screw pressing and then the water sent to a larger storage area. It is then used for irrigation of the maize crops and hay cropping areas and any grain produced.

The capital expenditure here has been huge. There is no cheese factory in this operation and milk is transported to other processors.

The second farm visited was the **Agricola Casaeria Lupara** (translates as sawn-off shotgun) with a black wolf on their logo at Pastorano which had a combined breeding herd, feedlot and cheese factory. The businesses were run by three brothers with family name Iemma with two farms supplying milk to the factory.
The two farms have 500 milking cows, total herd of 1600 head and supply an average of 7.5 litre head day for a 240 day lactation. Milking is twice daily with 70 per cent from this farm 30 per cent from the brother's farm.
The 2 storey residence dates back to the 15th Century.
The factory tests the milk delivered prior to unloading. It is unloaded into refrigerated storage to be used at 3–4 am the next morning to make into Mozzarella. Tests for pH (6.6 – 6.85) and it is also tested for mastitis. They produce 3 tonnes of product a day and approximately 40 per cent is exported. Retail prices are €13–14/ kg for Mozzarella and up to €20 /kg in cheese delicatessens. The shipping brine contains salt and lactic acid to preserve the product.
We were treated here to a magnificent Italian lunch, including bread, cheese, meat, pasta, vegetables and sweets (and red wine!). This farm was similar to the previous one with waste water used for irrigation to two large storage tanks to provide all the feed for the year. Also the dairy was a modern 10 per side herringbone. An interesting feature of this dairy was the washing arrangement in the holding yard before milking. There were many fine jets of water coming from the concrete pad which not only wash the udders but also provide gentle pre-stimulation to enable better milk let-down. The jets of water were about 600-800 mm high. We moved on to Frosinone for accommodation for two nights.

Day 3: Thursday 25th October 2007

Latina province.

The first visit for the day was to the Cistercian Abbey of Flossanova in Latinium, Lazio (South of Rome) where St. Thomas Aquinas (b. 1227 d. 1274) spent most of his lifetime. This building was huge and dated back to mid 1100s and was consecrated by Pope in 1208, with much beautiful stonework and architecture.
The first farm visited in the Latina region was that belonging to the Martini brothers. (Johnny and Angelo). Here milking is twice daily and they produce 2000 litre/day. I didn't like the condition that the calves were living in on this property.
They raise bulls for meat to 13 months of age, fed up from 6 month weaners. The ration balance of Ca : P = 16.5:15.5.
The dairy is a 14 per side with milking between the rear legs.
They use Milkline and Milkpro dairy equipment. The yards are cleaned twice weekly and their farm covers 100 ha. The value of this land is €30000 ha. Cows are run to 12-13 years of age some of the basic economics are €3.00 kg live weight for their meat animals = $5 Australian.
They get €1.00 litre for milk in the winter and €1.40 litre in the summer – mozzarella season. Daily feed costs are €4.00 cow. Cow milk yields need to be 3-4 litres/head/day to cover feed costs.
The second farm visited belonged to Sergio Boschetto. Their ration is 50 percent maize silage, ryegrass hay and straw. Heifers are fed 26 kg day (total as-fed weight) so as not to get over-fat. Lactating cows get 33 kg/head/day plus a vitamin mix. Calf feed is the same as for the milking cows. We noticed here and in many farms the high percentage of animals that have the swamp markings – referred to as "Aleardo". The herd is vaccinated against rotavirus, corona virus and clostridial bacteria infections. Conditions in the pens were less favourable here with many hoof problems from concrete. Some calves are reared on nurse cattle cows for 3 months – some multiple calves.
Cow milk yields average 7-10 litre/head/day or 2300 litre lactation
The best recorded in this farm is 4000 litre and the best first lactation is 2400 litre.

Between farms we visited the cheese factory "Maine Della Torre" which brings milk from Campania and they process fresh milk making the traditional Mozzarella di buffalo Campania. They also make a range of hard cheeses which was very impressive. They also had a very good display of vacuum packed buffalo meat cuts that were very well presented.

In the afternoon we had a lunch stop which lasted from 1400 – 1700 hours with four large courses and plenty of seconds of each course. Again the food was fabulous and unstoppable.

Early evening was spent at the Pofi Museum which was set up in 1916 – and delves into the Prehistory of the area. Bones etc have been dug up in old sediments up to 1 million years old. They have mammoth tusks 300000 years old. Elephant teeth are very interesting in that they have a row of molar teeth all fused together. When they wear out, they are replaced by the next set. There are 24 sets of molars available in the elephants lifetime, waiting to get pushed up for action.
They have a large section tracing the evolution of man from the first hominoids which appeared between 4 million – 16 million years ago.
We still managed to fit in another three course dinner at the hotel after 9 pm!

Day 4 Friday 26 October 2007

Frosinone Region.

The farms we visited first today were in more hilly country with poorer soils. This area has a fair number of farms despite the rockiness of the soils – Buffalo are kept here but more feed is generally transported in than in most other areas in Italy. This farm is owned by the local president of the ANASB (Italian Buff Breeders Association) in the Amazallo Valley. Rina Farm runs 300 head of breeders. The main calving periods are in March and August. August is the best natural mating period for buffalo, but the March period is preferred for the summer demand for Mozzarella. Automatic calf milkers were here as well.
Heat lamps are required for calf survival in the winter on this farm. Calves are weaned off milk after three months of age – solid feed is introduced early. They inseminate cows using Ovisynch Protocol.
Dry cows are fed 13 kg DM of feed with a 1:3.Ca:P ratio, protein 9 per cent and fat 2 per cent.
Lactating cows are fed 17-18 kg dry matter with 13-14 per cent protein, 3 per cent fat.
The milking parlour here was only 6 per side herringbone – much smaller than other farms visited – reflecting the smaller numbers milked. The pens here were quite wet and with a lot of manure. Calves in particular didn't look as comfortable in these conditions as other farms where straw bedding was used.
The next stop was another cheese factory (Caseificio) where the morning's mozzarella manufacture was in full swing. They had a direct sales outlet. This factory had a large buffalo out on the main road verge (in their car park) – A black version of the Darwin Pink buffalo, but a little less anatomically correct. The mozzarella was hand made, not machine made, with lots of people in the factory.
They had a factory-direct sales outlet similar to most others with a very good range of buffalo cheeses and butter and also had some goat cheeses as well. Their meat display was also quite good – colour was nice and light. They also have a lot of delicatessen type lines to go with the cheese such as olives, dried tomatoes and herbs. Prices ranged from €12 to €24 kilo for the cheese (€23 for one of the goat cheeses). The meat which looked like veal sold for €17 kg for cuts that looked like blade. Smoked and Ricotta cheeses were also well displayed.
Once departed from this factory, we had a very long drive to the northern city of Sirmione, Lake Garda, punctuated by lunch at a very nice restaurant "Borgo San Faustino" in a very hilly agricultural setting overlooking a valley full of horticultural farms. Another superb three course Italian meal.
A long drive to the lakeside Best Western Hotel with an arrival at 2100 hours, followed by another great meal.

Day 5: Saturday 27th October 2007

Lombardia Region.

Bussed to the city of Cremona and spent four hours walking about the exhibits of 62nd International Dairy Show. This featured mainly Freisian cattle but there was also judging of local buffalo. Unfortunately I failed to pack a battery in the camera after overnight recharging, so had a photo free day.
There was a massive amount of machinery and dairy equipment on show plus the live animal exhibits and judging. Artificial Insemination supply companies, cheese making equipment, yard equipment, cleaning equipment, and milking machinery (computerised) were all on display. Irrigation equipment was also on display. Food and wine was also a prominent feature in every Company's displays. It appears that good food and wine attract Italian customers and other countries for that matter. A lot of literature and pamphlets were collected on dairy and cheese equipment.
Italy seems to produce a lot of agricultural and manufacturing equipment particularly in this northern region.
For lunch we were ushered into the Lombardia region exhibit where we were treated to a range of cheeses, meats and bread (and wine) from their region. The cheeses were given on a specific order for tasting from mildest to strongest and included:

a) Bocconcini di Mozzarella di bufala
b) Bufalino bianco (cow/buffalo blend)
c) Bufalino (cow/buffalo blend)
d) Quadrello 200 per cent buffalo
e) Casatica 100 per cent buffalo
f) Caciocavallo di bufala 100 per cent buffalo (15 months)
g) Sovrano (Cow/buffalo blend) 18 months maturity minimum
h) Desiderio (Cow/buffalo blend) 18 months maturity minimum
i) Blu di bufala 100 per cent buffalo

All with a range of processed smallgoods, pastrami and sausage.

We left for the last farm of the tour in Lombardia. Again this farm was a dairy and cheese factory/retail outlet combined.
This farm is fairly recent in comparison with many of the others visited and is in a rapid expansion phase.
At present they have 250 cows lactating and want to have 500 by next year. They have a 10 side herringbone dairy and the stock here looked to be in very good health.

The dry cow ration is:
3-4 kg maize silage
3-4 kg grass hay (first cut)
1 kg maize grain
Vitamin/mineral mix
1 : 1 Ca : P ratio
10 kg fibre

The lactating cow ration is:
16 kg maize silage
3 kg maize grain
2 kg soya grain
1.5 kg cottonseed meal
4 kg ryegrass hay
Vitamin/Mineral mix
12.5 -13 per cent protein

Prolapses are still a problem – there were five head in the one hospital pen.
Bulls are kept in the cow group all year round, and rotated on a regular basis. They don't use AI at this stage. They have a large number of calves at present, (autumn) and into winter.
In cheese production from the factory, they produce 80 per cent into mozzarella, 20 per cent others in summer and 50 per cent mozzarella, 50 per cent other cheeses in winter.
The cows average 9 litre head day for 210-230 day lactation. This would appear credible considering the feeding levels. They do use some beef cows for nursing some of the buffalo calves but you need breeds with higher fat percentage such as Brown Swiss or Hereford.
We tasted their Mozzarella and Caciotta cheeses.
One interesting feature of this farm not seen in any of the others was the biogas plant.
A high steel tank (like a water reservoir) has an internal gas collecting bladder. Manure from the feedlot is pumped to the tank plus any other usable animal waste. The gas from the reservoir is fed to two Deutz V12 diesel engines running Stanford alternators which provide power to the cheese factory and dairy/feedlot and also feeds power into the grid. Dead animals from the feedlot and from the local abattoir can be minced up and put in the tank to provide methane gas.
One kg of carcase produces four kg of gas – which can produce 12 kW of energy.
They have to autoclave to 80°C for 55 seconds to reduce the risks of spreading animal diseases.
The effluent pH range and bacterial composition needs to be monitored closely, in order to obtain good methane production. Other by-products are the solids which can be used by worms to produce humus and organic fertiliser. The liquid waste goes to the paddock and is used for irrigation plus the methane can be used to provide steam heating for the cheese factory.
The whole set up capital cost has been € 3.5 million of which 30 per cent has been provided by the Government.
It will take 3 – 4 years to pay it off at 4.5 per cent interest with positive cash flow for the whole venture within 10 years.
We bussed back to the Lake Garda Hotel for the final tour dinner which was another splendid affair with good food and wine and live trio playing great music.

Sunday 28th October 2007

We were taken by minibus to Linate Airport Milan (Milan has three airports for international flights) in the morning.
I had a long wait till the 7pm British Airways flight to Heathrow – spent the night in Heathrow Terminal One and left the next day for Darwin via Dubai, Brunei (no wait in Brunei). The Brunei to Darwin flight had only 24 people – plenty of room to stretch out. Arrived Tuesday at 4pm in Darwin, thanks to our + 9.5hrs for CST time.

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Australian Water Buffalo

Genetic and reproduction improvements

by Barry Lemcke
RIRDC Publication No. 08/189

This report is the culmination of five years work carried out in three states of Australia for the Australian buffalo industry. It discusses research completed to bring the Buffalo Industry into line with other mainstream industries such as cattle.

This research demonstrates that it is important for the buffalo industry to use the latest technologies available in order to bring about rapid genetic progress in an animal that has had mostly only feral slaughter or capture value in Australia since its introduction in the 1820s into the Northern Territory.

Rapid expansion over all states of Australia has occurred over less than 20 years which has been hampered by the latest long running drought. A critical mass for long term industry survival has still to be achieved.

The report is targeted at producers and potential producers of water buffalo in Australia for both the meat and dairy industries. It shows that the required background equipment has now been put in place that will allow the industry to become a lot more competitive.

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