CHAPTER 4

4. Factors affecting post partum milk production and ovarian activity of small scale dairy cows in the Western Highlands of Cameroon

4.1. Validation of a human progesterone Enzyme Immunoassay (Eia) Kit for use on serum of cattle in Cameroon

Published in Tropical Animal Health and Production

Bayemi P. H., Munji V. N., Cavestany D., Perera B. M. A. O., Webb E. C. 2007.
Validation of a human progesterone EH511 Enzyme Immuno Assay kit for use in cattle serum at Bambui, Cameroon. Tropical Animal Health and Production 39: 335-338.

4.1.1. Abstract

A study aimed at validating a human progesterone enzyme immuno assay kit was carried out on cattle at Bambui, Cameroon. Progesterone ELISA Kits (EH-511) were obtained from Clinpro International. Fourty one cows were selected from of which 19 were pregnant and 22 within 14 days post-partum. Blood samples were analyzed and progesterone levels deduced from a curve obtained from standards absorbance values (A_{450}). Results show that 95.5% of post-partum cows had progesterone levels below 1ng/ml with the highest level being 0.75 ng/ml. The mean level was 0.5±0.26 ng/ml. The cows in the 'pregnant group' had progesterone levels ranging from 3.5 to 17.5 ng/ml. This kit can be used for measuring progesterone levels in cattle. Cows with 1ng/ml for two consecutive samples or one sample at or above 3ng/ml are an indication of the presence of corpus luteum while cows below 1ng/ml will be in anoestrus.

Key words: Cameroon, cattle, ELISA, Progesterone

4.1.2. Introduction

Progesterone is a reproductive hormone produced by the Corpus Luteum (CL) of the ovary and the placenta. Its concentration in blood increases in the body after ovulation and during pregnancy and declines during follicular growth after luteolysis and parturition. Its principal sites of production are the ovaries and the placenta during pregnancy. Thus progesterone is commonly measured to determine whether a cow is cycling or pregnant, using Radio Immuno Assay (RIA) or Enzyme Linked Immunosorbent Assay (ELISA) in milk, blood or serum (Plazier, 1993; Ovucheck, 2003).

One of the main constraints hindering dairy cow production in the developing countries is poor reproductive performance of cows (Eduvie *et al*, 1993; Mbaye and Ndiaye, 1993; Makkar, 2002). Although other factors such as health and nutrition also affect reproduction, they usually do this by influencing the functions of the ovaries or uterus, and result in changes of circulating hormone levels. The determination of progesterone (P4) levels to monitor the major reproductive events such as failure or inappropriate oestrus detection, missed heats, early embryonic mortality, and pregnancy estimation gives more accuracy to the evaluation of the reproductive efficiency of dairy farms (Edgerton and Hafs, 1973). Therefore, a common method to assess the reproductive functions of cows has been to monitor progesterone profiles.

In the developing countries, particularly in Cameroon, these methods are not always available. And even when the equipment is even found, the reagents may not be at hand to be able to carry out appropriate tests. Some of the kits developed for humans, when available, could also be used for cattle because progesterone is not species specific (Wakeman, http://www.showdogsupersite.com/kenlclub/breedvet/vfes1.html). EIA has the advantage over RIA of not requiring a radioisotope (Plazier, 1993). Therefore this study aims at validating the use of Human Progesterone Enzyme Immunoa Assay kits (obtained from Clinpro International Co.LLC.) for measuring progesterone in the blood serum of cattle.

4.1.3. Material and methods

4.1.3.1. Animals

Fourty one cows were selected for this study, with 19 being diagnosed and confirmed pregnant by rectal palpation and 22 within two weeks post partum. They were Holstein (*Bos taurus*), Red Fulani (*Bos indicus*) and crossbred (*Bos taurus x Bos indicus*). Holstein cows were kept in stalls and fed in a zero grazing system. Grass was chopped and provided in feeding troughs. Crosses grazed on *Brachiaria spp, Pennisetum purpureum* or *Trypsacum laxum* dominant pastures while Red Fulani cows grazed on communal *Sporobolus africanus* pastures.

Cows were considered pregnant by farmers who declared them bred and did not show external signs of return to oestrus. These pregnancies were subsequently confirmed by rectal palpation. One weekly blood sample was collected for each cow within 2 weeks post-partum.

4.1.3.2. Blood collection and analysis

Blood samples were collected in tubes kept in ice and taken to the laboratory. They were centrifuged at 1200 rpm for 15 mn and the serum stored in a freezer until analysis with the EIA kits obtained from Clinpro International Co. LLC.(Union City, USA, CA 94587, EH-511). It is designed for measurement of total progesterone in human serum or plasma. The minimum detectable level of progesterone in the run comprising post-partum cows was 0.05ng/ml and in the run of pregnant cows was 0.3ng/ml (Clinpro International, 2004 and 2005). Intra-assay precision (coefficient of variation) by replicate determination of four different serum samples in one assay varied from 2.4% to 7.1% while inter assay precision of six different serum samples over a series of individually calibrated assays varied from 2.6% to 12.6%. The assay could detect progesterone levels of up to 50ng/ml above which there is a need of a diluent (but this diluent was not needed as cows will not get that high). The low control had values ranging from 3.5 to 7.5ng/ml and the high control 20 to 39.5ng/ml. Samples, standards and controls were duplicated and the mean absorbance value (A_{450}) calculated in nm. The six standards had the following progesterone concentrations: 0; 0.5; 3; 10; 25 and 50ng/ml. A standard curve was constructed by plotting the mean absorbance for each reference standard against its concentration in ng/ml. This standard graph was used to determine the concentration of progesterone in each sample.

4.1.4. Results and discussion

The assay was considered valid as controls fell within the manufacturer's specified range. The low and high controls had respectively values of 4ng/ml and 34 ng/ml. Twenty one cows out of 22 representing 95.5% of post-partum cows had progesterone levels below 1ng/ml. The mean level was 0.5±0.25. Intra assay precision was 10.4%. Progesterone average level in the 'pregnant group' was 7±4.3 ng/ml with values ranging from 3.5 to 17.5 ng/ml.

These results confirm the normal usage of the tested kit specifying that progesterone concentrations of more than 3ng/ml will be a strong presumptive evidence of the presence of a CL (Clinpro International, 2005) even in cattle. The International Atomic Energy Agency (IAEA) milk standard used in solid phase RIA at Seiberdorf Austria recommended 3nmol/L levels as reflecting luteal phase while less than 1nmol/L were related to anoestrus or follicular phase of the oestrus cycle (Cavestany *et al*, 2001). Progesterone levels in milk reflect those in blood (Ovucheck, 2003). Other studies have demonstrated a strong correlation between EIA and RIA. Plazier (1993) found this correlation to vary from 0.8 to 0.93 while Nagy *et al* (1998) found a correlation of 0.9. The IAEA (1997) also adapted a human progesterone kit to be used in determining P4 in domestic animals including cattle, sheep, goat, buffalo, camelids and yaks. Similarly, Eckersall and Harvey (1987) validated a bovine plasma progesterone ELISA kit to be used in equine, ovine and canine. EIA kits used in one species must be validated for use in other species. Consequently this kit can be confidently used for measuring progesterone levels in cattle. The cow with the highest progesterone level (1.5 ng/ml) in the post-partum group may have had an early return to ovarian activity. Otherwise this could be explained by assay variation. Unfortunately the sample was not reassayed.

This work agrees with the practice that concentrations over 1ng/ml for two consecutive samples or one sample at or above 3ng/ml are an indication of presence of corpus luteum (Cavestany *et al*, 2001; Msangi *et al*, 2004). Owing to the fact that EIA compares very well with RIA provided the assay is carried out by an experienced technician able to standardize each step, the lack of appropriate technicians in developing countries may be a problem if they are not well trained. In which case large differences in duplicate will lead to unacceptable results. This training can even be organized among developing countries as many laboratories are now used with EIA techniques.

Intra assay precision in this work was good (10%). Plazier (1993) stated that intra assay precision in EIA use must be below 15%. Low precision, above this value may happen because of the product not being sufficiently stable during transportation as in the case of long delays in customs clearance. In Cameroon, this kit needs to be ordered from abroad and will be received within a week after shipping. It is sold in the country at $200 per kit and at the Bambui laboratory charged at $10 per sample. Therefore laboratories ordering this kit in Cameroon must ensure quick clearance at the airport and protection from external factors such as high temperatures and exposure to light. Low precision may also be due to a large number of pipetting steps. Laboratories in developing countries will also gain in precision by having mutichannels pipettes of 8 and 12 channels.

4.1.5. References

Cavestany, D., Galina, C. S., Cinoles Vinoles, C., 2001. Effect of the characteristics of the post-partum onset of ovarian activity on the reproductive efficiency of Holtein cows in pastoral conditions. Archivos de Medicina Veterinaria (Chile); 33: 217-226.

Clinpro International Co.LLC., 2004. Progesterone Enzyme immunoassay test kit. No EH-511. Handbook.

Clinpro International Co.LLC., 2005. Progesterone Enzyme immunoassay test kit. No EH-511. Handbook.

Eckersall, P.D., Harvey, M.J., 1987. The use of bovine plasma progesterone ELISA kits to measure progesterone in equine, ovine and canine plasmas. Vet. Rec. 120(1): 5-8.

Edgerton, L.A., Hafs, H.D., 1973. Serum luteinizing hormone, prolactin, glucocorticoid, and progestin in dairy cows from calving to gestation. J. Dairy Sci. 1973;56(4):451-8.

Eduvie, L.O., Bawa, E.K., Dawuda, P.M., Oyedipe, E.O., Olorunju, S.A.S., Bale, J.O., Sekoni, U.O., 1993. Factors affecting the reproductive performance of Bunaji cattle under different pastoral Management systems in the guinea savanna zone of Nigeria. Improving the Productivity of Indigenous African Livestock. Results of FAO/IAEA/DGIS coordinated Research programmes organized by the joint FAO/IAEA division of Nuclear Techniques in Food and Agriculture.

IAEA (International Atomic Energy Agency), 1997. Applied research in Methods development. Activity report.
www.iaea.or.at/programmes/rial/agriculture/animalprotection/animalprotection_body.htm.

Makkar, H.P.S., 2002. Development and field evaluation of animal feed supplementation packages. Proceeding of the final review meeting of an IAEA Technical Cooperation Regional AFRA Project organized by the FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and held in Cairo, Egypt, 25-29 November 2000: Project summary.

Mbaye, M., Ndiaye, M., 1993. Etude de l'activité ovarienne cyclique chez les génisses prépubères et chez les vaches en postpartu, de race zébu Gobra au Sénégal. Improving the Productivity of Indigenous African Livestock. Results of FAO/IAEA/DGIS coordinated Research programmes organized by the joint FAO/IAEA division of Nuclear Techniques in Food and Agriculture.

Msangi, B.S.J., Bryant, M.J., Nkya, R., Thorne, P.J., 2004. Effect of a short term increase in supplementation on the reproductive performance in lactating crossbred dairy cows. Tropical Animal Health and Production Vol. 36, No. 8, pp. 775-787.

Nagy, P., Solti, L., Kulcsar, M., Reiczigel, J., Huszenicza, G., Abavary, K., Wolfling, A., 1998. Acta. Vet. Hung 46(4): 501-13.

Ovucheck, 2003. Measurement of Progesterone in Bovine Milk. The OVUCHECK® MILK ELISA, Product #C006. Technical services: support@biovet-inc.com www.biovet.ca.

Plazier, J.C.B., 1993. Field validation of an EIA kit for progesterone measurement in milk and blood plasma. Improving the Productivity of Indigenous African Livestock. Results of FAO/IAEA/DGIS coordinated Research programmes organized by the joint FAO/IAEA division of Nuclear Techniques in Food and Agriculture. 157-165.

Wakeman, M.C., Fresh extended semen breeding. Ashford Animal Clinic, Canine Fertility Center. http://www.showdogsupersite.com/kenlclub/breedvet/vfes1.html

4.2. Post partum ovarian activity and milk production

Paper presented at 3R conference Paris

P. H. Bayemi, D. Cavestany, M. J. Bryant, V. N. Munji, E. C. Webb, M. Ngo Lihep, B. M. A. O. Perera , D. Whitaker, J. N. Mbanya, 2007. Factors affecting return to oestrus and milk production in lactating cows of small holder dairy farms of Bamenda, Cameroon. 3R , Rencontres-Recherche-Ruminants, conference Paris, 14e Journée, Dec 2007. http://www.journees3r.fr/texte.php3?id_article=2553

4.2.1. Abstract

Thirty five pregnant dairy cows were selected for studies on post partum return to ovarian activity and milk production. They consisted of 13 Holstein, 13 local Gudali (*Bos indicus*) and 9 crosses (*Bos taurus x Bos indicus*). Holstein cows were stall fed in a zero grazing system with Guatemala grass *(Trypsacum laxum)*, *Desmodium spp* and Elephant grass (*Pennisetum purpureum*). Concentrate feeding was provided during milking. Crosses and indigenous cattle grazed either on *Brachiaria spp.,* on native *Sporobolus africanus* or on marginal pastures. Monthly records were kept on body condition score (BCS) at calving and milk production from the time of calving to 12 weeks after calving. Blood samples were analyzed using ELISA progesterone kits. Results show that Holstein cows produced more milk than other breeds (P<0.05), with an average of 12.52 ± 4.34 litres per cow per day vs 9.59 ± 2.3 for crosses and 3.38 ± 0,94 litres per cow per day for traditional zebu breeds. There was no evidence that parity, system of feeding and calving season affect milk production (P >0.05) though primiparous cows produced 13.2± 2 litres per cow per day and multiparous 7.4± 5 litres per cow per day. Milk production was 13.31 ± 4.34 litres per cow per day in the feeding system where cows received more that 3kg concentrate, 8.14 ± 4.49 litres per cow per day and 6.2 ± 3.5 litres per cow per day respectively in feeding systems of less than 3kg concentrate and grazing without concentrate respectively. No factor seemed to influence resumption of ovarian activity (P>0.05). The period of post partum first ovulation was 41±2 days in Holstein compared to 38 ±2 days for crosses and 51±20 days for local cows. The mean interval to first service was 58 days. It was found that an increase in one litre of milk production of cows producing between 2 to 20 litres will reduce the anoestrus period by 2.25 days. This finding suggests that in this area, cows producing more milk tend to take less time to resumption to ovarian activity.

Key words: Post partum, oestrus, progesterone, ELISA kits, small holder, dairy, Bamenda, Cameroon.

4.2.2. Introduction

The republic of Cameroon has a population of 6 million cattle of which 4% is dairy (FAO, 2004). More than 90% of this population is found in the northern and northwestern regions. Traditionally, milk production in Cameroon is based mainly on the transhumance system using local zebu cows of the breeds, Gudali, White Fulani, Red Fulani. Quantitatively this production has been low, yielding an average of only 3 liters of milk per cow per day. Subsequent improvement in milk production was made possible through importations of high yielding dairy breeds such as Holstein Friesian, Jersey and others. Although improvements were observed in terms of milk yields in other semi intensive and intensive production systems of 12.8kg per annum per capita (Bayemi *et al*, 2005), milk production has not satisfied the demand and is still below the annual per capita consumption standing currently at 15.3kg.

Reproduction is the key factor affecting the efficiency of production and it is influenced by the environment. Previous studies in the Western Highlands of Cameroon reported an inter-calving period of 448 days in cows whose breed was not stated (Njoya *et al*, 1999). Yet a 12-month calving interval is generally considered the most economically desirable situation for dairy cows (Cavestany *et al*, 2003). An early resumption of the normal post-partum ovarian activity is important in order to maximize reproductive efficiency (Butler and Smith, 1989). It is necessary to have information on the reasons for long periods of resumption of ovarian activity in order to devise interventions aimed at correcting factors that delay reproduction in different breeds. The aim of this research was to study the factors that influence post-partum anoestrus and milk production of cattle breeds used for dairy production in the Western Highlands of Cameroon.

4.2.3. Materials and methods

4.2.3.1. Study site

The North West Region of Cameroon is part of the Western Highlands. It is located in the mid and high altitude zone of the country which lies between latitudes 5°20’ and 7° North and longitude 9°40’ and 11°10’ East of the Equator. The surface area of the Region is 17,910 km^2 covering 1/6 of the country’s land area. Altitudes range from 300 to 3000 m above sea level. There are two main seasons: a dry season from November to mid March and a rainy season from mid March to October. Rainfall ranges between 1300-3000 mm with a mean of 2000 mm. Minimum and maximum temperatures range between 15.50°C and 24.5°C respectively although temperatures can exceed 30°C.

4.2.3.2. Selections of farmers

The farmers who participated in the study were selected by means of the Economic Opportunity Survey (Nordlund *et al*, 2007). Fifteen farmers were chosen on the basis of commitment to long-term collaboration in the present research project. They were from a peri-urban area of Bamenda and had at least one pregnant cow.

4.2.3.3. Animal management

Thirty Five cows were chosen for the study, which consisted of 13 Holstein, 13 local Gudali (*Bos indicus*) and 9 crosses (*Bos taurus x Bos indicus*). Holstein cows were stall fed in a zero grazing system. Forage was cut, chopped and fed to cows in feeding troughs. It consisted mainly of Guatemala grass *(Trypsacum laxum)*, *Desmodium spp* and Elephant grass (*Pennisetum purpureum*). Water was provided daily in iron buckets or in drinking troughs made from iron drums. Concentrate feeding was provided during milking. Crosses and indigenous cattle grazed either on *Brachiaria spp.,* on native *Sporobolus africanus* or on marginal pastures. The feeding systems practiced at those farms were described in order to possibly link reproduction to feeding. This description took into consideration the quantity of concentrate given, the estimated nutrient composition of the feed in terms of crude protein, metabolizable energy, calcium and phosphorus, the type of forage fed, the frequency of feeding, the grazing behaviour and water supply. Three feeding systems were described: Forage without supplementation (A), small level supplementation with forage and at most 2kg supplement (B) and High level supplementation in zero grazing with 3 to 5kg concentrate (C). Monthly records were kept on body condition score (BCS) at calving and milk production from the time of calving to 12 weeks after calving. Milk production was recored daily.

4.2.3.4. Data collection and laboratory analysis

Information such as cow identification, lactation number and breed were obtained prior to the beginning of the study. Pre-partum BCS was recorded using the 1 to 5 scale (Edmonson *et al*, 1989). Daily milk production was recorded after calving. Starting from 7 days after calving, and until 12 weeks, weekly blood samples were collected in 10 ml sample tubes and kept on ice during transportation to the laboratory. They were centrifuged and the serum frozen at -20 degrees Celsius until analysis. Progesterone was analyzed by means of standard ELISA Progesterone kits from Clinpro International Co. LLC.(Union City, USA, CA 94587, EH-511) to determine reinitiation of ovarian activity. This kit has been validated for use in cattle (Bayemi *et al*, 2007). The minimum detectable level of progesterone in the assay was 0.05ng/ml (Clinpro International, 2004). Intra-assay precision varied from 2.4% to 7.1% while inter assay precision varied from 2.6% to 12.6%. The assay could detect progesterone levels of up to 50ng/ml above which there is a need of a diluent. The low control had values ranging from 3.5 to 7.5ng/ml and the high 20 to 39.5ng/ml. Serum samples were analyzed in duplicate.

4.2.3.5. Ovarian activity and milk production

The influence of factors such as age, parity, feeding system, month of calving, breed and body condition score at calving on milk production and ovarian activity was investigated. It was considered that samples with P4 over 3ng/ml or two consecutive samples with more than 1ng/ml indicated ovulation.

4.2.3.6. Statistical analysis

Calving was subdivided into three periods, early (June to July), mid (August to September) and late (October to November) rainy season. The effect of the following factors on milk production and ovarian activity was investigated: feeding system, parity, season of calving, breed, age and body condition score. This analysis used regressions and general linear models procedures (GLM) in SAS.

4.2.4. Results and discussions

4.2.4.1. Milk production

The model tested accounted for 87% of the variation in average daily milk yield. As expected, Holstein cows produced more milk than other breeds and their crosses (P=0.004: Table IV.2.1.). There were individual Holstein cows which produced up to 20 litres per day meaning that even in this small holder system, well managed cows can produce a significant amount of milk. Cows concerned in this study had milk production recorded only for 3 months and cannot efficiently be compared with complete lactation records. The challenge will be for the farmers to strive to maintain good production during the whole lactation. Exotic breeds of Uganda produce 7-10kg per cow per day (ILRI, 1996) over the whole lactation.. In Kenya, a survey found that dairy cows produce 5.9± 4.4 litres per cow per day (Stall *et al*, 2001) while it was 5.5 litres per cow per day in intensive production systems in Tanzania (MOAC, 1998). This higher milk production of the exotic breed in Cameroon may be explained by the fact that cows concerned in this study had been chosen among the small scale farmers accepting to apply technologies aimed at improving their farms. In such, they had started applying these interventions that probably improved their production.

BCS did influence milk production (P=0.002, Table IV.2.1). Although a number of animals was not well fed (BCS < 2), they still managed to produce a substantial amount of milk. BCS is usually well correlated with feeding and was thus expected to have a similar effect. BCS 3 gave less milk because most traditional cows (*Bos indicus*) were in this group.

Table IV.2.1. Effects of different factors on milk production and resumption of ovarian activity

Variables	Factors	Average daily milk production in liters (mean ±std)	Days to first ovulation
Breed	Traditional cows	3 ± 1^a	51 ± 20^a
	Crosses	10 ± 2^b	38 ± 25^a
	Holstein	12 ± 4^c	41 ± 24^a
Body Condition Score	2	13 ± 2^a	38 ± 12^a
	3	6 ± 3^b	48 ± 26^a
	3.5	15 ± 3^a	23 ± 8^a
Season of calving	Early rainy	9 ± 5^a	51 ± 20^a
	Mid rainy	7 ± 5^a	41 ± 24^a
	Late rainy	10 ± 5^a	38 ± 25^a
Feeding	Grazing	8 ± 5^a	41 ± 24^a
	less than 3kg concentrate	6 ± 4^a	51 ± 26^a
	More than 3kg concentrate	13 ± 5^a	31 ± 12^a
Parity	Primiparous	8 ± 6^a	43 ± 23^a
	Multiparous	9 ± 4^a	40 ± 24^a

[a, b] Means of the same variable with the same superscript are not significantly different

There was no evidence that period of calving, parity and system of feeding influence milk production (P>0.05) although as expected feeding system C with more concentrate seemed to lead to more milk production. Sawadogoo et *al* (1999) in Senegal rather found a difference in feeding systems. In this zero grazing system where restricted cows are undernourished because they do not receive enough forage, supplementation will help them to receive more energy and therefore produce more milk. However farmers tend to neglect the forage feeding of zero grazing cows and therefore this group does not produce as much milk as expected.

4.2.4.2. Post partum ovarian activity

There was no evidence that any factor studied in this work had an influence on post-partum ovarian activity (P>0.05). However, the interval to the first post-partum ovulation seemed shorter in Holstein and crosses compared to traditional cows (Figures 4.2.1, 4.2.2 and 4.2.3). The fact that traditional cows did not take a very long time to return to ovarian activity is an indication that they are well adapted to this challenging environment.

The post-partum anoestrus period in this study is shorter compared to that reported by Cavestany *et al*, (2001) of 80.8±8.6 days in Holstein and 104.8±7.6 days in crosses. It is also shorter than that found by Msangi *et al* (2004) in crossbred cows of Tanzania (74±33 days). Furthermore, Njoya *et al* (1999) found the period to be 172 ± 16 days in local cows of the Western Highlands of Cameroon. Approximately 20% of cows returned to ovarian activity within 30 days post-partum and 42.8% between 30 and 60 days post partum. The interval from calving to first service in cows which had an early first ovulation was nearly half compared to those with a later first ovulation (Table IV.2.2). This is an indication that early P4 rise results in early service.

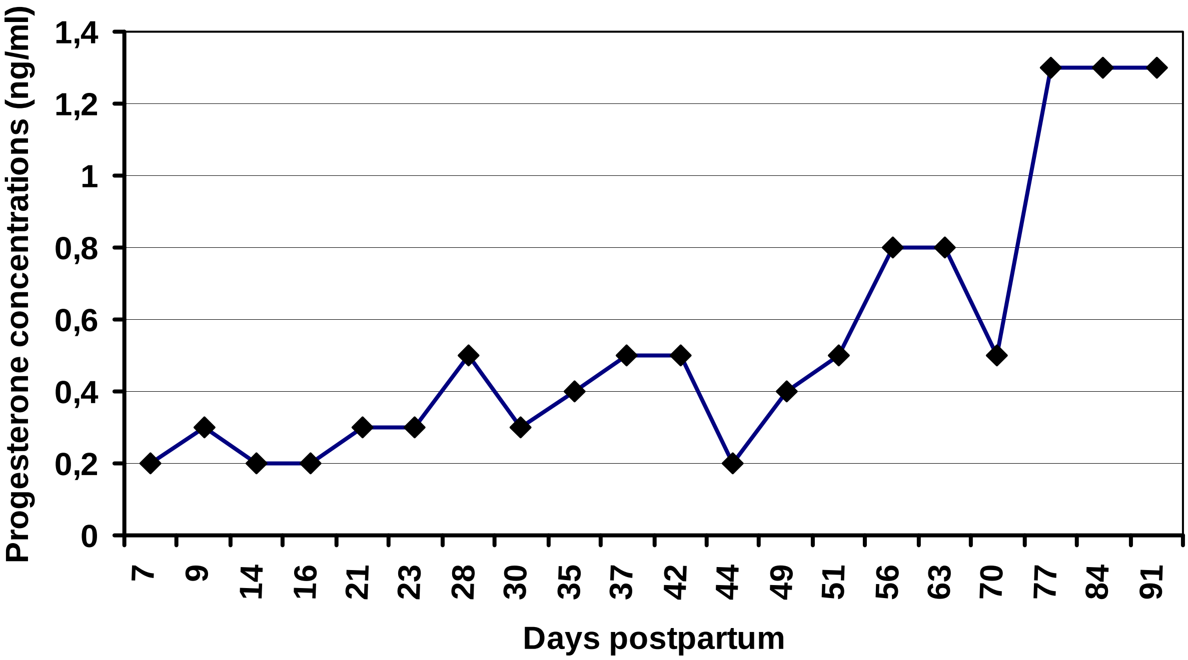

Figure 4.2.1. Progesterone profile of a local cow showing a long interval to the first post partum ovulation

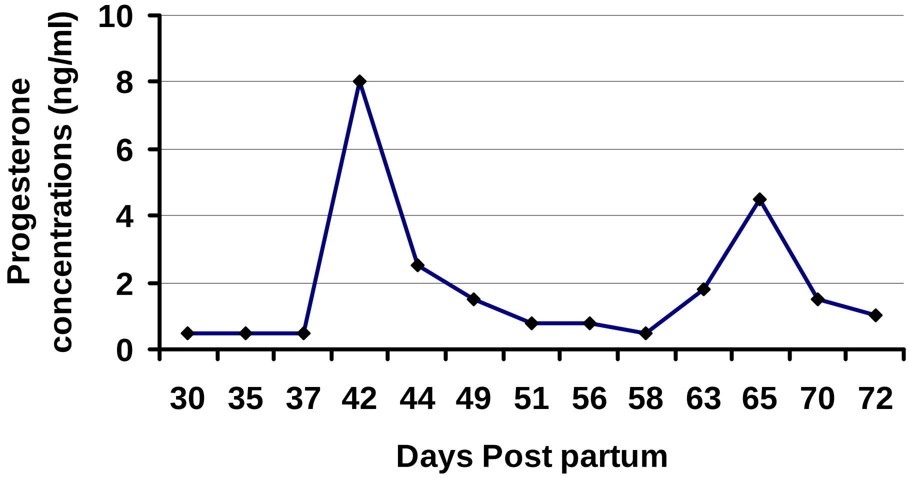

Figure 4.2.2. Progesterone levels of a Holstein x Gudali cross (one short cycle followed by cessation of ovarian activity and another short cycle)

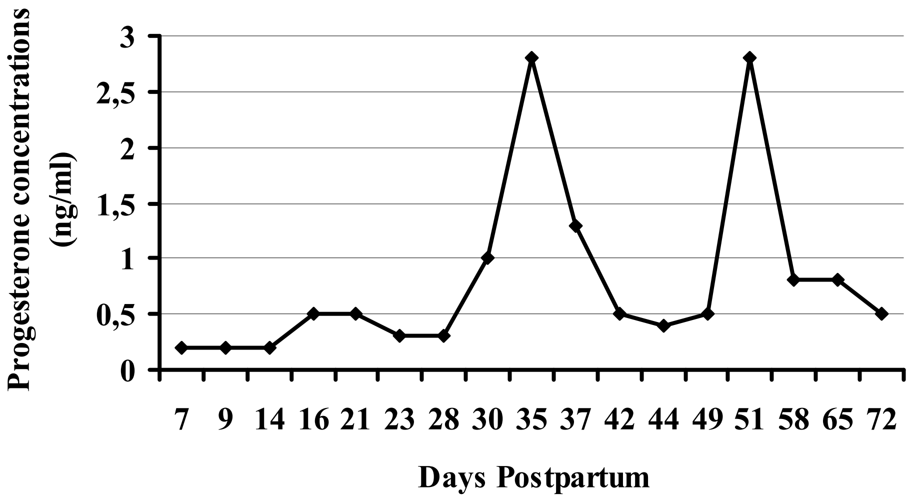

Figure 4.2.3: Progesterone levels of a Holstein cow showing early resumption of post partum ovarian activity

Table IV.2.2. Interval from calving to first P4 rise and first service

	Early return (1st P4 before 30 days)	**Late return (1st P4 rise after 30 days)**
Calving to 1st P4 rise (days)	23.66 ± 5.4	53.11± 19.87
Calving to 1st service (days)	41.33 ± 12.81	70.75 ± 24.52

There was no evidence that feeding systems in this study influenced the return to ovarian activity (P>0.05). However, cows in system C showed a shorter post-partum anoestrus period (Figure 4.2.4). These results are similar to those of Nkya *et al* (1999) in Tanzania who found no effect of supplementation on post-partum return to ovarian activity though animals receiving more concentrate showed a slightly shorter post-partum anoestrus period.

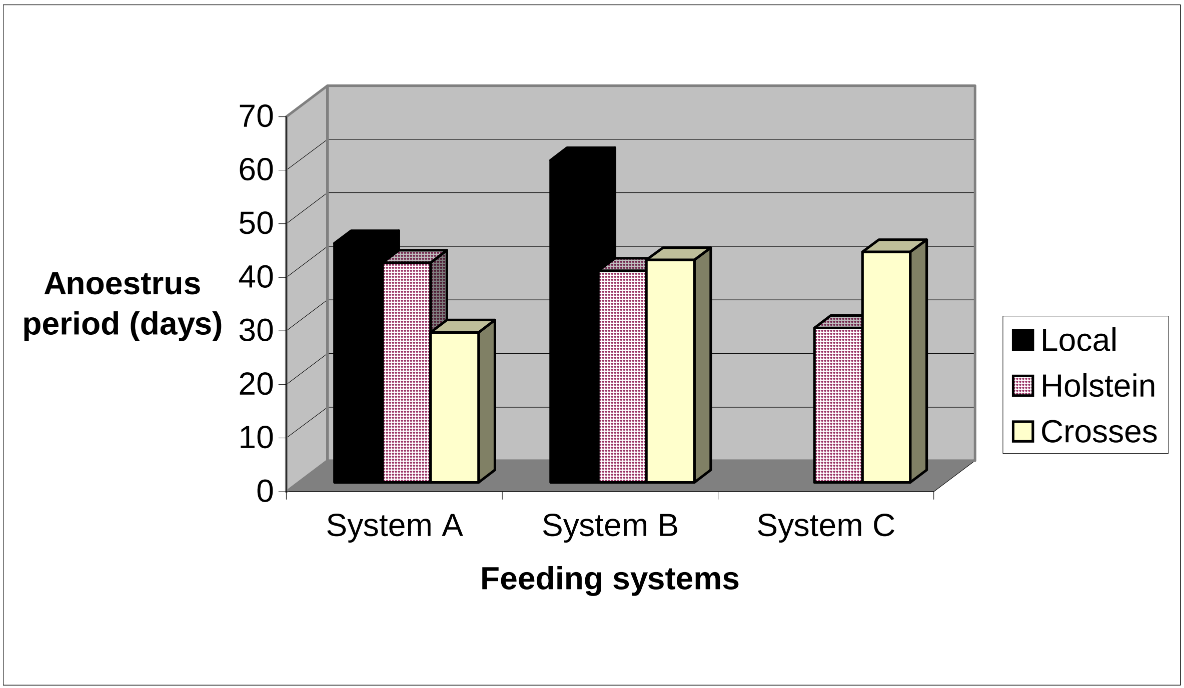

Figure 4.2.4. Effect of feeding systems on ovarian activity

The relationship between milk production and post partum ovarian activity was investigated. It was found that interval to first ovulation could be predicted by milk production ($P < 0.05$). An increase in one litre of milk production of cows producing between 2 to 20 litres will reduce the anoestrus period by 2.25 days. This finding suggests that in this area, cows producing more milk tend to take less time to resume ovarian activity, which agrees with the findings of Dhaliwal *et al* (1997) and Cavestany *et al* (2001) who reported that cows producing less milk have a prolonged anoestrus. There is a cumulative gain in well managed cows which produce more milk and resume reproductive activities earlier. However below a threshold weight, the cows are expected to show a delay in the rate at which they returned to oestrus because of being in a negative energy balance (Butler, 2000).

In conclusion, this study indicates that even though Holstein cows are not managed to their full potential, they are still better than local cows in terms of milk production and reproductive efficiency under the zero grazing system practiced in this region of Cameroon.

4.2.5. References

Bayemi, P.H., Bryant, M.J., Perera, B.M.A.O., Mbanya, N.J., Cavestany, D., Webb, E.C., 2005a. Milk production in Cameroon: A Review. Livestock research for rural development 17 (6). www.cipav.org.co/lrrd/lrrd17/6/baye17060.htm

Butler, W. R., Smith, R. D., 1989. Interrelationships between energy balance and post-partum reproductive function in dairy cattle. Journal of Dairy Science 72: 767-783.

Butler, W. R., 2000. Nutritional interactions with reproductive performance in dairy cattle. Animal Reproduction Science, 60-61, 449-457.

Cavestany, D., Corea, E., Silva, D., Meikle, A., 2003. Dairy cattle reproduction in tropics. Breed differences and metabolic profiles. In IAEA. Regional coordination meeting, Asuncion, Paraguay 21st – 26th July 2003

Cavestany, D., Galina, C.S., Vinoles, C., 2001. Effects of the characteristics of the post partum onset of ovarian activity on the reproductive efficiency of Holstein cows in pastoral conditions. Archivos de Medicina Veterinaria (Chile) 33: 217 – 226.

Clinpro International Co.LLC., 2004. Progesterone Enzyme immunoassay test kit. No EH-511. Handbook.

Clinpro International Co.LLC., 2005. Progesterone Enzyme immunoassay test kit. No EH-511. Handbook.

Dhaliwal, J. J., Skidmore, A. L., Lloyd, J. W., Kaneene, J. B., 1997. Relationship between body condition scores and conception at first artificial insemination in a large dairy herd of high yielding Holstein cows. *Journal of Dairy Science* 80: 113-120.

Edmonson, A.J., Lean, L.J., Weaver, L.D., Farver, T., Webster, G., 1989. A body condition scoring chart for Holstein daily cow. Journal of Dairy Science. 72 : 68-78.

FAO., 2004. Base de données Agriculture. http://www.fao.org.

ILRI (International Livestock Research Institute), 1996. The Ugandan dairy subsector. A Rapid Appraisal, Appendices. Pp10.

MOAC (Ministry of Agriculture and Cooperatives), 1998. The Tanzanian dairy subsector. A rapid Appraisal. Vol3. Main report. Dar-es-Salaam, Tanzania. Pp 23.

Msangi, B.S.J., Bryant, M.J., Nkya, R., Thorne, P.J., 2004. The effects of a short-term increase in supplementation on reproductio9n performance in lactating crossbred dairy cows. Tropical Animal Health and Production Vol. 36**,** No. 8**,** pp. 775-787.

Njoya, A., Mbanya, N. J., Nguemdjom, A., Kamga, P., Ndi, C., Kameni, A., Nfi, A., 1999. Cattle productivity on smallholder farms in the western highlands of Cameroon. In Development of feed supplementation strategies for improving the productivity of dairy cattle on small holder farms in Africa. Proceedings of Research Coordination of a Coordinated Research Project organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture held in Vienna, Austria, 7-11 September1998.

Nkya, R., Aboud, A. A., Kessy, B. M., 1999. Improving the productivity of smallholder dairy cattle in peri-urban Morogoro, United Republic of Tanzania. In Development of feed supplementation strategies for improving the productivity of dairy cattle on smallholder farms in Africa. Proceeding of research coordination of a Coordinated Research Project Organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture held in Vienna, Austria, 7-11 September 1998.

Nordlund, K.V., Goodger, W.J., Bennett, T.B., Shamsuddin, M, Klos. R.F., 2007. Methods for Conducting an Economic Survey in Small-Holding Dairy Farms, Tropical Animal Health and Production 39:557–566.

Sawadogo, G. J., Belemsaga,D. M. A., Yameogo, N., Manirarora, J. N., Toukour, M., 1999. Improvement of Zebu cattle productivity in the Sahel region :Feed supplementation on smallholder farm in peri-urban Dakar. In Development of feed supplementation strategies for improving the productivity of dairy cattle on smallholder farms in Africa. Proceeding of research coordination of a Coordinated Research Project Organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture held in Vienna, Austria, 7-11 September 1998.

Staal, S. J., Owango, M., Muriuki, H., Kenyanjui, M., Lukuyu, B., Njoroge, L., Njubi, D., Baltenweck, I., Musembi, F., Bwana, O., Muriuki, K., Gichungu, G., Omore, A., Thorpe, W., 2001. Dairy systems characterisation of the greater Nairobi milk Shed. SDP Research report. Smallholder Dairy (R&D) project, Nairobi, Kenya. Pp 31.

4.3. Effect of pre-partum feed supplementation on post partum ovarian activity, milk production and calf growth of dairy cattle of small scale dairy systems of the Western Highlands of Cameroon

Accepted in TECDOC, International Atomic Energy Agency (IAEA), Vienna, Austria.

Bayemi, P. H., Webb, E. C. , Mbanya, J. N., Cavestany, D. , Bryant, M. J. and Nsongka, V. M., 2008. Effect of short term Pre-partum Feed Supplementation on Post Partum Ovarian Activity, Milk Production and Calf Growth of Dairy Cattle of small scale dairy systems of the Western Highlands of Cameroon. Accepted in TECDOC, small scale market oriented dairy systems, International Atomic Energy Agency (IAEA), Vienna, Austria.

4.3.1. Abstract

Thirty seven cows were selected for an on-farm study on the effect of feed supplementation before calving on milk production, ovarian activity and calf growth of Holstein, indigenous Red Fulani cows and their crosses. Pre-partum feed supplementation, 30 days before calving, was done using cotton seed cake (80%), maize (18%), bone meal (1%) and kitchen salt (1% NaCl). Supplementation levels consisted of a LOW supplementation fed at 1kg per animal per day and HIGH supplementation fed at 2kg per animal per day. In addition, Red Fulani cows received the supplements in two different ways namely a pre-partum supplementation consisting of 1kg per cow per day and pre-and post-partum supplementation consisting of 1kg per cow per day before calving and 1kg per cow per day post partum up to 30 days after calving. Blood samples were analyzed using ELISA Progesterone kits to determine the length of post-partum anoestrus. Results show that pre-partum levels of feeding did not have any effect (P>0.05) on body condition score (BCS) at 12 weeks after calving, calf birth weight, average daily weight gain of calves, milk production and resumption of ovarian activity. High BCS at calving was shown to influence BCS at 12 weeks of lactation. Holstein cows had bigger calves (P<0.01) at birth (45kg) compared to traditional cows (36kg) and crosses (34kg). There was little benefit of pre-partum supplementation on the parameters investigated in this study.

Key words: Cameroon, small holder dairy, pre-partum supplementation, post-partum

4.3.2. Introduction

Supplementation with concentrates is a key aspect to improve productivity in tropical dairy production. Not only does supplementation help sustain high milk yields of cows, but it also improves the fertility of these cows (Mukasa-Mugerwa, 1989). In developing countries, low income farmers do not always supplement milking cows, yet they expect them to produce adequate quantities of milk and also complete involution and return to the reproductive phase in the shortest possible time. Most farmers who supplement their cows do so by giving a small quantity of concentrate during milking. Studies have shown that cows have a critical body condition score at mating, below which conception rates are reduced if the animals are still in a negative energy balance (Haresign, 1980). The energy status of the cow, seen by the change in the body condition score, also affects fertility (Chamberlain and Wilkinson, 1996). These studies were carried out in high yielding cows in commercial farms. There is little information on the effect of pre-partum supplementation and body condition score at calving on production parameters in small holder dairy systems. The purpose of this study was to investigate the effects of pre-partum supplementation, breed and body condition score on the resumption of ovarian activity, milk production and calf growth of milking cows in small holder dairy systems in Cameroon.

4.3.3 Materials and methods

4.3.3.1. Study site

The North West Region of Cameroon is located in the mid and high altitude zone of the country, which lies between latitudes 5°20' and 7° north and longitude 9°40' and 11°10' east. The surface area of the is 17,910 km2 covering 1/6th of the country's land area. Altitudes range from 300 to 3000 m above sea level. There are two main seasons: a dry season from November to mid-March and a rainy season from mid-March to October. Rainfall ranges between 1300-3000 mm, with a mean of 2000 mm. Minimum and maximum temperatures range between 15.50°C and 24.5°C, respectively, although temperatures above 30°C are not uncommon.

4.3.3.2. Animals and experimental design

Thirty seven cows from 24 farms were randomly selected from dairy farms in the region. They were bred by natural mating and confirmed pregnant by rectal palpation at about 6-8 months of gestation. The experimental animals consisted of 26 Red Fulani (*Bos indicus*), 28 Holstein (*Bos taurus*) and 18 crosses (*Bos taurus x Bos indicus*). The potential effects of diffences in forage availability were assumed to be reflected in the body condition score of cows.

Holstein cows were stall fed in a zero grazing system. Forage was cut, chopped and fed to cows in feeding troughs. This forage consisted mainly of Guatemala grass (*Trypsacum laxum*), *Desmodium spp* and Elephant grass (*Pennisetum purpureum*). Crosses and indigenous cattle grazed either on *Brachiaria spp.,* on native *Sporobolus africanus* or on marginal pastures. The supplement (340 g/kg crude protein and energy level of 11MJ/kg) consisted of cotton seed cake (80%), maize (18%), bone meal (1%) and kitchen salt (1% NaCl). Holstein and crosses were randomly allocated to a 2 × 2 factorial design, breed x level of feeding.

Supplementation levels consisted of a LOW fed at 1kg per animal per day and HIGH fed at 2kg per animal per day (T1 to T4). Red Fulani cows were fed in two different ways, namely, pre-partum supplementation consisting of 1kg per cow per day (T5) and pre-and post-partum supplementation consisting of 1kg per cow per day before calving and 1kg per cow per day post partum up to 30 days after calving (T6). The supplement for this last period was given during milking, whereas for other groups the supplement was given in the morning from 30 days before the expected calving date until calving (Figure 4.3.1). When a cow calved after the expected day, it did not receive any additional supplement other than the 30 or 60kg planned. And when it calved before the expected day, experimental supplementation stopped. Some farmers in the zero grazing and semi-extensive systems continued to supplement their cows after calving. This variable was included in the statistical analysis. Information such as cow identification, lactation number and breed were obtained prior to the beginning of the study. Body condition scores (BCS) were done using the 1 to 5 scale (Edmonson *et al,* 1989). Daily milk production, calf birth weight and weekly weight of calves were recorded after calving. Red Fulani cows were not milked and so their milk production is not considered in the study. But calf weight was noted in order to have an idea of milk production at the early age; furthermore it is expected that this supplementation influences early ovulation.

4.3.3.3. Progesterone levels analysis

Starting from 4 days post-partum until 12 weeks post-partum, weekly blood samples were collected in 10 ml sample tubes and kept on ice during transportation to the laboratory. Blood samples were centrifuged and the serum frozen at -20 degrees Celsius until analyzed. Progesterone was analyzed by means of standard ELISA progesterone kits from Clinpro International Co. LLC. (Union City, USA, CA 94587, EH-511) to determine re-initiation of ovarian activity. The minimum detectable level of progesterone in the assay was 0.05 ng/ml (Clinpro International, 2005). Intra-assay precision varied from 2.4% to 7.1% while inter-assay precision varied from 2.6% to 12.6%. The assay could detect progesterone levels of up to 50 ng/ml, above which a diluent is required. The low control had values ranging from 3.5 to 7.5 ng/ml and the high 20 to 39.5 ng/ml.

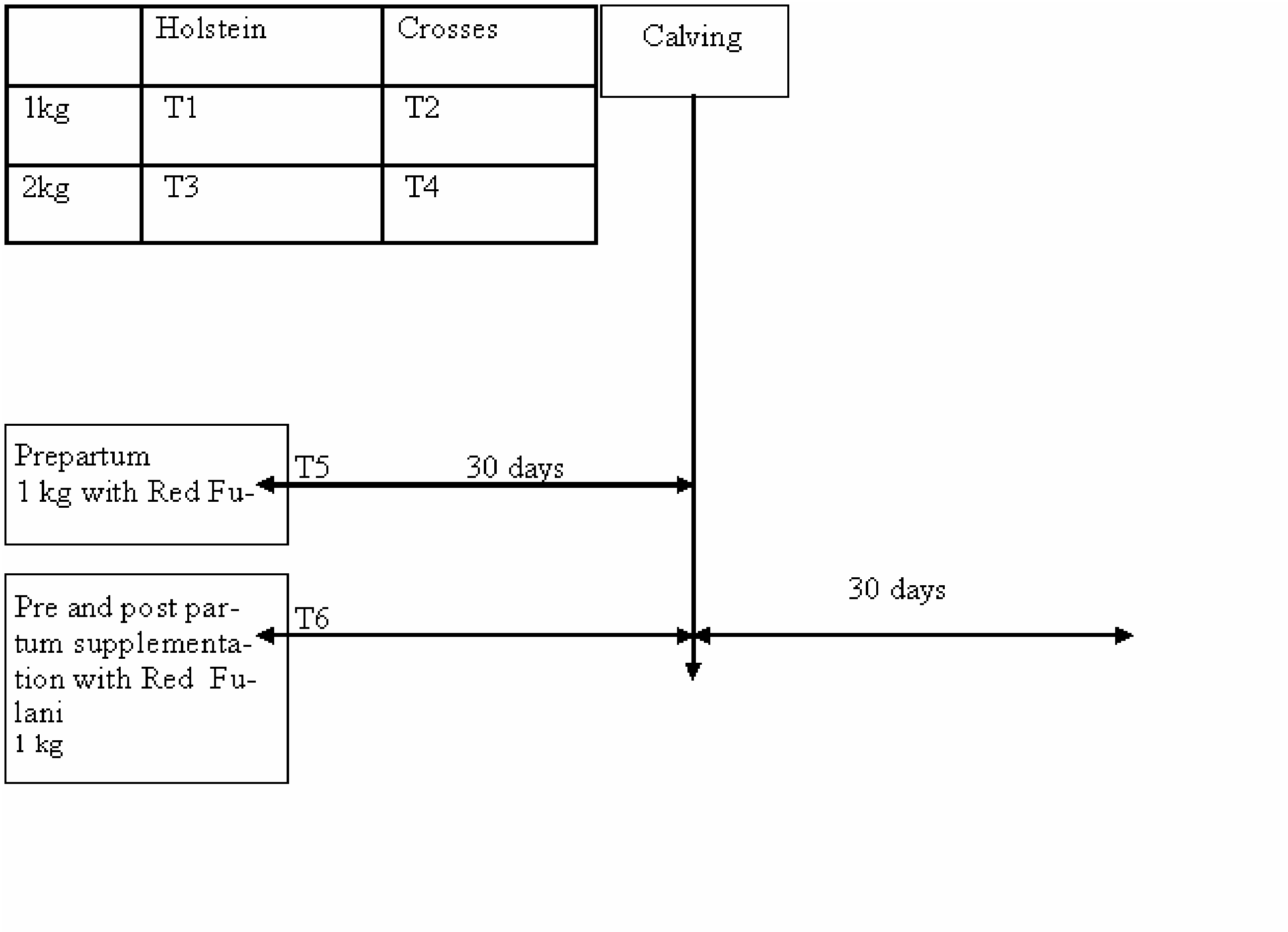

Figure 4.3.1. Diagrammatic representation of the six experimental treatments

4.3.3.4. Statistical analysis

A General Linear Model (GLM) in SAS was used to test the effect of the four variables listed below on calf birth weight, average daily weight gain of calves up to 12 weeks of age, milk yield within the same period, days required to resumption of ovarian activity and body condition score of milking cows at 12 weeks post partum. The variables included in the model were: level of pre-partum feeding, body condition score at calving, breed and post-partum supplementation. Body condition score at calving was divided into two groups: less that 3 and from 3 upwards. Post-partum level of feeding was also considered in two groups: ≤3kg per cow per day and >3kg per cow per day, up to a maximum of 6kg per cow per day.

Within breed comparison of the effect of the level of feeding on the variables studied was done. Means were compared using the Student-Newman-Keuls Test. The statistical models were as follows:

$\gamma_{ijkl} = \mu + F_i + B_j + C_k + S_l + e_{ijkl}$, Where γ_{ijkl} is the dependant variable (milk yield, daily weight gain of calves, length of post-partum anoestrus and body condition score at 12 weeks post-partum). μ is the overall mean, F_i is the level of pre-partum feeding, B_j is the cow breed, C_k is the body condition score at calving, S_l is level of post-partum supplementation and e_{ijkl} is the random error.

$\gamma_{ijk} = \mu + F_i + B_j + C_k + e_{ijk}$, Where γ_{ijkl} is the calf birth weight, μ is the overall mean, F_i is the level of pre-partum feeding, B_j is the cow breed, and C_k is the body condition score at calving, e_{ijk} is the random error.

For the Red Fulani, $\gamma_{ijk} = \mu + F_i + B_j + C_k + e_{ijk}$, F_i is the level of feeding either prepartum or throughout up to 12 weeks, B_j is the cow breed, and C_k is the body condition score at calving, e_{ijk} is the random error.

4.3.4. Results

Results show that there was no significant effect (P>0.05) of pre-partum level of feeding on the variables studied in this experiment, (Table IV.3.1). Similarly the birth weights of calves were slightly higher when cows were fed more concentrates before calving, although this result was not statistically significant (P>0.05). The results confirm that BCS at calving significantly influenced BCS at 12 weeks after calving (P=0.014). Cows in a better body condition managed to maintain their condition (BCS at 12 weeks) more stable during the period of investigation (Table IV.3.2). The condition score at calving did not affect the other variables as expected in this study (P>0.05).

Breed significantly influenced (P < 0.001) calf birth weights, although crosses did not have significantly higher birth weights than traditional breeds (Table IV.3.3). Average daily weight gains of calves were slightly higher for the Holstein breed. Traditional cows suckled their calves and as such, they were not milked. Furthermore, it was found that their return to ovarian activity was similar to the other breeds (P>0.05).

On the other hand, there was no evidence (P> 0.05) that post-partum supplementation had any influence on BCS at 12 weeks post partum, average daily weight gain of calves, milk yield or time required to post-partum return to ovarian activity (Table IV.3.4). However there were indications that high levels of concentrate feeding of lactating cows let to marginally higher weight gains of calves (P=0.08). Supplementation of newly calved cows tended to increase milk production although this was not statistically significant (P> 0.05). This result was to be expected, because supplementation is supposed to help the cow get more nutrients not found in pastures.

Table IV.3.1. Effect (lsmeans±sem) of pre-partum level of feeding on BCS, calf weight, weight gain, milk production and ovarian activity

	BCS at 12 weeks	**Calf birth weight (kg)**	**Average daily weight gain (g)**	**Average daily milk production (litres)**	**Postpartum resumption of ovarian activity (days)**
Low Level of feeding (n=10)	2.6±1.42[a]	37±1.4[a]	124±14[a]	8.3±1.6[a]	79±13[a]
High Level of feeding (n=9)	2.3±1.38[a]	40±1.4[a]	91±19[a]	8.1±1.5[a]	70±15[a]

[a] Means with the same letter are not significantly different.

Table IV.3.2: Effect (lsmeans±sem) of body condition score at calving on BCS at 12 weeks calf weight, weight gain, milk production and ovarian activity

	BCS at 12 weeks	Calf birth weight (kg)	Average daily weight gain (g)	Average daily milk production (litres)	Postpartum resumption of ovarian activity (days)
BCS <3 (n=9)	2.4±0.16[a]	38±1.3[a]	105±14[a]	8.9±1.2[a]	69±11[a]
BCS>3 (n=10)	2.9±0.19[b]	40±1.6[a]	110±19[a]	8.7±1.5[a]	68±13[a]

[a,b] Means with the same letter are not significantly different.

Table IV.3.3. Effect (lsmeans±sem) of breed on cow and calf production and reproductive performances

	BCS at 12 weeks	Calf birth weight (kg)	Average daily weight gain (g)	Average daily milk production (litres)	Postpartum resumption of ovarian activity (days)
Traditional (n=7)	2.9±0.4[a]	36±1.7[a]	106±39[a]	Not milked	76±12[a]
Holstein (n=10)	2.3±0.13[a]	45±1.6[b]	134±12[a]	12±0.9[a]	55±9[a]
Crosses (n=2)	2.8±0.18[a]	34±1.9[a]	83±17[a]	9±1[b]	56±9[a]

[a] Means with the same letter are not significantly different.

Table IV.3.4. Effect (lsmeans±sem) of post partum supplementation on BCS, calf weight gain, milk production and post-partum ovarian activity

	BCS at 12 weeks	**Average daily weight gain (g)**	**Average daily milk production (litres)**	**Postpartum resumption of ovarian activity (days)**
< 3kg (n=10)	2.5±0.15[a]	106±14[a]	7±0.8[a]	61±6[a]
3 to 6kg (n=9)	2.9±0.24[a]	109±23[a]	9±1.2[a]	64±11[a]

[a] Means with the same letter are not significantly different.

In order to investigate the variability within breeds, analyses similar to the ones above were done within each breed to investigate the effect of the level of feeding on the variables considered in this work. The results were not different compared to the between breed comparisons.

4.3.5. Discussion

Condition score is related to the level of feeding. In this experiment, BSC at calving did not affect return to ovarian activity. This result agrees with that reported by Bourchier *et al* (1987) who showed that condition score at calving did not affect conception rates and therefore resumption of ovarian activity until condition score was below 1.5. This level of BSC was not observed for the cows in the present study. Although not significant, there were indications that cows with greater BCS at calving produced less milk. Cavestany *et al* (2003) showed a negative correlation between BCS and milk production. However BCS at calving strongly influenced BCS at 12 weeks lactation. Stalker *et al* (2006) also found that feeding supplement pre-partum improved BCS precalving and prebreeding and increased the percentage of live calves at weaning. These results indicates that within the last third of gestation, cows should be fed adequately in order to ensure a sound BCS at calving which correlates with a good nutritional state of the animal even after calving.

The effect of supplementation before calving yielded no additional improvements. However, logic would suggest that feeding some supplement before calving will help the cow to increase forage digestibility and therefore feed efficiency especially with low quality feeds commonly found in the tropics. Perhaps one explanation for a lack of observed effect was the relatively small difference between treatments. It seems that it would have been better to have levels of 0kg and 2kg, rather than 1kg and 2kg. That would have possibly increased the chance of observing an effect. But farmers were not willing to participate as a 0kg control as they would have gained nothing from the experiment. Farmers regarded participation in the experiment as a way to get concentrates for free and would not have adhered to an experimental design with 0kg concentrate.

Chamberlain *et al* (1996) suggest that 10 days before calving, the cows should be put on a diet similar to that for the medium-yielding cows in the milking herd, as two weeks before and two weeks after calving are the most important of the productive cycle. Holstein cattle have a large frame and they generally produce bigger calves even in challenging environments. Although the birth weights of calves are not expected to be as high as in their original region, they still outweigh traditional Cameroonian breeds and their crosses. No evidence of heterosis was observed in birth weights, as crossbred animals had lighter weight offspring than did local animals. This may be because these crosses are not from the F1 generation. Also, the breed of sire was not considered in the analysis and perhaps indigenous animals were carrying a greater proportion of calves sired by Holsteins. The small difference in birth weights may show that these can adequately be used in crossing with the Holstein breed without fear of an increasing problem of calving difficulties.

Traditional cows were not milked. They were therefore able to suckle their young as naturally as possible. Their production was therefore not recorded. Meanwhile crossbred cows, although they grazed, were partially milked. Their calves could not receive enough quantities of milk and tended to show lower weight gains. It is worth mentioning that Holstein calves were bucket fed and their weight gains will not adequately reflect milk production of their mothers but milk offered by the farmers. As such, average daily weight gains of calves will not adequately reflect the effect of treatments.

Post-partum supplementation in *Bos indicus* Red Fulani cows, as expected, tended to lead to higher weight gain of calves and therefore, higher milk production as early calf weight was assumed to be reflective of mother's milk production. But in this study the tendency was not significant ($P>0.05$). Stalker *et al* (2006) also found that calves born to dams fed supplement pre-partum had similar birth weight but greater weaning weight.

The overall mean length of post-partum anoestrus was 64 days. However 25% of cows showed a persistent high progesterone concentrations. This is an indication of infections and it shows the need for veterinary intervention for many cows in these systems. Post-partum anoestrus was lower in this study compared to the work of Msangi *et al* (2004) with Low supplementation leading to a period of 110±9 days and High supplementation 87±7.6 days. Similar results were reported by Cavestany *et al* (2003) with Holstein cows (49.9±7.1days). Fagan and Roche (1984) found an early resumption of ovarian activity in Holstein cows in a pasture based production system.

Though there is evidence that good pre-partum nutrition shortens the length of post-partum anoestrus (Selk *et al*, 1988; Wright *et al*, 1992), there are also conflicting reports which may be reflected in the interactions among pre- and post-partum nutrition, negative energy balance, BCS, milk yield and suckling, as well as other environmental factors that influence duration of post-partum anestrus (Montiel and Ahuja, 2005). Therfore, post-partum resumption of ovarian activity may not depend exclusively on BCS at calving but on other factors such as BCS post-partum and health status. Wiltbank *et al* (1964) found that breeding cows must be in an improving body condition during the breeding period. This was confirmed by Wiltbank (1977) and Haresign (1984). Animals losing weight between calving and breeding usually have decreased conception rates (Anon, 1984). Therefore, adequate feeding at the early stage of lactation is crucial even in small-scale dairying.

4.3.6. References

Anon, 1984. Dairy herd fertility Ref Book 259. London, HMSO., 1984.

Bayemi P. H., Munji V. N., Cavestany D., Perera B. M. A. O., Webb E. C. 2007. Validation of a human progesterone EH511 Enzyme Immuno Assay kit for use in cattle serum at Bambui, Cameroon. Tropical Animal Health and Production 39: 335-338.

Bourchier, C.P., Garnsworthy, P.C., Hutchinson, J.M., Benton, T.A., 1987. The relationship between milk yield, body condition and reproductive performance in high yielding dairy cows (Abstr). Animal Production **44:** 460.

Cavestany, D., Corea, E., Silva, D., Meikle, A., 2003, Dairy cattle reproduction in tropics. Breed differences and metabolic profiles. In IAEA. Regional coordination meeting, Asuncion, Paraguay,21st – 26th July.

Chamberlain, A.T., Wilkinson, J.M., 1996. Feeding the dairy cow. Chalcombe publications, UK, pp 151.

Clinpro International Co.Llc., **2005**.Progesterone Enzyme immunoassay test kit. No EH-511. Handbook, .

Edmonson, A.J., Lean, L.J., Weaver, L.D., Farver, T., Webster, G., 1989. A body condition scoring chart for Holstein daily cow. Journal of Dairy Science **72:** 68-78.

Fagan, J.G., Roche, J.F., 1984. Reproductive activity in post partum dairy cows. 11th Int. Congr. Anim. Rep. A. I., Illiniois, USA 2:28.

Haresign, W., 1980. Body condition score, milk yield and reproduction in cattle. Recent advances in animal nutrition. London, Butterworths, P. 107.

Haresign, W., 1984. Underfeeding and reproduction: Physiological mechanisms. In: C Chemineau, D Gauthier and J Thimonier (eds), *Reproduction des ruminants en zone tropicale*. INRA Publication No. 20. INRA (Institut national de la recherche agronomique), Paris, France, pp. 339-365.

Montiel, F., Ahuja, C., 2005. Body condition and suckling as factors influencing the duration of post-partum anestrus in cattle: a review. Animal Reproduction Science 85: 1–26.

Msangi, B.S.J., Bryant, M.J., Nkya, R., Thorne, P.J., 2004. The effects of a short-term increase in supplementation on reproductio9n performance in lactating crossbred dairy cows. Tropical Animal Health and Production Vol. 36**,** No. 8**,** pp. 775-787.

Mukasa-Mugerwa, E., 1989. A review of reproductive performance of female Bos Indicus (Zebu) cattle. ILCA Monograph No 6, Addis Abba**.**

Selk, G.E., Wettemann, R.P., Lusby, K.S., Oltjen, J.W., Mobley, S.L., Rasby, R.J., Garmendia, J.C., 1988. Relationship among weight change, body condition and reproductive performance of range beef cows. J. Anim. Sci. 66, 3153–3159.

Stalker, L. A., Adams, D. C., Klopfenstein, T. J., Feuz, D. M., Funston, R. N., 2006. Effects of pre- and post-partum nutrition on reproduction in spring calving cows and calf feedlot performance. J Anim Sci 2006. 84:2582-2589.

Wiltbank, J. N. Rowden, W. W. Ingalls, J. E., Zimmerman, D. R., 1964. Influence of post-partum energy levels on reproductive performance of Hereford cows restricted in energy intake prior to calving. Journal of Animal Science **23:**1049-1053.

Wiltbank, J. N., 1977. Effect of level of nutrition on growth and reproduction of beef females. Georgia Nutrition Conference, 16-18 February. pp. 138-146.

Wright, I.A., Rhind, S.M., Whyte, T.K., Smith, A.J., 1992. Effects of body condition at calving and feeding level after calving on LH profiles and the duration of the post-partum anoestrous period in beef cows. Anim. Prod. 55, 41–46.