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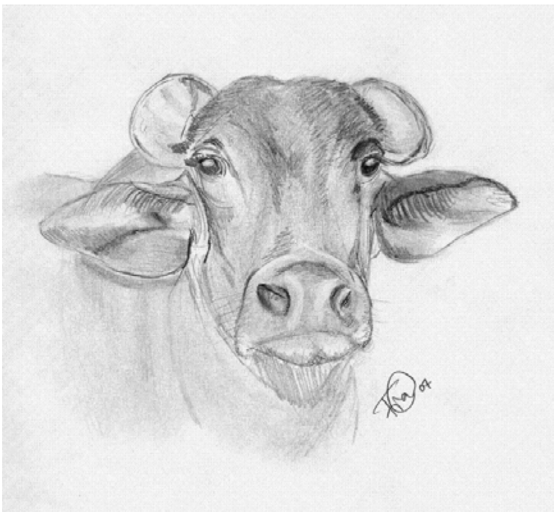

**Institutionen för husdjurens utfodring och vård**

**Examensarbete 235**

***Swedish University of Agricultural Sciences***
***Department of Animal Nutrition and Management***

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## ABSTRACT

Water buffaloes are very important dairy animals in many developing countries, for example India. One of the greatest problems with buffalo milk production is the poor reproduction efficiency. Silent heat is common among buffaloes, which means that ovulation occurs without any visual heat signs. This makes it hard to detect heat and causes economical losses for the farmers. One method used for heat detection in cattle is measurements of the physical activity.

The aim with this study was to document if activity tags (measurements of the physical activity) can be useful in water buffaloes. The milk production and behaviour during heat were followed to see if deviation in those could be used for heat detection as well.

Eight Murrah buffaloes were selected for the heat study. Four of them were expected to come in heat during the experimental period and four were induced to heat with hormonal treatment. The heat induced buffaloes were also followed during their next natural heat. Altogether thirteen heats were followed. In addition to this two control groups with five pregnant buffaloes and three pregnant cows were selected. All animals were equipped with activity tags (DeLaval, Sweden) during the entire study time. The animals in the control groups were not exposed to any other observations than the activity measurements. The animals were kept indoors in a lose housing system and were milked twice daily. The activity and milk yield were recorded daily by ALPRO®. The strip yield was measured with a measuring cylinder and milk samples were taken every milking to determine fat content in the strip yield. Milk samples were taken once daily to determine progesterone concentration. The fat was removed before running RIA (radioimmunoassay).  Five days before expected heat and until the heat had passed the buffaloes were examined with ultra sound (7.5 Mhz) to see when ovulation occurred. At the same time the external genitalia were checked for heat signs (congestion, relaxation and swelling). The behaviour study started as well five days before expected heat with two 30-minutes observations daily (before milking in the morning and the evening). Three days before expected heat the study increased with two more observations, at 14.30 and 22.30.

The ultrasound measurement together with heat signs expression in the external genitalia determined the actual heat dates. Statistical evaluations of the activity data showed that it was only possible to correctly detect one heat in one buffalo. This buffalo had a significant high activity during the heat day. The other buffaloes in the study had too many false alarms and the heat day would not have been possible to distinguish. The progesterone analysis was not useful in this study due to inefficient fat removal. A trend in decreased morning milk yield and increased evening strip yield during heat was observed. The behaviour observations showed that the buffaloes spent more time standing and less time standing eating roughage before morning milking during heat. This might be indications of restlessness and decreased appetite. There were no differences in numbers of social interactions during heat. There were no expressions of the heat signs standing when mounted by herd mates and no attempts to mount herd mates.

The conclusion with this study is that the today's activity meter system is not useful for heat detection in buffaloes, but there were indications that activity meters can be useful after adjustments and further improvement.

## SAMMANFATTNING

Vattenbufflar är ett mycket viktigt mjölkproducerande djur och har en stor betydelse för mjölkproduktionen i många u-länder, framförallt Indien. Ett av de största problemen med buffelmjölkproduktion är den låga reproduktionseffektiviteten. Tyst brunst är vanligt hos bufflar, vilket innebär att ägglossning sker utan brunstsymtom. Det gör att det är svårt att upptäcka brunsten vid rätt tidpunkt, vilket orsakar ekonomiska förluster för bönderna. En metod som används för att upptäcka brunst hos kor är att mäta den fysiska aktiviteten.

Syftet med den här studien var att undersöka om aktivitetsmätare (mätning av fysisk aktivitet) kan användas för att hitta brunst hos vattenbufflar. Mjölkproduktionen och beteendet följdes under brunsten för att se om avvikelser i dessa också kan användas för att hitta brunst.

Till brunststudien valdes åtta bufflar av rasen Murrah. Fyra av dem förväntades komma i brunst under försöksperioden och fyra inducerades till brunst med hormonbehandling. Bufflarna som inducerats till brunst studerades även under den nästkommande naturliga brusten. Sammanlagt studerades tretton brunster. Utöver detta valdes två kontrollgrupper med fem dräktiga bufflar och tre dräktiga kor. Alla djur försågs med aktivitetsmätare (DeLaval, Sverige) under hela studieperioden. Djuren i kontrollgrupperna studerades inte med något annat än aktivitetsmätare. Bufflarna hölls inomhus i en lösdrift och mjölkades två gånger om dagen. Aktiviteten och mjölkmängden registrerades dagligen med ALPRO®. Mängden strippmjölk mättes med en mätcylinder och mjölkprover togs varje mjölkning för att bestämma slutfetthalten. Mjölkprover för analys av progesteronhalt togs en gång om dagen. Fettet avlägsnades innan mjölken analyserades med RIA (”radioimmunoassay”). Fem dagar innan förväntad brunst tills dess att brunsten inträffat undersöktes bufflarna med ultraljud (7.5 Mhz) för att se när ägglossning hade ägt rum. Samtidigt undersöktes de yttre genitalierna för att notera förekomsten av brunsttecken (rodnad, svullnad och avslappning). Beteendestudien påbörjades också fem dagar innan brunst med två 30 minuters observationer dagligen (innan mjölkning morgon och kväll). Tre dagar före brunst utökades beteendestudien med ytterligare två observationstillfällen, kl. 14.30 och 22.30.

Ultraljudsundersökningarna tillsammans med brunsttecken i de yttre genitalierna låg till grund för att fastställa brunstdatum. Statistiska analyser av aktivitetsdatan visade att det bara var möjligt att hitta en brunst hos en buffel. Den buffeln hade en signifikant aktivitetshöjning på brunstdagen. De övriga bufflarna i studien hade för många falsklarm för att aktivitetsdatan skulle vara användbar för att hitta brunstdagen. Analyserna av progesteronhalten i mjölk var inte användbar i den här studien på grund av metodfel då fettet inte togs bort tillräckligt effektivt. En möjlig trend i minskad mjölkmängd på morgonen och ökad stripmängd på kvällen under brunsten kunde ses. Beteendestudien visade signifikanta skillnader i ståtid och tid som spenderades på att äta grovfoder på morgonen på brunstdagen. Detta kan vara indikationer på rastlöshet och minskad aptit. Det var inga skillnader i antalet social interaktioner under brunsten. Inga primära brunsttecken, som att stå när andra bufflar gjorde försök att bestiga eller att försöka bestiga andra bufflar, uppvisades.

Slutsatsen från den här studien är att dagens aktivitetsmätare inte är användbara för att hitta brunst hos vattenbufflar, men det finns indikationer på att aktivitetsmätare kan vara användbara efter vidareutveckling och förbättring av systemet.

## INTRODUCTION

The buffalo is a very important dairy animal in many developing countries. The buffaloes are suitable for this, because of their high disease resistance and the opportunities of milk production despite feeding with low quality roughage (Thomas, 2004). The buffaloes are multipurpose animals and have been used for milk production, meat production and draft power for more than 5000 years (Nanda and Nakao, 2003).

Reproduction management is a major concern for the dairy industry, because inefficient heat detection causes significant economic losses (Karir *et al.,* 2006). The dairy water buffaloes have poor reproduction efficiency attributed mainly to poor expression of oestrus symptoms especially during hot summer months and long calving interval. The marked seasonality of buffalo breeding handicaps the commercial milk production which alternate between surplus and shortage (Gupta and Prakash, 1990).

Improvement of reproduction efficiency also relies on the farmers' skills and capabilities as well as the quality of for example the artificial insemination and disease control. These factors vary greatly depending on the production systems and ecological conditions. It is also important to identify the specific limiting factors under the different situations, the improvements and interventions have to be sustainable with available local resources. The application of modern reproduction technologies in buffaloes requires knowledge about their biology and reproduction physiology as well as the limitations and potentials under each production system (Oswin Perera, 1999).

The knowledge in milk production including reproduction management is a driving process and to develop better methods for heat detection in dairy buffaloes gives advantages for both big and small scale farmers. If the heat detection is less depending on the breeding season it is possible to provide a more equal milk production and thereby also higher income for buffalo milk producers.

## LITTERATEUR REVIEW

### Buffalo world production

The world population of buffaloes is approximately 153 million animals. India is the country with the largest population; approximately 94 million (table 1). The buffalo is an important dairy animal in many other Asian countries as well (FAOSTAT, 2003).

*Table 1. Current buffalo population and its yearly contribution to milk production in some Asian countries (FAOSTAT, 2003).*

| Country | Population | Milk (t) |
|---|---|---|
| India | 94 132 200 | 48 000 000 |
| China | 22 253 550 | 2 650 000 |
| Pakistan | 24 000 000 | 22 500 000 |
| Nepal | 13 700 864 | 806 694 |
| The Philippines | 3 115 090 | * |
| Vietnam | 2 819 400 | 30 000 |
| Myanmar | 2 552 020 | 116 018 |
| Indonesia | 2 300 000 | * |
| Thailand | 2 100 000 | * |
| Laos | 1 060 000 | * |
| Bangladesh | 830 000 | 22 400 |
| Sri Lanka | 661 200 | 68 240 |
| Cambodia | 626 016 | * |
| Iran | 523 500 | 225 950 |
| Malaysia | 140 000 | 6 900 |
| Turkey | 138 000 | 63 327 |
| Iraq | 65 000 | 26 600 |
| Russia | 15 700 | * |
| Syria | 2 900 | 820 |
| Bhutan | 1 800 | 320 |

* denotes lack of information

Depending on the geographic situations and the purpose with keeping buffaloes the management systems are different all over the world. In India for example 99 % of the buffaloes are kept in so called backyard systems with herd sizes of 2-10 animals in average. Buffalo farms with milking machines and other technology also exist, for example in Italy (Ståhl-Högberg and Lind, 2003).

The domesticated buffalo (*Bubalus bubalis*) is divided into two distinct classes; swamp buffalo and river buffalo. The swamp buffaloes are mainly used for meat and draft power while the riverine breeds are used for milk production. Twelve of the eighteen riverine breeds are used for milk production. The main breeds are Murrah, Nili-Ravi, Sutri, Mehsana, Nagpuri and Jafrabadi. Murrah is the best known breed in the world because of its high milk production (For review see Thomas 2004).

**Milk yield and composition**

The riverine breeds that are mainly kept for milk production have a daily milk yield between 7 – 10 litres (Thomas, 2004). The Murrah buffaloes in India have an average lactation length of 319 to 331 days with a total milk yield of 1866 to 2304 kg per lactation (Falvey and Chantalakhana, 1999).

There are some differences between cow milk and buffalo milk (see table 2). Buffalo milk contains less water, more total solids, fat and protein and slightly more lactose. Buffalo milk lacks or only contains traces of β-carotene; precursor for vitamin A. β-carotene is the pigment which makes the slight yellow shade in cow milk and since buffalo milk lacks this substrate the milk has a whiter colour. Despite the absence of β-carotene, buffalo milk contains almost as much vitamin A as cow milk. (Ståhl-Högberg and Lind, 2003)

*Table 2. Composition of buffalo and cattle milk (Walsta et al., 1999).*

| Species | Fat (%) | Protein (%) | Lactose (%) | Total solids (%) |
|---|---|---|---|---|
| Buffalo | 7.5 | 4.3 | 4.8 | 17.5 |
| European cow (*Bos Taurus*) | 3.9 | 3.2 | 4.6 | 12.7 |
| Zebu cow (*Bos indicus)* | 4.7 | 3.2 | 4.9 | 13.5 |

The biggest difference between cow- and buffalo milk is the fat. Both the content and the fatty acid composition differ. Buffalo milk contains lower levels of phospholipids and cholesterol and the fat has a higher proportion of saturated fatty acids which leads to a higher melting point (Ståhl-Högberg and Lind, 2003).

## Milking

The milking management is a big issue of the milk production, because of the simple reason that's why we keep dairy animals. In general buffalos are known to be difficult to milk and they are sensitive to changes in the environment during milking. Hand milking is still dominant, even though it is possible to machine milk buffaloes (for review see Thomas 2004).

Udder anatomy and physiology differs slightly between dairy buffaloes and cows. The cisternal area and milk fraction are smaller in buffaloes than in cows. The teats of buffaloes are in general longer than cows' and the teat canal is longer and more compact in buffaloes. The cisternal milk fraction in buffaloes is about 5 %, in contrast to cows which have more than 20 % of the milk in the cisternal area. It is therefore important with a proper pre-stimulation to get a good milk ejection and a complete milking in buffaloes. This makes it therefore harder to machine milk buffaloes than cows (Thomas *et al.,* 2005; Thomas *et al.,* 2004). When milking buffaloes with machines it is therefore important to consider the differences and not implement the cattle milking routines directly to the buffaloes. Research is ongoing how to improve buffalo machine milking (Thomas *et al.,* 2004; Friberg, 2005).

## The reproductive cycle in buffaloes

The female reproductive tract normally lies in the pelvic cavity and consists of the external genitalia; vulva and vagina and the internal reproductive organs; cervix, uterus, the oviducts with the two ovaries and their supporting tissue (figure 1). The uterus structure of the cow is showed in figure 2. The main function of the uterus is to protect the foetus and supply it with nutrients. The uterus also supply information about the state of pregnancy to the rest of the reproductive system and has the function to transport the spermatozoa from the ejaculation site to the oviducts. During parturition the uterus transports the foetus out of the maternal body (Sjaastad *et al.,* 2003).

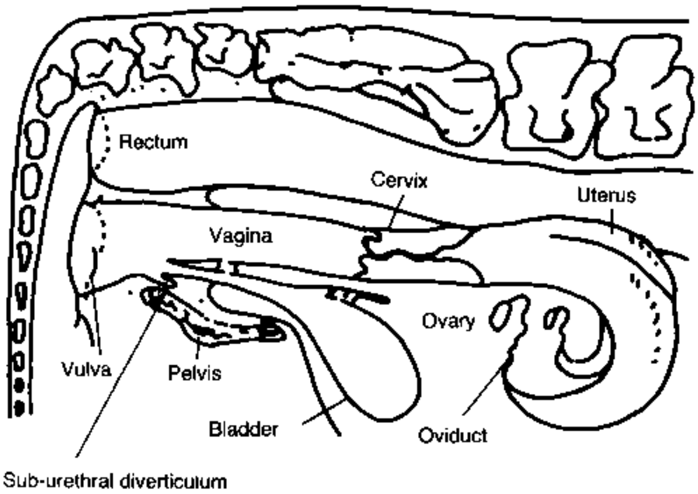


*Figure 1. Reproductive tract of the cow (lateral view)*
(*http://www.fao.org/Wairdocs/ILRI/x5442E/x5442e04.htm#2.1%20anatomy*)

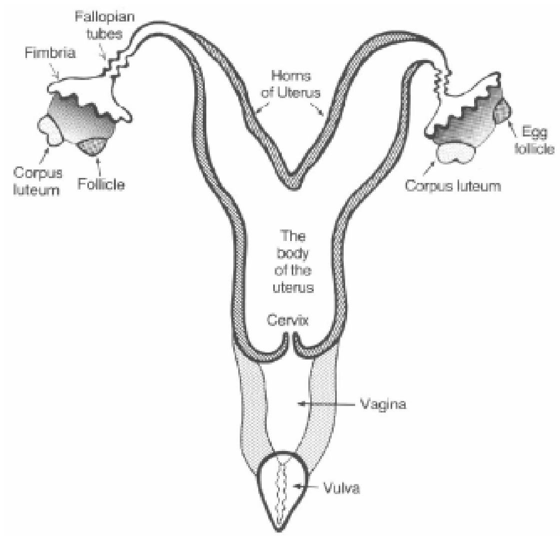


*Figure 2. Schematic drawing of the characteristics of the cow uterus and ovaries.* (ALPRO® ver 6.30/DeLaval activity meter system)

The postpartum period in buffaloes, like in most animals, starts with parturition and ends with complete uterine involution, resumption of the cyclic ovarian activity and normal oestrus expression (El-Wishy, 2006). The oestrus cycle can be divided into different phases. Follicular phase 0-6 days after oestrus, when the egg matures and the ovulation occurs. Luteal phase, 7-20 days, when the embryo development starts if the animal is pregnant. In non pregnant animals the luteal phase follows with luteolysis, which is regression of corpus luteum and return to oestrus. (Gupta and Prakash, 1990; Sjaastad *et al.*, 2003). The oestrus cycle is shown in figure 3.

The most important hormones of the reproductive cycle are estradiol, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and progesterone. The egg development and release is depending on the presence of FSH. During the luteal phase progesterone from the corpus luteum is the dominant hormone whilst estradiol from the ovarian follicles is the dominant hormone during the follicular phase. The follicle produces oestrogen which causes the signs of heat and also prepares uterus to receive a fertilized egg. (Sjaastad *et al.*, 2003).

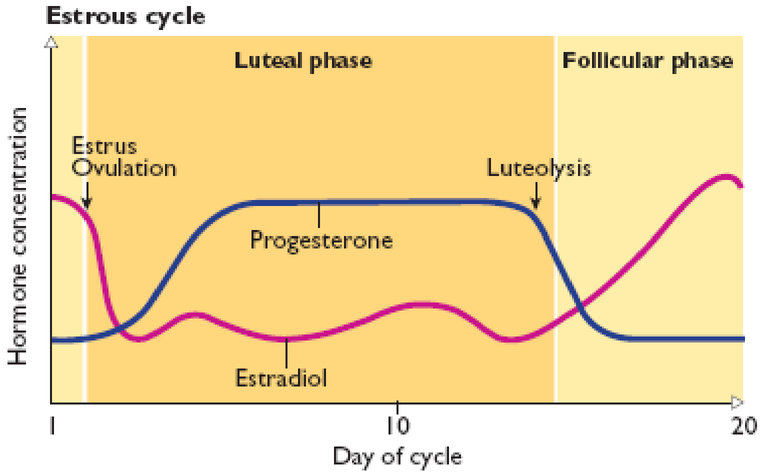


*Figure 3. The oestrus cycle in domestic animals. Day 1 refers to the first day of oestrus, when the female is ready to mate (http://www.scanvetpress.com/pdf/18_Reproduction.pdf).*

## Problems with reproduction in buffaloes

Buffaloes are said to be seasonal breeders, which is a simplification of the truth. They are polyestral animals and are able to breed the whole year (For review see Ståhl-Högberg and Lind, 2003). Buffaloes have poor reproduction efficiency attributed mainly to poor expression of oestrus symptoms especially during hot summer months and due to long calving interval. Buffaloes that calve during the cool season have better reproductive performance than them calving during the hot season. The marked seasonality of buffalo breeding handicaps the commercial milk production which alternate between surplus and shortage (Brakawi *et al.*, 1998; Gupta and Prakash, 1990; Sastry, 1983). Buffaloes may have silent oestrus even in non-stressful periods of the year (Kamboj and Prakash, 1993). Successful breeding must take place within 85 – 115 days after parturition to maintain a calving interval of 13 – 14 months in buffaloes (El-Wishy, 2006).

The incidence of anoestrus in buffaloes varies between 20-80 %, depending on season. Most female buffaloes which exposed to extreme hot conditions during the summer season cease ovarian activity. During this time the milk production and reproductive efficiency are strongly negative affected, probably due to the combined effects of nutrition, environment and management (Nanda *et al.*, 2003; Sastry, 1983).

Silent oestrus is a common problem among buffaloes even under good management (Abdalla, 2003). A study performed by Brakawi *et al.*, (1998) shows that approximately 90 % of the buffaloes resume their ovulatory and oestrus activities within 60 days post-partum.
The silent oestrus period is a big problem and because of this it is difficult to determine the exact time for artificial insemination or servicing. Average calving intervals for Indian buffaloes ranged from 15 to 18 months. The dry period has been reported to be 60 to 200 days, average gestation period was 308-318 days and the average lactation length ranged from 252 to 270 days. As a result of these factors the productive life of a buffalo is only 39 % compared to 52 % in developed dairy breeds. Due to these facts, an important factor to consider in improving milk production in dairy buffaloes is heat detection (Ganguli, 1981; Sastry, 1983).

**The production and effects of progesterone**
The corpus luteum (CL) develops on the ovarian site where the follicle ruptured and the egg was released around the time of oestrus. The CL produces high levels of the hormone progesterone ($P_4$) which is needed to maintain the pregnancy. The CL lacks the enzymes to metabolize progesterone to other steroids and it will therefore diffuse to the plasma and subsequently into the milk (Sjaastad *et al.,* 2003; Karir *et al.,* 2006).

Increased concentration of LH in plasma induces ovulation and the synthesis of progesterone is stimulated by this ovulatory LH peak. When the luteolysis is induced the secretion and concentration of progesterone in plasma falls rapidly. Therefore the progesterone levels can be used for diagnosis of pregnancy. An animal that is not pregnant will have very low levels of progesterone in the plasma and vice versa. Since the range of progesterone concentration in the luteal phase overlaps with the pregnancy concentration repeated measurements are required to determine if the animal is pregnant or not (Sjaastad *et al.,* 2003).

The function of progesterone is to create favourable conditions for the fetus and neonate. Increased levels of progesterone influence the epithelium in the uterus. The glands in the endometrium grow and secrete nutrients, the effects of that are to prepare the uterus to have the right conditions for the development of the embryo. Progesterone prevents uterus contractions that can lead to abortion or too early parturition. Progesterone also contributes to growth and differentiation of the mammary tissue and prepare for milk synthesis (Sjaastad *et al.,* 2003).

**Progesterone levels in blood and milk**
In pregnant buffaloes the progesterone levels increase up to day 20 after insemination. It's therefore easy to set a limit when the buffalo is pregnant or not (Gupta and Prakash, 1990).

The progesterone levels in milk are approximately four to five times higher than in plasma (Batra *et al.,* 1979; Kamboj and Prakash, 1993). According to the study in buffaloes done by Kamboj and Prakash (1993) the milk and plasma levels of progesterone are highly correlated in the same buffalo during cyclicity.

Since milk samples are easier to collect than blood samples, the progesterone levels in milk can be used routinely for the diagnosis of early pregnancy and oestrus. The test is based on the expected hormonal changes during the reproductive cycle (Karir *et al., 2006*).

**Methods for determination of progesterone in milk**
There are different methods that can be used to determine progesterone concentration in milk. Radio immunoassay (RIA) is one frequent used method. This method is used for blood samples and needs to be developed for milk samples. One study performed by Karir *et al.,* (2006) showed that it is essential to have an efficient defatting of the milk since the fat layer interfered with the antibody used in the assay system. Using the developed RIA method for estimation of progesterone concentrations in milk Karir *et al.,* (2006) also showed in their experiments that the test is well correlated with clinical diagnosis of the reproductive status of the buffalo.

Enzyme immunoassay (EIA) is another method of estimating milk progesterone concentration by using an enzyme label and an anti-serum. Prakash *et al*., (1990) did a comparative study between EIA and RIA. They indicated that EIA is about 10 times more sensitive than RIA in buffalo milk**.**

The differences in milk composition between species influence the performance of determining for example progesterone in milk. Small variations in the progesterone concentration in plasma may occur during the follicular and luteal phase. These small variations were not reflected in the milk progesterone values due to the day to day milk fat variations. Cows in heat sometimes have inhibited milk ejection leading to a lower fat content in milk. It is well documented in cows that variations in milk progesterone are associated with changes in milk fat percentage and it is therefore necessary to take samples more than one day. Fat content in buffalo milk appears to be influenced by environmental factors in the same way as for cattle. Most probably these problems are even higher when determining progesterone in buffalo milk, since the content of milk fat is higher (Kamboj and Prakash, 1993; Romagnolo and Nebel, 1993; Shah *et al.,* 1983).

**Progesterone levels in milk as a reproduction management tool**

Several studies on milk progesterone levels in cattle milk have showed that it is a useful and accurate reproduction management tool for heat detection, pregnancy test and incidences of atypical ovarian patterns. However, the milk progesterone test is not justified in many countries because of the high costs (Lamming and Darwash, 1998; Stevenson and Pursley, 1994).

Three different tests with milk progesterone performed on buffaloes by Karir *et al.,*(2006), Singh and Puthiyandy (1980) and Gupta and Prakash (1990) showed that there is a 100 percent accuracy determining non-pregnant buffaloes by using milk progesterone concentrations after day 20 post insemination. These studies showed different accuracy in early pregnancy diagnosis, depending on which day after insemination the determination of progesterone level is performed. The most accurate testing will be after day 24 post insemination (Gupta and Prakash, 1990; Kaul and Prakash 1994, Singh and Puthiyandy 1980). Variable cycle length among buffaloes can be the reason for false positive pregnancy tests (Gupta and Prakash 1990).

Kaul and Prakash (1994) compared buffaloes with zebu and cross bred cattle and found that the accuracy of determining positive pregnancy status was higher in cows than in buffaloes. The different accuracies may depend on that there is a greater variability in oestrus length in buffaloes than in cattle and that the embryonic mortality is higher in buffaloes than in cattle. This study agrees with other studies that day 24 post insemination has the highest accuracy in determining pregnancy status in buffaloes.

All studies indicated that milk progesterone values have the potential to provide the basis for a useful reproduction management tool, particularly for detection of non-pregnant animals. Furthermore, some studies have also showed that progesterone can be a good method to find silent heat since that is common among buffaloes (Gupta and Prakash, 1990; Kamboj and Prakash, 1993). Milk progesterone determinations can be useful for monitoring the cyclicity of buffaloes and also for determination of oestrus because the progesterone levels are low during oestrus (Kamboj and Prakash, 1990).

**Milk production during oestrus**

The relative day to day variation in milk yield among cattle when milking twice daily is 5.3 % (Larsson, 1998). Studies have been done to investigate whether the variation in milk yield is a good indicator of heat. Studies in dairy cow have shown that approximately 30 % of the cows have a decreased milk yield in the beginning of the oestrus cycle (Walton and King, 1986; Larsson, 1998). Decreased milk yield may be a useful oestrus indicator, but has to be combined with other visible oestrus signs such as changes in the behaviour and physical changes in the external genitalia (Walton and King, 1986).

A study performed in cattle by Cowan and Larson (1979) showed that milk yield and sodium and magnesium concentrations of the milk vary with days of the estrous cycle. Mean milk yield was highest and sodium and magnesium concentrations were lowest on day 1 of the estrus cycle (day 0 = estrus). In this study, sodium concentration of the milk was the only component that varied significantly during the 3 days centered on oestrus (days -1, 0, and 1). No other components changed significantly during the oestrus cycle. Although milk yield and composition varies throughout the estrous cycle, none of these components appeared to be a practical indicator of estrus.

Lopez *et al.,* (2004) showed that there is a correlation between milk production and the duration of oestrus in Holstein dairy cows. It was concluded that high milk production decreased the duration in oestrus, probably due to the circulating concentrations of estradiol during oestrus.

To sum up, the changes in milk yield during oestrus may be an indicator of oestrus but has to be combined with other indications of oestrus as well.

**Oestrus behaviour**

Sexual behaviour is activated by hormones from the time of puberty throughout the animal's life. The increasing amounts of reproductive hormones during oestrus affect parts of the brain in female animals. This result in behavioural changes directed towards mating which varies considerably between species (Sjaastad *et al.,* 2003).

Changes in the amount of estrogen secreted from the follicles are the main cause of variations in the visible signs of oestrus. From two to three days before until the onset of oestrus, estrogen causes increased blood supply to the uterus and increased uterine tone. Furthermore estrogens relax the cervix, increases cervical mucus production and the number of endometrial glandular cells. Estrogens also initiate oestrus behavior (Sjaastad *et al.,* 2003).

The primary oestrus sign in cattle is standing to be mounted by herd mates. Apart from the primary signs there are some secondary oestrus signs showed by cattle such as mounting herd mates, decreased appetite and milk yield and restlessness. Also swelling, relaxation and congestion of the vulva, clear mucus discharge and bleeding after oestrus are secondary oestrus signs (Yoshida and Nakao, 2005). These authors also observed that cows in standing oestrus have longer duration of expression of secondary oestrus signs than cows that not showed standing oestrus.

One of the features in oestrus behavior in dairy cows is marked increase in walking activity. The mean daily activity is higher on the day of oestrus than during any of the three days before or after oestrus (Moore and Spahr, 1991).

The buffaloes have an oestrus cycle that is in its main events similar to the cattle's. The buffaloes' oestrus cycle is differentiated by a greater variability in length, oestrus to ovulation interval and oestrus behaviour. Furthermore the buffaloes have a reduced follicle reservoir compared to cattle (Presicce *et al.,* 2003).

## Methods for oestrus detection

Reproduction management is a major concern for the dairy industry, because inefficient oestrus detection causes significant economic losses (Karir *et al.,* 2006). There are some different visual and non-visual methods to detect oestrus in both buffaloes and cows. Examples of visual signs are as mentioned before standing to be mounted by herd mates and the secondary signs of oestrus. To be able to see these signs of oestrus it is required that the cows express the signs for a sufficient time period and the signs need to be carefully observed by the farmer in order to achieve an acceptable oestrus detection rate in the herd (Yoshida and Nakao, 2005).

The tail-paint-mark system where a strip of paint is applied on the rump of the cow can be used for oestrus detection. The purpose with this system is to see if the cow has been mounted, because when the cow is mounted the paint becomes scuffed or cracked. The animals should be examined at least daily to determine whether they have been mounted or not. Although several special paints or pastes are commercially available, ordinary high gloss enamel, paste or water-based paint can be used successfully. Where ordinary household paint is used it should be applied every 3 or 4 days. The tail painting method was found to be 88% accurate in a cattle study in which oestrus was also determined by regular progesterone assay (Kerr and McCaughey, 1984). Another method also with the purpose of using paint to see whether the cows are in heat or not, are the use of chin ball markers or harnesses worn by a bull or a vasectomised bull. The marker leaves a strip of paint on the back of the cow after mounting (Elmore *et al.,* 1986).

The non-visual methods are for example rectal palpation of the ovaries and uterus, changes in electrical resistance of the reproductive tract tissue, monitoring the follicular development by using ultra sound technique, vaginal pH measurements and measurements of the physical activity and hormonal changes during oestrus. The two latest methods are described in more detail further down in this text. This kind of methods relies on technical equipment or has to be done by a veterinarian. They are very often effective but might be difficult or impossible to use in small scale systems in developing countries.

## Ultra sound for monitoring the ovarian function

The use of ultra sound technique for monitoring the ovarian function in buffaloes as well as in other mammals has improved the knowledge and understanding of the follicular development and regulation (Manik *et al., 2002*; Garcia *et al.,* 1999). In several reproduction studies done in both cattle and buffaloes ultra sound has been used to confirm the ovarian activity and number of follicles (Presicce *et al.,* 2003; Taniguchi *et al.,* 2006; Awasthi *et al.,* 2006; Baruselli *et al.,* 1997).

To be able to use ultra sound technique to follow the follicular growth and see when ovulation occurs would be a very good method to determine the right time for artificial insemination or natural service. It is not useful on farm level as a method to find heat, because of the equipment and professional knowledge that is required.

### Measurements of the physical activity for oestrus detection

The purpose with using measurements of the physical activity as oestrus detection is to alert the farmer when an animal becomes more active than usual, which normally indicates that she is in oestrus. The system is assuming that each cow has her own individual motion pattern and that it does not differ much from day to day. During oestrus this pattern differs due to the fact that many cows become more restless and move around more than usual. Physical activity increases at the time of oestrus; studies have showed that cows were about four times as active during oestrus as they were when not in oestrus. Different studies have shown that recording the cows' physical activity are useful tools in reproduction management because there is a close link between increased walking activity and heat (Kiddy, 1977; Liu and Spahr, 1993; Moore and Spahr 1991; López-Gatius *et al.,* 2005).

Lewis and Newman (1984) performed experiments with cows equipped with pedometers. In 75 % of the cases peaks of physical activity coincided with oestrus. Furthermore their study shows that the physical activity was maximal on the first day of oestrus in 73 % of the cases. Increased activity of cows in commercial dairy herds agreed well with the farmers' own diagnosis of oestrus and the conclusion from this was that measurements of physical activity with pedometers could be a valuable indicator of oestrus in cattle.

## AIM

The aim of this study was to document if activity tags (measurements of animal activity) can be used as a method for heat detection in dairy buffaloes. The buffaloes' behaviour and milk production during heat were followed and tested if deviations in those parameters could be used for heat detection as well.

## MATERIAL AND METHODS

### Time period

This study was planned to start in the beginning of June 2006, but due to technical problems with the experimental equipment the study was delayed. This period was however useful as a preparatory time.

The study was performed during the monsoon season 2006; July 22 to August 21.The monsoon starts in the south part of India (Kerala) moves upwards at a rate of 1-2 week per state. The monsoon reaches Maharashtra in the middle of June and is ongoing until September. During the monsoon season it is common with very heavy rainfall and the temperature ranges from 20 to 28ºC.

### The experimental farm

The study took place at M/s B.G. Chitale Dairy Pvt. Ltd, Research and development wing located in Bhilawadi station (Sangli district), 230 km from Pune in the State of Maharashtra, India (photo 1). This commercial farm is equipped with laboratory facilities for reproduction studies in buffalo cows and bulls. The farm had about 150 dairy buffaloes (including recruitments) both of the Murrah and Nili-Ravi breed and about 50 jersey cows. The average milk yield of the buffaloes was 3500 kg/lactation.

*Photo 1. Chitale Dairy, where the study took place.*

**Housing and management routines**

The buffaloes were kept indoors in loose-housing barns with four groups of approximately 24 animals (photo 2). Each lot had 26 resting places (1.2 x 2 meters) on one side of the barn and a manure alley with a Delta Master® manure scraper (DeLaval, Sweden), on the other side there was a feed rack. The barn had micro sprinklers that sprayed water on the animals during the hottest hours of the day. The feed rack had 26 standing places (each 1.2 m wide) without locking system. Each lot had access to 11 water bowls and one automatic concentrate feeding station (AFS). Concentrate feeding in the barn and at milking was controlled with the ALPRO® system (DeLaval, Sweden) where a central processor received the milking and feeding data of all the animals. Animals provided with activity meters were also controlled with the ALPRO® system. The manure scrapers were turned on twice a day, before milking in the morning and afternoon. The barn was illuminated at night with two tube lights on either side of the barn.

*Photo 2. The barn and the feed rack.*

**Milking routines**
The buffaloes were milked twice daily at 06.30 in the morning and at 16.30 in the evening, in a DeLaval 2x5 low-line Vario Tandem® parlour with Duovac milking machines (DeLaval, Sweden). There were three persons working in the milking parlour and usually one person that got the buffaloes to the parlour. Before entering the collection yard the buffaloes passed a shower.

The pre-stimulation routine began with washing the udder with water and then hand stimulation for about half a minute before the cluster was put on. When the milk flow stopped the cluster was automatically removed. After the machine milking was finished the buffaloes were hand stripped. If the buffaloes had problem with milk let-down and no ejection occurred they were given an oxytocin injection.

**Feeding routines**
The ordinary feeding routine in the barn was to feed roughage three times a day;

07:30 Sorghum straw
09:30 Green fodder (marvel grass, alfalfa, elephant grass, maize) mixed and chopped
16:00 Alfalfa and sorghum straw

The roughage consisted of 40 % green fodder (20 % dry matter) and 60 % dry fodder (90 % dry matter).

Concentrate ("Milk More"; 16% protein 2.5% fat) according to actual milk yield was fed through the AFS in the barn and about 0.5 kg in the milking parlour during milking. The amounts were 1.5 kg for body maintenance and 0.4 kg / litre milk.

If the calculated amount was not consumed the leftovers were transferred to the next feeding. Residual amounts at the end of each 24-h period were transferred to the next 24-h period. Total amount feed given (roughages and concentrate) was 2.5 kg dry matter / 100 kg body weight.

### Animals participating in the experiment

Sixteen clinically healthy animals from the herd of M/s B.G. Chitale Dairy Pvt. Ltd, Research and development wing were used in the experiment. Of these animals thirteen were Murrah buffaloes and three were Jersey cows. The status of the experimental animals is showed in table 3.

*Table 3. Status of the experimental animals the first day of the experiment.*

| Animal id | Group | Species | Date when started exp. | No of lactations | Days in milk | Average milk yield 7 days (kg) |
|---|---|---|---|---|---|---|
| 18 | C | Buffalo | 22-jul | 8 | 298 | 5.5 |
| 65 | A | Buffalo | 22-jul | 7 | 424 | 5.6 |
| 142 | A | Buffalo | 22-jul | 5 | 220 | 3.4 |
| 163 | C | Buffalo | 24-jul | 5 | 210 | 5.9 |
| 204 | D | Cow | 31-jul | 5 | 627 | Dry |
| 222 | A | Buffalo | 23-jul | 2 | 75 | 7.7 |
| 229 | D | Cow | 31-jul | 3 | 282 | 6.2 |
| 283 | B | Buffalo | 22-jul | 5 | 63 | 7.4 |
| 432 | B | Buffalo | 22-jul | 6 | 61 | 10.2 |
| 434 | C | Buffalo | 22-jul | 5 | 255 | 6.9 |
| 626 | D | Cow | 31-jul | 2 | 263 | 6.6 |
| 706 | C | Buffalo | 22-jul | 5 | 205 | 8.5 |
| 713 | A | Buffalo | 22-jul | 5 | 92 | 9.2 |
| 715 | C | Buffalo | 22-jul | 5 | 254 | 3.5 |
| 721 | B | Buffalo | 22-jul | 5 | 107 | 9.8 |
| 753 | B | Buffalo | 22-jul | 4 | 45 | 9.1 |

### Experimental design

The animals were divided into four groups (see table 4), one group of buffaloes that were expected to come in heat during week 29-34 (group A) and one group of buffaloes that was induced to heat (group B). Group C; randomly chosen buffaloes that were pregnant (not late). However these buffaloes were not exposed to other treatments than use of activity meters. This gave control values of normal activity. Group D: randomly chosen pregnant cows (same as for group C).

*Table 4. The groups of animals participating in the experiment.*

| Group | "Treatment" | Animal number |
|---|---|---|
| A | Natural heat | 65, 142, 222, 713 |
| B | Induced + natural heat | 283, 432, 721, 753 |
| C | Control | 18, 163, 434, 706, 715 |
| D | Control (cow) | 204, 229, 626 |

**Study description**
Activity tags were placed on each animal participating in the study. The animals were milked twice daily and milk yield was recorded daily by the ALPRO® system. During the entire study, milk samples were taken from strip milk every milking for analyses of fat content to be used as an indicator for udder emptying and once daily (morning) to determine the concentration of progesterone. Strip milk yield was measured every milking by using a 1000 ml measuring cylinder.

Oxytocin treatment during milking was noted and taken into account when analysing the results.

The buffaloes in group B were treated with hormones to induce heat according to the ovsynch protocol described by Vijay and Prakash (2005), which performed an experiment with Murrah Buffalos in India:
Day 0: 10 µg GnRH Analouge (Buserelin-Acetate Inj. Receptal® VET., Intervet International GmbH, Unterschlieisheim, Germany)

Day 7: 25 mg $PGF_{2\alpha}$ (Iliren® for veterinary use. Intervet International GmbH, Unterschlieisheim, Germany)

Day 9: 10 µg GnRH Analouge (Buserelin-Acetate Inj. Receptal® VET., Intervet International GmbH, Unterschlieisheim, Germany)

The animals were supposed to ovulate within 96 hours after being induced to heat according to this protocol (Vijay and Prakash, 2005).

All treatments were done by a veterinarian and the hormones were given as i.m. injections in the neck.

After the Group B buffaloes had participated in heat-induced study they were continuously followed during their next cycle (natural heat). The expected date of heat for the next cycle was assumed according to the date of induced heat.

The expected date of heat for the buffaloes in group A were assumed according to breeding records in the ALPRO® system.

Five days before expected heat:

- The buffaloes were painted on the horns and with numbers on their back.
- The behaviour study started with two observations daily, before morning milking and before evening milking. The buffaloes were observed in the barn before they went to the milking parlour. Each observation period lasted for 30 minutes.
- Ultra sound scanning of the ovaries through rectum, for confirmation of the ovarian activity and to confirm the actual date of heat. The measurement was performed and evaluated by a veterinarian using an Aloka SSD-900 (7.5 MHz). With Aloka SSD-900 it was also possible to print out pictures.
- To determine the actual date of heat the external genitalia were checked for heat symptoms, such as congestion, swelling and relaxation. The observations were done by a veterinarian.

Three days before expected heat the behaviour study increased with two more registrations. One was done in the afternoon around 14:30 and one in the late evening, around 22:30, until the day of heat and one day after heat had past. Depending on when the buffaloes came in heat, the total days of behaviour studies varied (table 5).

*Table 5. Total number of days with behaviour studies and the number of behaviour studies each day for the experimental animals.*

| **Animal id** | **Group** | **Expected heat** | **Start date** | **Final date** | **Total number of days** | **Behaviour study twice daily** | **Behaviour study four times daily** |
|---|---|---|---|---|---|---|---|
| 65 | A | 05-aug | 31-jul | 05-aug | 6 | 2 | 4 |
| 142 | A | 04-aug | 30-jul | 05-aug | 7 | 2 | 5 |
| 222 | A | 27-jul | 23-jul | 28-jul | 6 | 1 | 5 |
| 222 | A | 17-aug | 12-aug | 20-aug | 9 | 2 | 7 |
| 283 | B | 31-jul – 01-aug | 27-jul | 02-aug | 7 | 2 | 5 |
| 283 | B | 21-aug | 16-aug | 20-aug | 5 | 1 | 4 |
| 432 | B | 31-jul – 01-aug | 27-jul | 02-aug | 7 | 2 | 5 |
| 432 | B | 21-aug | 16-aug | 20-aug | 5 | 1 | 4 |
| 713 | A | 04-aug | 30-jul | 05-aug | 7 | 2 | 5 |
| 721 | B | 31-jul – 01-aug | 27-jul | 02-aug | 7 | 2 | 5 |
| 721 | B | 21-aug | 16-aug | 20-aug | 5 | 1 | 4 |
| 753 | B | 31-jul – 01-aug | 27-aug | 02-aug | 7 | 2 | 5 |
| 753 | B | 21-aug | 16-aug | 20-aug | 5 | 1 | 4 |

**Ethogram for behaviour study**

*General behaviours (observed every 5 minutes for 30 minutes)*
Standing – standing with all four feet on the ground
Standing and ruminating
Standing and eating roughages
Lying – lying down
Lying ruminating
Walking

*Frequently observed:*
Drinking
Urinating
Scratching against the interior
Defecate
Visit automatic feeding station - entering the AFS with or without getting access to feed.

*Frequently observed heat behaviours:*
Cajoling (flehmen)
Sniffing the vagina of herd mate

Mounted (or attempt) by herd mate – standing
Mounted (or attempt) by herd mate – not standing
Mounting (or attempt to mount) herd mate
Chin resting of herd mate
Vocalization

*Social interactions:*
Nose – body
Nose – nose
Head – head
Others (for example: push another buffalo with the body, licking (tongue roll) at another buffalo, scratching against another buffalo, push with head or horns against another buffalo's body, shake the head against another buffalo, nose –horn, horn-body and other social behaviours)

**Determination of the heat date**

The actual date of heat was determined by a veterinarian by using the results from ultra sound and from the status of the external genitalia. By using ultra sound the numbers and size of follicles in the left and the right ovary were determined. Ovulation was able to be seen according to the changes in number and size of follicles in the ovaries. The animals were said to be in heat if ovulation had occured, this was able to see by counting the number of follicles left in the ovaries. To get a higher accuracy of the status the conditions of external genitalia was also considered.

During the examination of the status of external genitalia (congestion, swelling and relaxation of the vagina) a three-grade scale was used; yes, no or slight. "Yes" was fully expression of the sign, "slight" mediate expression and "no" no expression at all.

**Activity meters**

Basis for the activity system is the registrations of activity. The activity meters were fastened with a neckband around the neck of the buffaloes. The position of the activity meter is showed on photo 3.

*Photo 3. The position of the activity tag around the buffalo's neck.*

The main components of the activity system are (figure 4):

1. Activity tag (fastened around the neck of the animal)
2. Receiver with an antenna
3. Processor that are connected to a computer in order to run the ALPRO windows program.

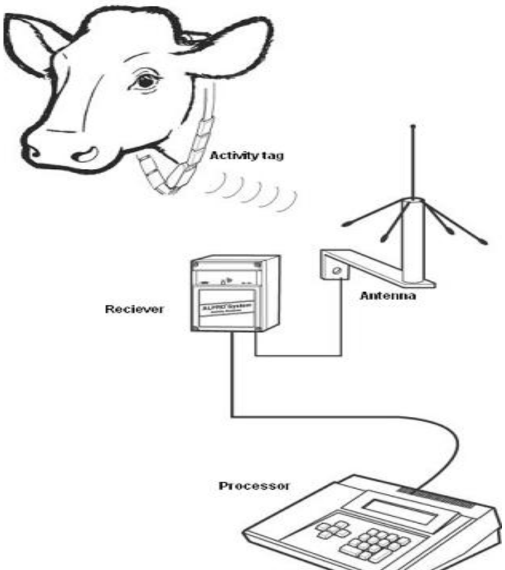

*Figure 4. The main components of the activity meter system (ALPRO® ver 6.30/Delaval activity meter system)*

The activity tag contains a sensor which detects movements. The sensor consists of a magnetic ball that moves in a cavity that is surrounded by two copper coils. An imposed movement is thus transferred into an electrical potential. The impulses are registered, stored and transmitted on an hourly basis to the antennas installed in the stable, onto the ALPRO® processor and finally stored in a PC program. The activity is then presented as an activity list and the data can be showed as an activity graph.

The activity pattern is individual for each animal and there are therefore no specific and fixed control values for all animals. The processor makes a statistical calculation on each individual animal's mean value, based on the previous daily activity every hour. The rolling once-per-hour activity value is matched against its previous values and when the processed data reaches a certain level the program announces that via the activity alarm list, the animals with high activity that can be close to heat. The activity levels +, + +, + + + (table 6) indicates when the animals activity differs from the predicted activity according to the filter.

*Table 6. Explanation of the activity levels used in ALPRO®*

| Activity level | Indication of high activity |
|---|---|
| + | Weak |
| + + | Medium |
| + + + | Strong |

The selection of animals showing high activity is made broad by the activity meter system. The ones with high activity are grouped according to their activity level. The limit values can be changed according to the individual herd.

With a low limit value the system is more precise in recognizing the animals in oestrus, but also more animals that are not in oestrus will be presented on the alarm list. This will have the effect that the farmer has to make larger selections among the alarm list.

With higher limit value both the incorrect and correct oestrus alarms reduces. Thus the farmer will miss a few of the animals in oestrus and the calving intervals may be prolonged. This shows that it is important with an optimal limit on each farm. (ALPRO® ver. 6.60/Delaval activity meter system; personal communication Elfgren)

The animals in the study were equipped with DeLaval's transponders and activity meters that is a part of the ALPRO® system. In the study ALPRO windows version 6.42 was used. The default limit values for high activity in the study were + (40), + + (50) and + + + (70).

### Milk composition analysis

Milk samples were collected twice daily from the strip milk and the composition were analysed using Mid-infrared spectroscopy technique (Milko Scan 93, Foss Electric, DK-3400 Hillerød, Denmark).

### Progesterone concentration analysis

Milk samples were defatted and frozen to be analyzed later by authorized laboratory personal using a radioimmunoassay kit; ACTIVE® Progesterone RIA DSL-3900 (Diagnostic Systems Laboratories, Inc. Webster, Texas, USA). The kit is intended to be used for quantitative measurements of progesterone in serum or plasma. Therefore the milk had to be prepared according to the method described by Karir *et al.,* (2006).

The basic principle for a radioimmunoassay test is a competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The amount of [I-125]-labelled progesterone bound to the antigen is inversely proportional to the concentration of unlabeled progesterone present. The separation of the bound and the free antigen is achieved by decanting the test tubes. The test tubes are then counted in a gamma counter (Diagnostic System Laboratories, Inc., 2005).

**Statistical analysis**

All statistical analysis on the collected data was performed by using the SAS software (SAS-institute).

On the basis of collected data on milk yield (morning and evening), strip milk yield (morning and evening) and strip milk fat percentage (morning and evening), day 0, day -1 and day +1 were compared separately with the other days. Day 0 referred to the actual day of heat, day -1 one day before and day +1 one day after heat has passed. Variation within the individual buffaloes as well as a day-wise comparison between group A and B were done. Following mixed model was used:

$$Y_{ijk} = \mu + \alpha_i + b_{ij} + \delta_k + (\alpha\delta)_{ik} + e_{ijk}$$

where $\mu$ is the overall mean, $\alpha_i$ is the fixed effect of group i (i = 1,2), $b_{ij}$ is the effect of the individual buffalo (j= 1,2,3,4) in group i, $\delta_k$ is the fixed effect of day k (k=-1,0,+1), $(\alpha\delta)_{ik}$ is the fixed effect of group * day interaction and $e_{ijk}$ are the random errors.

The same model was used on the collected data for general and social behaviour.

For statistical evaluation of the activity data, the raw activity data every hour from each individual animal in group A and B were accessed from ALPRO® by using sql-reports provided from software engineers at DeLaval. The raw data from every hour the whole experimental period were evaluated in SAS by using a seasonal ARMA (SARIMA)[1] model.

With a fit ARMA($p$,q) model $ø(B)t = \theta(B)Z_t$ to the differenced series $Y_t = (1 - B^s) X_t$, then the model for the original series is $ø(B)(1 - B^s)X_t = \theta(B)Z_t$. Differencing the series $\{X_t\}$ at lag $s$ is a convenient way of eliminating a seasonal component of period $s$.
B is a back step operation meaning:

$$(1-B) X_t = X_t - X_{t-1}$$
$$(1-B)^2 X_t = (1-2B+B^2)X_t = X_t - 2 X_{t-1} - X_{t-2}$$
$$(1-B^2) X_t = X_t - X_{t-2}$$
$$(1-B^{24}) X_t = X_t - X_{t-24}$$

This is a special case of the general SARIMA model defined as follows:

If $d$ and $D$ are nonnegative integers, then $\{X_t\}$ is an SARIMA $(p,d,q)$ x $(P,D,Q)_s$ process with period $s$ if the differenced series $Y_t = ø(B)\, \Phi(B^s)\, Y_t = \theta(B)\, \Theta(B^s)\, Z_t$, $\{Z_t\} \sim WN(0, \sigma^2)$, Where $ø(z) = 1 - ø_1 z - \ldots - ø_p z^P$, $\Phi(z) = 1 - \Phi_1 z - \ldots - \Phi_p z^P$, $\theta(z) = 1 + \theta_1 z + \ldots + \theta_q z^q$, and $\Theta(z) = 1 + \Theta_1 z + \ldots + \Theta_Q z^Q$.

For the activity data in this study an adjustment of an ARMA-model was done after $1 - B^{24}$ according to (ARMA (3,24)):

[1] ARIMA=Autoregressive integrated moving average process

$$(1 - ø_1B – ø_2 B^2 – ø_3 B^3 ) X_t = (1 - θB^{24} ) Z_t$$
$$X_t - ø_1X_{t-1} – ø_2X_{t-2} - ø_3X_{t-3} = Z_t - θ Z_{t-24}$$
(Brockwell and Davis, 2002).

## RESULTS

### Heat date

The difference between actual and the expected dates of heat were not bigger than 2 days (table 7). Three of the animals in group A were observed during one heat and one was observed during two heats. All animals in group B were observed during the first induced heat and thereafter observed during the next coming natural heat. Altogether among the eight selected animals in group A and B, 13 occasions of heat were observed and evaluated.

*Table 7. Actual and expected dates of heat for the buffaloes in group A and B.*

| Animal id | Group | Expected heat | Actual heat | Difference (actual – expected) | Type of heat |
|---|---|---|---|---|---|
| 65 | A | 05-aug | 04-aug | -1 | Natural |
| 142 | A | 04-aug | 04-aug | 0 | Natural |
| 222 | A | 27-jul | 28-jul | +1 | Natural |
| | | 17-aug | 19-aug | +2 | Natural |
| 283 | B | 31-jul – 01-aug | 31-jul | 0 | Induced |
| | | 21-aug | 20-aug | -1 | Natural |
| 432 | B | 31-jul – 01-aug | 01-aug | 0 | Induced |
| | | 21-aug | 20-aug | -1 | Natural |
| 713 | A | 04-aug | 04-aug | 0 | Natural |
| 721 | B | 31-jul – 01-aug | 01-aug | 0 | Induced |
| | | 21-aug | 20-aug | -1 | Natural |
| 753 | B | 31-jul – 01-aug | 01-aug | 0 | Induced |
| | | 21-aug | 20-aug | -1 | Natural |

One part of the heat date determination was in addition to the ultra sound measurements, examination of the external genitalia to see the expression of heat signs such as congestion, relaxation and swelling. All buffaloes had congestion on their heat day, also swelling of the external genitalia were noticeable in all buffaloes even though one had a slight swelling. The relaxation of the external genitalia was not to same great extent as the other signs (table 8).

*Table 8. The extent of heat sign expression (congestion, relaxation and swelling) of the external genitalia on the day of heat.*

| Animal id | Group | Date | Congestion | Relaxation | Swelling | Type of heat |
|---|---|---|---|---|---|---|
| 65 | A | 04-aug | Y | S | Y | Natural |
| 142 | A | 04-aug | Y | N | Y | Natural |
| 222 | A | 28-jul | Y | Y | Y | Natural |
| | | 19-aug | Y | N | Y | Natural |
| 283 | B | 31-jul | Y | N | S | Induced |
| | | 20-aug | Y | N | Y | Natural |
| 432 | B | 01-aug | Y | Y | Y | Induced |
| | | 20-aug | Y | N | Y | Natural |
| 713 | A | 04-aug | Y | N | Y | Natural |
| 721 | B | 01-aug | Y | Y | Y | Induced |
| | | 20-aug | Y | N | Y | Natural |
| 753 | B | 01-aug | Y | Y | Y | Induced |
| | | 20-aug | Y | N | Y | Natural |

Y=yes, N=no, S=slight

**Ultra sound measurements**
Pictures from ultra sound scanning were used as confirmation of the heat date. Since the animals are moving a little bit during the ultra sound examination it is very hard to take pictures of the ovaries from exactly the same angle every time (personal communication, Mazgori). When ovulation had occurred the buffaloes were said to be in oestrus (see photos 4-6).

*Photo 4a. Ultra sound picture on the day of heat. Animal number 713, group A (natural heat). The approximate place of the ovary is marked by an ellipse and the follicle is marked by an arrow.*

*Photo 4b. Animal number 713, group A (natural heat) when ovulation has occurred. The approximate place of the ovary is marked by an ellipse.*

*Photo 5a. Ultra sound picture on the day of heat. Animal number 283, group B (induced heat). The approximate place of the ovary is marked by an ellipse and the follicle is marked by an arrow.*

*Photo 5b. Animal number 283, group B (induced heat), when ovulation has occurred. The approximate place of the ovary is marked by an ellipse.*

*Photo 6a. Ultra sound picture on the day of heat. Animal number 721, group B (induced heat). The approximate place of the ovary is marked by an ellipse and the follicle is marked bv an arrow*

*Photo 6b. Animal number 721, group B (induced heat), when ovulation has occurred. The approximate place of the ovary is marked by an ellipse.*

**Activity**

The statistical evaluations showed that in one buffalo the activity on the day of heat differed from the other days in the study and the heat would have been possible to predict (figure 5). The other animals in the study had several days with high activity which all could have been indicators of heat (figure 6). This means that there were lots of false alarms.

The plots shows when the animal’s activity level has been higher than expected when comparing the previous eight hours (Yseq). If the value is 1 the activity has been higher and if the value is 0, there is no true ongoing high activity that hour.

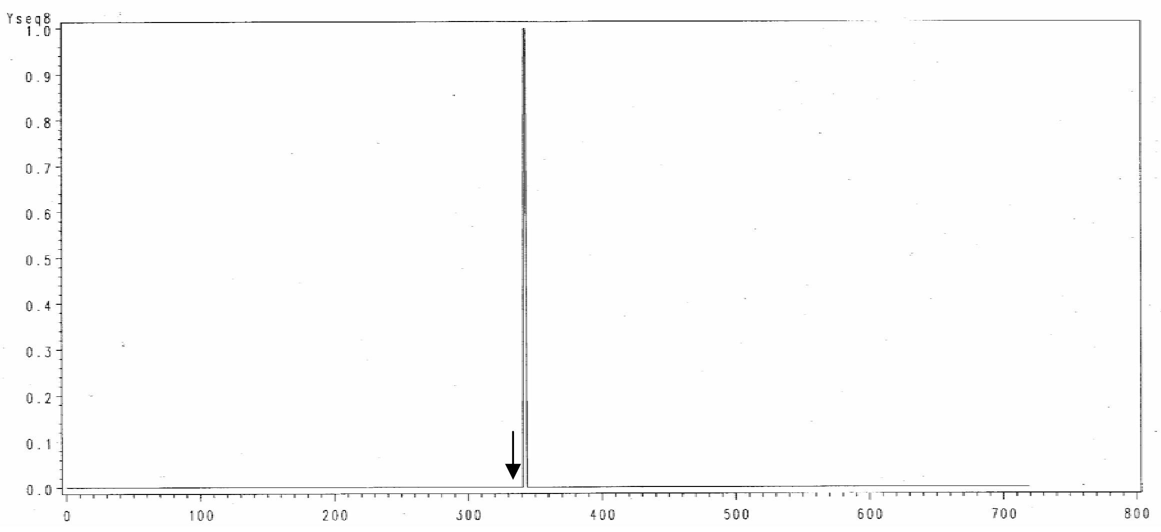


*Figure 5. Hours with high activity, buffalo number 65. Activity level has been higher than expected when comparing the previous eight hours (Yseq). If the value is 1 the activity has been higher and if the value is 0, there is no true high activity that hour. The arrow marks the start hour of the heat day.*

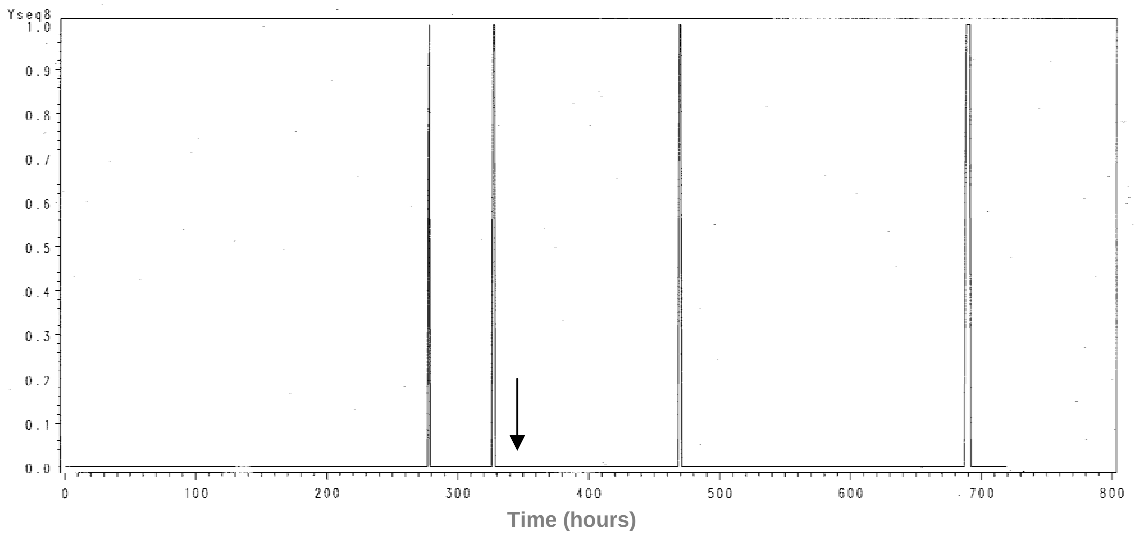


*Figure 6. Hours with high activity, buffalo number 142. Activity level has been higher than expected when comparing the previous eight hours (Yseq). If the value is 1 the activity has been higher and if the value is 0, there is no true high activity that hour. The arrow marks the start hour of the heat day.*

When using the default limit settings + (40), + + (50) and + + + (70) in ALPRO® during the experimental period none of the buffaloes in group A and B were having a strong indication (+ + +) of high activity on the actual day of heat. However it was possible to see weak and medium (+ and + +) indications of high activity on the heat days in one buffalo (table 9). With sql reports provided from DeLaval it was possible to check which animals that gave high activity alarms at different default limit settings (table 10).

*Table 9. Dates when high activity alarms were given for the buffaloes in group A and B during the entire experimental period.*

| Animal id | Group | Date | High activity level | Heat day |
|---|---|---|---|---|
| 65 | A | 30-jul | + + | 04-aug |
| | | 05-aug | + + | |
| 142 | A | 02-aug | + | 04-aug |
| | | 03-aug | + + + | |
| | | 10-aug | + | |
| | | 18-aug | + | |
| | | 19-aug | + + | |
| 222 | A | 07-aug | + | 28-aug (1); 17-aug (2) |
| | | 17-aug | + | |
| 283 | B | 14-aug | + | 31-jul (1); 20-aug (2) |
| 432 | B | 02-aug | + | 01-aug (1); 20-aug (2) |
| | | 05-aug | + + + | |
| | | 20-aug | + | |
| 713 | A | 12-aug | + + | 04-aug |
| 721 | B | 26-aug | + + + | 01-aug (1); 20-aug (2) |
| | | 01-aug | + | |
| | | 04-aug | + + | |
| | | 20-aug | + + | |
| 753 | B | 02-aug | + | 01-aug (1); 20-aug (2) |
| | | 09-aug | + + + | |
| | | 17-aug | + | |
| | | 18-aug | + + | |

+ = weak indication of high activity
+ + = medium indication of high activity
+ + + = strong indication of high activity
(1) = First heat, for animals in group B – induced heat.
(2) = Second heat, for animals in group B – natural heat.

*Table 10. Buffaloes in group A and B that would have given high activity alarm when using six different default limit values.*

| Date | Default limit values | | | | | |
|---|---|---|---|---|---|---|
| | 50 | 45 | 40 | 35 | 30 | 25 |
| 22-jul | | | | | | |
| 23-jul | | | | | | |
| 24-jul | | | | | | |
| 25-jul | | | | | | |
| 26-jul | | | | | | |
| 27-jul | 721 | 721 | 721 | 721 | 721 | 721 |
| 28-jul | | | | | | |
| 29-jul | | | | | | |
| 30-jul | | | | | | |
| 31-jul | | | 65 | 65 | 65,753 | 65,753 |
| 01-aug | | | | | | |
| 02-aug | | | 721* | 721* | 721* | 721* |
| 03-aug | | | | 753 | 142,432,753 | 142,432,753 |
| 04-aug | 142* | 142* | 142* | 142* | 142* | 142* |
| 05-aug | | | 721 | 142,721 | 142,721 | 142,721 |
| 06-aug | 432 | 432 | 65,432 | 65,432 | 65,432 | 65,432 |
| 07-aug | | | | | | 283 |
| 08-aug | | | | | 222 | 222 |
| 09-aug | | | | | 222 | 222 |
| 10-aug | 753 | 753 | 753 | 753 | 753 | 753 |
| 11-aug | 753 | 753 | 753 | 753 | 142,753 | 142,753 |
| 12-aug | | | | | 142 | 65,142 |
| 13-aug | | | 713 | 713 | 713 | 713 |
| 14-aug | | | | 713 | 713 | 142,713 |
| 15-aug | | | | | 283,713 | 222,283,713,721 |
| 16-aug | | | | | | |
| 17-aug | | | | 283 | 283 | 283 |
| 18-aug | | | | 753 | 222,753 | 222,753 |
| 19-aug | | | 142 | 142 | 142,753 | 65,142,753 |
| 20-aug | | | 142 | 142 | 142,753* | 65,142,753* |
| 21-aug | | | 721 | 432,721 | 432,721 | 222,432,721 |

*marking when the buffalo that was in heat

The activity data from the pregnant animals (group C and D) were not statistically evaluated. There were no differences in the activity pattern between pregnant and non pregnant buffaloes, only considering the graphs from ALPRO®, because the animals were in the same system with same routines. There were only three cows that had working activity tags in this study and even though the data was not statistically evaluated one could see from the ALPRO® graphs that their activity pattern in general were the same as the buffaloes but with a higher activity levels. The activity level were higher when the animals went to milking parlour, for example one of the cows (204) had at those times a top activity level of approximately 110 – 120, comparing with one of the buffaloes (65) that had approximately 50 – 60.

**Milk data**
The animals participating in the experiment showed day to day variation during the entire experimental period in milk yield, fat content in the strip milk and strip yield, examples are shown in figures 7 – 9.

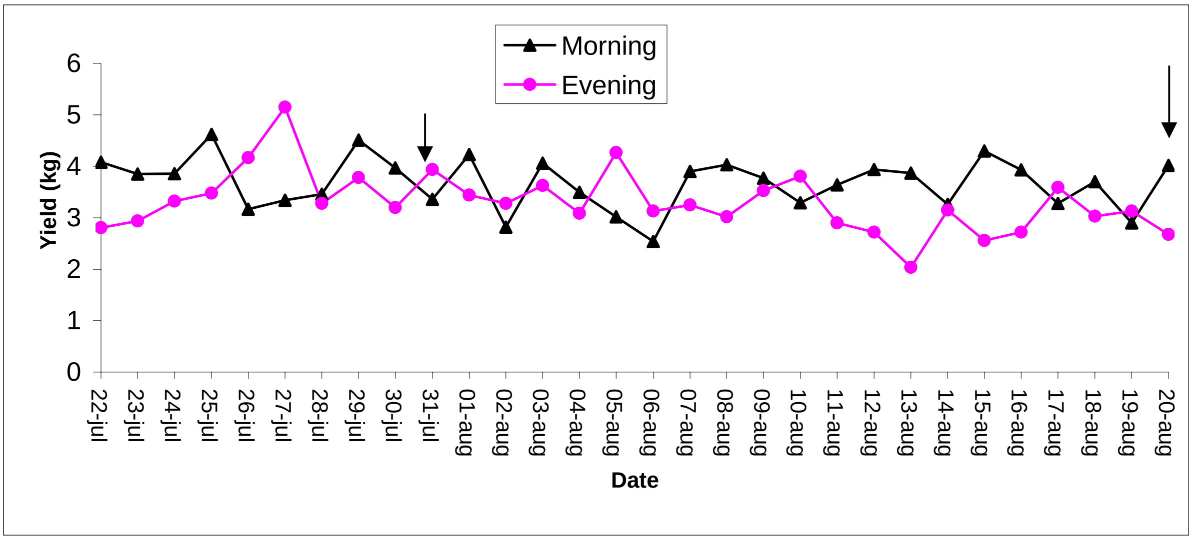


*Figure 7. Milk yield (kg) morning and evening, for buffalo number 283 group B (induced heat). The arrows mark the heat days.*

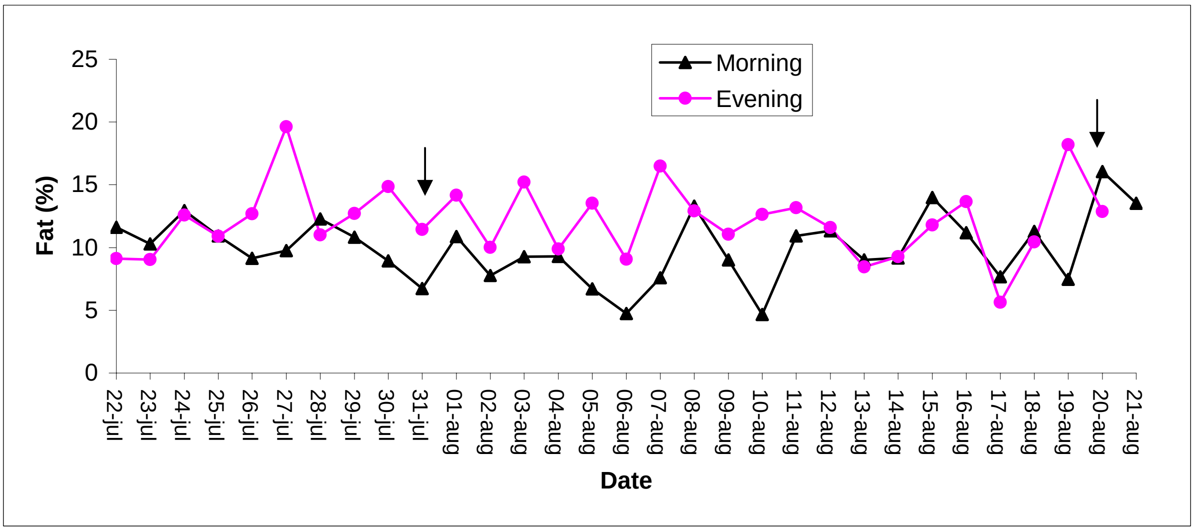


*Figure 8. Fat percent in strip milk morning and evening, for buffalo number 283 group B (induced heat). The arrows mark the heat days.*

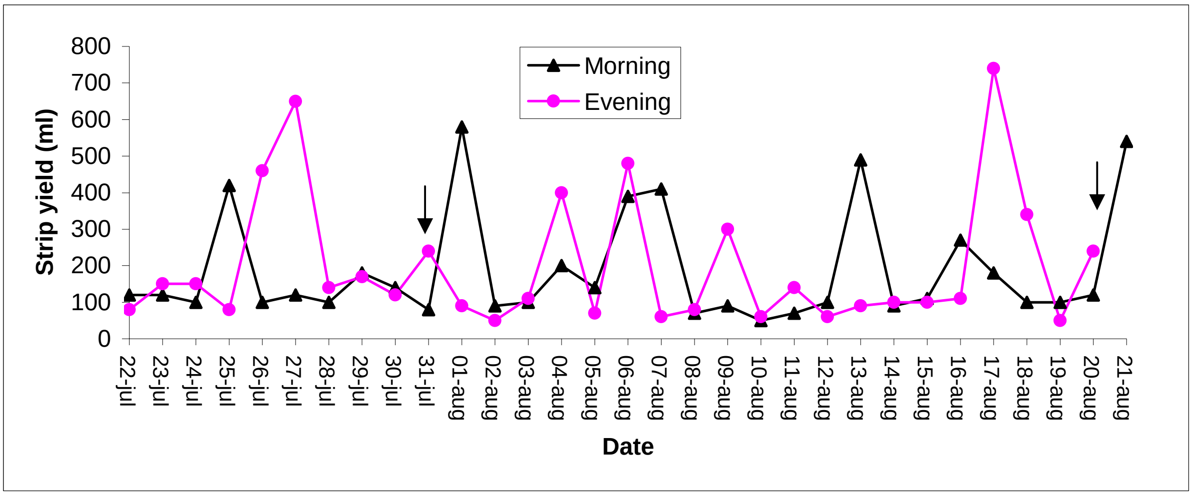


*Figure 9. Strip milk yield (ml) morning and evening for buffalo number 283 group B (induced heat). The arrows mark the heat days.*

There were no significant differences in milk yield fat percent or strip yield before, during and after heat (table 11). However a weak trend in decreased morning milk yield (pr 0.18) and increased evening strip yield (pr 0.17) was able to see.

*Table 11. Milk yield (kg), fat percentage in strip milk and strip yield (ml) before, during and after heat. Day 0 refers to the day of heat. The values are showed as least square means ± standard error, n=8 buffaloes.*

| | Before heat | During heat | After heat | Pr* day 0 | Pr* day-1 | Pr* day +1 |
|---|---|---|---|---|---|---|
| **Milk yield (kg) M** | 4.20±0.51 | 3.97±0.51 | 4.30±0.51 | 0.18 | 0.88 | 0.73 |
| **Milk yield (kg) E** | 3.41±0.37 | 3.36±0.37 | 3.40±0.37 | 0.68 | 0.53 | 0.57 |
| **Fat (%) M** | 10.33±1.48 | 10.63±1.48 | 10.95±1.48 | 0.58 | 0.42 | 0.79 |
| **Fat (%) E** | 12.18±1.18 | 10.59±1.18 | 10.87±1.18 | 0.37 | 0.46 | 0.67 |
| **Strip yield (ml) M** | 231.3±49.4 | 162.5±49.4 | 206.3±49.4 | 0.34 | 0.27 | 0.72 |
| **Strip yield (ml) E** | 172.5±60.1 | 272.5±60.1 | 188.8±60.1 | 0.17 | 0.22 | 0.42 |

*Probability that day 0, day -1 and day +1 were different than the other days.
M=morning
E=evening

## Progesterone

There were big variations between the milk progesterone profiles for all the animals participating in the experiment, two examples are showed in figure 10 and 11.

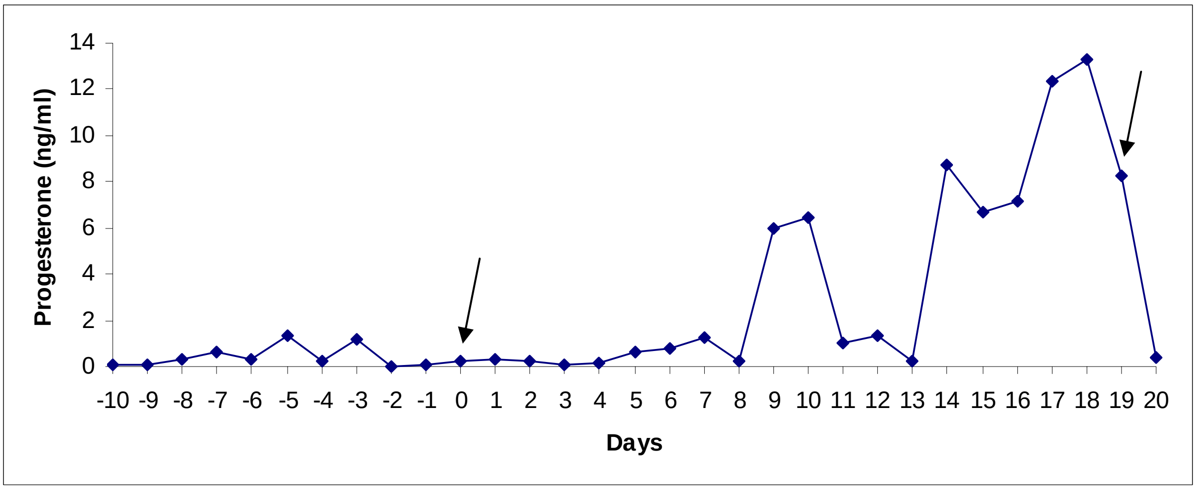


*Figure 10. Progesterone levels in the milk for buffalo number 432 group B. The arrows mark the heat days. Day 0 induced heat and day 19 natural heat.*

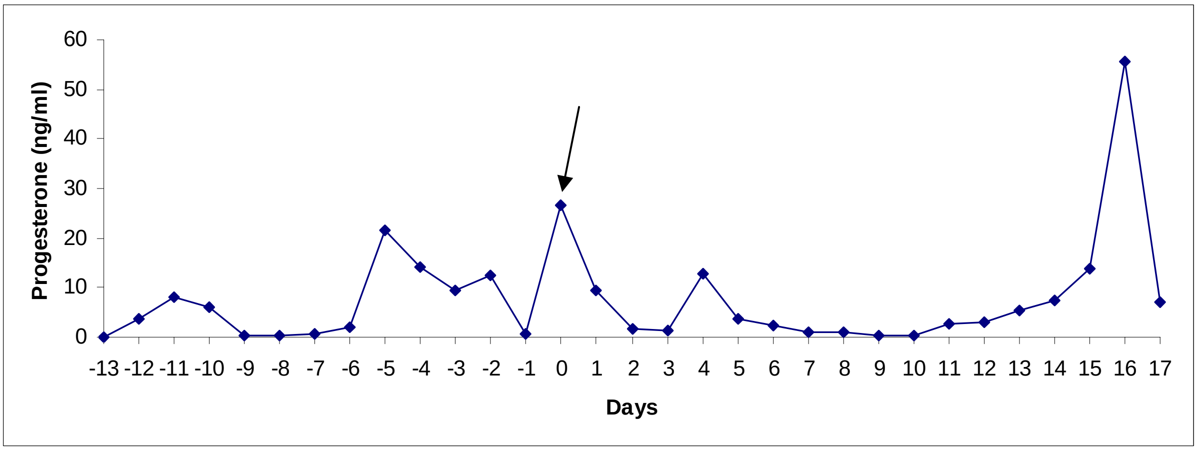


*Figure 11. Progesterone levels in the milk for buffalo number 713group A. The heat day (day 0) is marked by an arrow.*

## General behaviour

The time spent standing as well as standing and eating roughage, before morning milking were the only behaviours that significantly differed during heat compared to before or after. More time was spent on standing during the heat than before or after and less time was spent on standing and eating roughage (table 12).

*Table 12. Minutes spent on general behaviours during the observation periods, before during and after heat. The values are showed as least square means ± standard error, n=8 buffaloes.*

| Behaviour | Before heat | During heat | After heat | Pr* |
|---|---|---|---|---|
| **Standing (min)** | | | | |
| Before morning milking | 23.1±3.2 | 27.5±3.2 | 17.9±3.5 | 0.02 |
| Afternoon (at 14:30) | 8.1±2.6 | 6.3±2.6 | 6.0±2.8 | 0.73 |
| Before evening milking | 13.8±3.4 | 17.5±3.4 | 19.0±3.6 | 0.28 |
| Late evening (at 22:30) | 5.0±3.7 | 8.1±3.4 | 3.3±3.7 | 0.81 |
| **Standing and ruminating (min)** | | | | |
| Before morning milking | 6.9±2.3 | 3.1±2.3 | 0.59±2.5 | 0.38 |
| Afternoon (at 14:30) | 6.3±2.7 | 7.5±2.7 | 6.3±2.9 | 0.32 |
| Before evening milking | 16.9±3.6 | 6.3±3.6 | 8.8±3.9 | 0.22 |
| Late evening (at 22:30) | 3.1±3.0 | 4.4±3.0 | 11.7±3.2 | 0.98 |
| **Standing and eating roughage (min)** | | | | |
| Before morning milking | 1.9±1.8 | 0±1.8 | 14.2±1.9 | 0.002 |
| Afternoon (at 14:30) | 10.0±2.5 | 16.9±2.5 | 21.2±2.7 | 0.23 |
| Before evening milking | 0±2.3 | 3.1±2.3 | 0±2.4 | 0.84 |
| Late evening (at 22:30) | 3.1±0.8 | 0±0.8 | 0±0.9 | 0.42 |
| **Lying (min)** | | | | |
| Before morning milking | ** | ** | ** | ** |
| Afternoon (at 14:30) | 2.5±0.9 | 0±0.9 | 0±1.0 | 0.42 |
| Before evening milking | 0.6±2.8 | 3.8±2.8 | 4.1±3.0 | 0.70 |
| Late evening (at 22:30) | 6.9±4.7 | 11.3±4.7 | 10.7±5.1 | 0.68 |
| **Lying and ruminating (min)** | | | | |
| Before morning milking | ** | ** | ** | ** |
| Afternoon (at 14:30) | 3.8±1.8 | 0±1.8 | 0±2.0 | 0.59 |
| Before evening milking | 3.1±1.5 | 1.9±1.5 | 0±1.6 | 0.78 |
| Late evening (at 22:30) | 2.5±3.8 | 10.6±3.8 | 6.7±4.1 | 0.42 |
| **Walking (min)** | | | | |
| Before morning milking | 3.1±1.8 | 4.4±1.8 | 2.6±1.8 | 0.62 |
| Afternoon (at 14:30) | 4.4±1.5 | 4.4±1.5 | 1.7±1.6 | 0.61 |
| Before evening milking | 0.6±0.9 | 2.5±0.9 | 2.8±1.0 | 0.28 |
| Late evening (at 22:30) | 3.1±1.2 | 0.6±1.2 | 1.5±1.3 | 0.44 |

*****probability that the behaviour on the day of heat differs from the days before and after heat.
******stopped because of infinite likelihood.

## Frequently observed heat behaviours

These data have not been statistically analyzed. The frequency of all the heat behaviours from all preformed behaviour studies are showed in table 13.

*Table 13. The total frequency for heat behaviours the entire study period (all observations), n= 8 buffaloes.*

| Behaviour | Total frequency (numbers) |
|---|---|
| Flehmen | 34 |
| Sniffing the vagina of herd mate | 40 |
| Mounted (or attempt) by herd mate – standing | 0 |
| Mounted (or attempt) by herd mate – not standing | 3 |
| Mounting (or attempt to mount) herd mate | 0 |
| Chin resting of herd mate | 61 |
| Vocalization | 188 |

**Social interactions**

The social interactions did not differ significantly during heat compared with before or after heat (table 14).

*Table 14. The social interactions received or given before, during and after heat. The values are shown as least square means ± standard errors, n=8 buffaloes.*

| Social interactions | Before heat | During heat | After heat | Pr* |
|---|---|---|---|---|
| **Received from other individuals** | | | | |
| Before morning milking | 10.6±2.1 | 11.6±2.1 | 9.8±2.2 | 0.35 |
| Afternoon (at 14:30) | 8.5±1.8 | 8.8±1.8 | 6.6±1.9 | 0.58 |
| Before evening milking | 3.1±1.2 | 5.9±1.2 | 6.0±1.2 | 0.10 |
| Late evening (at 22:30) | 4.0±1.1 | 1.4±1.1 | 3.3±1.2 | 0.55 |
| **Given to other individuals** | | | | |
| Before morning milking | 11.6±2.0 | 11.4±2.0 | 8.0±2.1 | 0.22 |
| Afternoon (at 14:30) | 7.4±1.9 | 8.1±1.9 | 4.8±2.1 | 0.30 |
| Before evening milking | 3.5±2.2 | 4.5±2.2 | 5.4±2.3 | 0.97 |
| Late evening (at 22:30) | 3.4±1.4 | 1.5±1.4 | 2.2±1.5 | 0.96 |

*probability that the social behaviour differs on the day of heat compared with the days before and after heat.

## DISCUSSION

**Heat date and ultra sound measurements**

The expected heat dates for the buffaloes in group B agreed well with the actual heat dates for the induced heat as well as the second natural heat. Also for the animals in group A the expected heat dates agreed well with the actual. When ALPRO® assumes the heat date it relies on the fact that a normal cycle is 21 days long. If the animals show normal cyclicity and the heat is detected on the actual day this is a good system to remind the farmer.

The reason to why the system worked out so good in this case might be due to the fact that the detection of heat were done more carefully than normal routines. Using ultrasound is a very good method since the follicular changes characterizing the heat, are seen even though if the animal is not showing any other heat signs. In this study the ultra sound measurements were the key to find the exact heat date and it underlies the evaluations of the other parameters. The use of ultra sound for heat detection would be a really accurate method but is too costly, need technical equipment and experienced veterinarians.

All buffaloes in this study expressed clear heat signs in the external genitalia. In addition to the ultra sound results the expressions of heat signs in the external genitalia were useful for determination of the heat date.

**Activity data**

For one buffalo of eight in the study the activity data could be useful to find the heat date without any misleading false alarms. With the heat day key from ultra sound measurements it was possible to see if the days with higher activity than expected were heat days.
The ability to find one of thirteen heats with the statistical calculations shows that the activity meter system needs further adjustments and improvements to fit for buffaloes. More knowledge of the buffaloes' normal physical activity as well as the activity during heat is needed to make those improvements. This study was performed during a period of one month and the number of animals was small, this gives a small material both regarding time and occasions of heat. But the indications are that the activity meter system can not be applied on buffaloes without adjustments. It is the same scenario as when the machine milking for buffaloes started. The machine milking system today is different when comparing for buffaloes and cows (Thomas *et al.,* 2004).

When considering the alarms from ALPRO® one buffalo in the study had weak indication of high activity on one of the heat dates and medium indication of high activity on the other heat day. However, these indications were too weak and it was also possible to see indications of high activity on other days (false alarms) in the study for this buffalo.

Even though the filter settings in the activity system can be changed to be less sensitive and archive more strong high activity alarms, this study shows that it is still not useful for buffaloes. Lower filter settings will give a lot more false positives which makes the system unreliable.

What also need to be pointed out is that four of the animals in this study were followed during an induced heat. The aim with inducing heat is to achieve an ovulation with a high number of follicles and the animal might not show the same behaviour as during a normal heat (personal communication, Gustafsson).

When using activity meters in commercial herds it is possible to sort out not pregnant animals and only evaluate their activity alarms. To make a fair comparison between pregnant and non pregnant animals a study with greater number of animals for a longer time period is required.

The activity pattern is most probably the same for all animals in this study because the buffaloes and the cows were housed in the same system. When considering the graphs from ALPRO® it was possible to see that the cows had higher activity level, but studies with greater animal material are needed to get more accurate and valuable comparisons between buffaloes and cattle.

**Milk data**

There were big variations between the animals concerning all the different milk data that was evaluated in the study. Even though the small number of animals and the big variation, both day to day in individual animals and variation between the animals, there are indications for differences in the morning milk yield as well as the evening strip yield. These trends were able to see when the actual day of heat was compared with the days before or after.

Also the two days closest (day -1 and day +1) to the heat day were compared with the other days to see if they differed more. In those evaluations no significant differences or trends of difference were able to be seen. Due to the fact that some milk parameters showed a possible trend of difference on the heat day, it might be useful to consider the milk data as one indication in heat detection, but it has to be combined with other signs as well. This agrees with previous studies done in cattle (Walton and King, 1986; Cowan and Larson, 1979; Lopez *et al.,* 2004).

Buffaloes are known to be sensitive to changes in the environment or routines during milking and some parts of the variations in this study may therefore be due to the fact that the animals were participating in a study which also included new people being in the milking parlour.

**Progesterone in milk**

When taking milk samples for determination of the progesterone concentration in this study, strip milk was used even tough the high fat content. Strip milk has a higher fat content than the composite milk. Milk with higher fat are said to have a higher progesterone concentration, because the progesterone is bound to the milk fat (Karir *et al.,* 2006). In other studies performed with buffalo milk, the samples have been taking from whole milk (Batra *et al.,* 1979; Kamboj and Prakash, 1993; Gupta and Prakash, 1990). In studies done with cattle milk, the samples have been taken from the strip milk (Lamming and Darwash, 1998; Stevenson and Pursley, 1994).

When planning for the progesterone concentration determination in this study the decision was to perform as the normal routines used in Sweden. It is more practical taking samples from the strip milk when the animals are milked by machine, because it is not necessary to terminate the machine milking for taking samples. The second reason for using this method was due to the fact that the fat content is higher in the strip milk compared to the normal milk. This milk might therefore have higher progesterone content (personal communication, Gustafsson).

Radioimmunoassay with a 125 – I radioisotope uses a specific antibody and is therefore a very specific method of estimating the progesterone concentration in plasma (Karir *et al.,* 2006). The progesterone RIA DSL-3900 used in this study is optimized for estimations of plasma concentrations of progesterone. Before using it for milk analyses of progesterone concentration the milk must be defatted because the fat layer interfered with the antibody used in the assay system. This is probably due to the fact that progesterone is enclosed in the lipophilic fat layer (Karir *et al.,* 2006).

In the material used in this study there is a big variation between the individual animals and there is also a big day to day variation within the individuals. The abnormal milk progesterone profiles for all animals shows that this method was not useful at all. The method has to be more specified when using it for milk instead of plasma. It is also required with a very efficient removal of the fat before performing the RIA.

### General behaviour

There was a significant difference between the time spent standing before morning milking during oestrus compared to the days before and after. During heat the buffaloes spent more time standing in the morning than before and after. In addition to that there is also a significant difference in time spending on standing and eating roughage before morning milking. The buffaloes spent more time on standing and eating roughage after heat compared to the heat date. This might be an indication that the buffaloes are more restless during heat than before or after.

### Frequently observed heat behaviour and social interactions

None of the buffaloes showed what is said to be the primary oestrus sign in cattle; standing when mounted by herd mates, although three of them were mounted by herd mates. In addition to this, none of the buffaloes in the study mounted or attempted to mount any herd mate.

The frequency of vocalization seems to be high, but almost 80 percent of the vocalization frequency was caused by one buffalo in one day. It can be many reasons for this high frequency, since it was a day when the buffalo was not in heat.

The frequency of chin resting was also notable high. The most of the chin resting occurred during the observations before milking when it was cramped in the barns. The buffaloes were waiting to be milked and all of them were standing close together near the gate (photo 7). It is therefore hard to decide whether this is a heat sign or just because of lack of space. The fact that the buffaloes gather near the gate cause a higher frequency of social interactions as well. As this happened before every milking, it might be a reason that the numbers of social interaction did not differ when the animals were in heat.

*Photo 7. Buffaloes standing close to each other, near the gate in the barn.*

The behaviour towards herd mates changes when an animal is in heat, cattle for example become more restless and interact more with herd mates (Sjaastad *et al.,* 2003; Yoshida and Nakao, 2005).

There are differences between cattle and buffaloes in the behaviour during heat (Presicce *et al.,* 2003). The fact that there were no significant differences in numbers of social interactions during heat compared with before or after agrees with that statement.

**Thoughts**

Many of the buffalo farmers have their buffaloes in small back yard systems without any possibilities to have technical equipment such as activity meters. The activity meters can not be used in tied up systems.

Breeding is one point that could improve the ability to find heat among buffaloes. But before it is possible to set up breeding goals like that a proper recording is required. There is still a big difference between the modern dairy industry and buffalo farming. With knowledge and more intense research the buffalo milk production will be more effective.

**CONCLUSIONS**

- Ultra sound measurements are a good method to detect heat in water buffaloes, but can not be defended for practical use.
- There was expression of heat signs in the external genitalia during heat.
- The activity meters could detect one of thirteen heats in the study.
- The activity meter system needs further improvement and adjustments before it can be useful for heat detection in water buffaloes.
- There was a big day-to-day variation in milk yield, strip yield and strip milk fat content among the animals in this study.

- There are indications that the milk yield and the strip yield can be used as one part of the heat detection, although it has to be combined with other signs as well.
- Using RIA kit that is intended for plasma or serum was not useful in this study because of inefficient fat removal.
- The buffaloes in this study spend less time on eating roughage during heat than after which might be an indication of decreased appetite or restlessness. The standing time were increased during heat.
- There were no expressions of the heat signs mounting herd mates or standing when other herd mates tried to mount.

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