PERFORMANCE AND CYTOGENETIC ASPECTS OF SWAMP x RIVER CROSSBRED BUFFALOES

A. Na-Chiangmai[1] and V. Chavananikul[2]

[1]Department of Livestock Development, Bangkok 10400, Thailand.
[2]Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10400, Thailand.

SUMMARY

A crossbreeding program of buffalo between river (Murrah) and swamp types has been studied to help investigate the advantage of heterosis. The karyotype of crossbred buffaloes from various mating combinations were examined by the leukocyte culture method. Karyotypes showed that the diploid chromosome complements of the F_1 were always 2n=49, while the F_2 and F_3 resulting from backcrossing to Murrah were 2n=49 or 50, and F_2 from *inter se* mating were 2n= 48 or 49 or 50. The F_1 exhibited a higher potential to produce milk than other genotypes while maintaining growth characteristics. Milk and visual characteristics followed the % breed composition of the Murrah infusion. The offspring produced from F_1 *inter se* matings showed that the unbalanced chromosome complements (2n=49) did not cause infertility. A crossbreeding program of Murrah buffalo over swamp buffalo to improve milk while maintaining growth characteristics is therefore feasible.

Keyword: crossbreeding, karyotype, chromosome complements, buffalo.

INTRODUCTION

There are two types of water buffalo with different characteristics which classify them into two breed groups. The first group is the River buffalo (*Bubalus bubalis*) which can be found from India and further west to Egypt and some parts of Europe. This type of buffalo, which includes the Mediterranean buffalo, is primarily used for milk production. The other type is Swamp buffalo (*Bubalus carabanesis*) which is used for meat and work. It can be found mainly in Southeast Asia and China, and a century ago it was also introduced into the Northern part of Australia. The difference between these two types can be differentiated according to habitat and cytogenetically by the different chromosome complements. The diploid number of chromosomes in swamp buffalo is 48 of which five pairs are submetacentric and 19 pairs are acrocentric including sex chromosomes. The River buffalo is found to have 50 chromosomes of which five are submetacentric pairs and twenty are acrocentric pairs including sex chromosomes. In both types, the female has two of the larger X-chromosomes while the male has one larger X-chromosome and one second smaller Y-chromosome (Fischer and Ulbrich, 1968; Toll and Halnan, 1976; Di Berardino, 1981; Basrur *et al.*, 1988; Chavananikul *et al.*, 1994). According to the hypothesis of Wurster and Benirschke (1968), the occurrence of a tandem fusion translocation might have been responsible for the differentiation of the swamp buffalo from Murrah (a type of river buffalo). This was later supported by the report of Di Berardino (1981) that the Robertsonian translocation 1/29 is present in the Murrah as well as in the swamp buffalo. The study of crossbred buffaloes with several chromosomal genotypes has been reported in many Asian countries (Hilmi and Bongso, 1987; Harrisah *et al.*, 1989; Chavananikul *et al.*, 1994). Crossing Swamp and Murrah using different breeding plans produced offspring with various karyotypes (2n=48, 2n=49, 2n=50) (Chavananikul, 1989). Considerable chromosomal polymorphism exists within the crossbreds as reflected by loss and/or gain of genetic material but may also maintain genetic balance (Chavananikul, 1989; Harisah, 1989; Chavananikul, 1994). Gustavsson (1993) suggested that these unbalanced karyotypes may affect the process of gametogenesis in the male and may result in low fertility in the crossbred buffaloes. Low fertility is generally expected with multivalent chromosomal configurations undergoing degeneration (as observed when buffaloes of different karyotypes are mated) (Bongso *et al.*, 1982; Basrue *et al.*, 1988; Gustavssen *et al.*, 1993). Ramakrishnan *et al.*, (1989) reported that a study of semen evaluation in 2n=49 karyotype buffaloes showed low sperm motility, low number of sperm acrosomes and high percentage of abnormal sperm resulting in sub-fertile animals.

Buffalo crossbreeding programs are taking place in several countries including Thailand. To gain advantage of the heterosis of crossbreeding, the general procedure is to mate female swamp buffalo to male river buffalo and then the F_1 offspring are either back-crossed to river buffalo or *inter se* mating. Many researchers reported that weight gain and milk production of F_1 crossbred buffalo is higher than either swamp or river purebred (Bunyavejchiwin *et al.*, 1987-1988; Xiao, 1989; Na-Chiangmai *et al.*, 1990; Shrestha and Parker, 1992; Sanghuayprai *et al.*, 1994). Buffalo crossbreeding programs have operated in Thailand for over 40 years when the migrating Indians introduced Murrah buffaloes into the country to produce milk for home consumption. These *ad hoc* crossbreeding programs were conducted at a farmer level until 1978 when the Department of Livestock Development (DLD) imported Murrah buffaloes for structured crossbreeding programs. 10 breeding males and 90 pregnant females were imported from India to improve size (for draught), meat and milk production. The breeding design crossed female swamp buffaloes with Murrah bulls and offspring were selected for larger size and good milk production. Both first cross (50% Murrah) and backcross (75% Murrah) buffaloes have been studied. The crossbreeding program has been extended to include *inter se* matings of F_1 (Murrah-swamp) to generate the F_2. The F_2 have the characteristics of both Murrah and swamp types. The objectives of this study is to determine phenotypic appearance, growth, milk production and cytogenetic characteristics of these F_2 crossbred buffaloes.

MATERIALS AND METHODS

Study of environmental effect on growth traits

The Thailand Department of Livestock Development has a crossbred Buffalo project based at the Lamphyaklang Livestock Research and Breeding Center. Performance data on 1,237 crossbred buffaloes were collected during 1986-1996. Animals were with their dams until weaned at 8 months of age. They were grazed in pasture at 2.5 rai/head (1 rai = 0.16 ha) during the day. A feed supplement (cassava chip plus leuceana leaf meal in the ratio of 3:1) of 1.5 kg/head/day was supplied early in the morning. Animals were fed *ad lib* with hay upon returning to the barn in the evening. Mineral blocks and water were provided in the barn. Three crossbred types were evaluated - F_1, F_2 backcross (75% Murrah) and F_3 backcross (87.5% Murrah). These animals were weighed at birth, weaning (8 months) and at a year of age. Yearling animals were also visually assessed for coat hair, tail hair and chevron characteristics.

Study on cytogenetic aspects

Blood was collected in heparinised tubes from 160 animals randomly selected from the different breeding schemes. The blood was used to investigate karyotype by lymphocyte culture and Giemsa staining techniques. The lymphocytes from the buffy layer were cultured in RPMI 1640 medium to which were added L-glutamine, foetal calf serum, antibiotic and pokeweed and incubated at of 37 degrees Celsius. Colchicine solution was added at the 70th hour and incubation continued for 1 1/2 hours. The resulting cell suspension was treated with 0.56%KCL for 20 minutes in the incubator and fixed with a mixture of absolute methanol and acetic acid in the ratio of 3:1 for 12 hours. At least 30 well-spread and clear metaphases from each crossbred buffalo were stained, examined and photographed under the microscope. Chromosomes were numbered and rearranged in order of decreasing size from submetacentric autosomes, followed by acrocentrics and then sex chromosomes according to Di Beradino *et al.* (1979). Data was analyzed in groups of breed percentages as F_1 (50%Murrah-50%swamp), F_{2I} from *inter se* mating (F_1 x F_1), F_{2S} backcross to swamp (75%swamp-25%Murrah) , and F_{2M} (75%Murrah-25%swamp) and F_{3M} (87.5%Murrah-12.5%swamp) backcross to Murrah . The level of statistical significance was tested using the Chi-square method.

Study on growth and milk production

Growth characteristics of different chromosome complements. Statistical analysis of growth was carried out on the 160 animals selected for cytogenetic study. Analysis used the least squares General Linear Model procedures for unequal sub-class variables using the Statistical Analysis System (SAS®, 1991) to test the significant effects by fitting independent variables of season, year, sex and breed (%Murrah) as fixed effects.

Fattening potential. Thirty two heads of crossbred buffaloes were selected from the 160 animals assigned to the cytogenetics study. Animals were selected from the same calf drop (year, season) divided into subclasses of sex (steer, female) and breed types (50% Murrah and 75% Murrah) resulting in 8 animals in each subclass combination. The average weight of the animals was 340 kg and each animal was kept individually penned and fed a concentrate ration at the amount of 2% of body weight per day. Concentrate ration contained 30% rice bran, 50% cassava meals, 16.5% dry luecean leaf, 1% salt, 1.5% urea, 0.1% sulphur powder and .9 % di calcium phosphate giving approximately 14% protein. After feeding all animals were grazed together on improved pasture during the day with free access to water and mineral blocks. Weights of the animals were recorded at onset and then monthly until the animals reached 450 kgs. Analysis of variance was done on the data and a comparison of means was conducted using Duncan's new multiple range test.

Milk yield and composition. Female progeny retained as breeders in the overall crossbreeding program were measured for milk yield and composition. Calves were separated from their mothers after 3 days. The cows were hand milk twice daily with the calf being visible to the cows to stimulate milk flows. Each individual cow's milk was weighed after each milking and milk samples collected once per month for composition analysis.

RESULTS AND DISCUSSION

General appearances

The 50% Murrah-swamp buffaloes are usually dark grey, have good conformation, are large in size showing good meat type. They have well developed udders for easier milking ability. Chevron is a specific visual character existing in swamp buffaloes and is not observed in the F_1. It only occurs in 60% of the 25% Murrah-swamp crossbreds and 25% in the 75% Murrah-swamp crossbreds. The coat hair of the swamp buffalo is light grey or white while the coat hair of Murrah is black. In the crossbred buffalo, it was found that the coat hair depends on the blood level of each breed. As the Murrah blood level increases, the color of the coat hair tends to be darker. The tail hair is white in Murrah, which is dominant, and tends to increase in crossbreds as the percentage of Murrah increases. There is no significant correlation between phenotype and karyotype for coat hair, chevron and tail hair of crossbred buffaloes but there is a tendency to correlation with the percentage of each breed. This observation supports a previous study reported by Chavananikul *et al.* (1994).

Results of the cytogenetic study

The results of the karyotypes of F_1 (50%Murrah-50%swamp), F_{2M} (75%Murrah), F_{2S} (25%Murrah), F_2 (F_1 x F_1) and F_{3M} (87.5%Murrah) is shown in Table 1. The F_1 crossbreds only exhibited diploid chromosome complements of 2n=49. Due to a tandem fusion translocation, the 4th pair of chromosomes in F_1 is formed by the large metacentric chromosome 4 of Murrah buffalo and the submetacentric chromosome 9 of swamp buffalo. The various backcrosses from the F_1 showed a ratio of diploid chromosome complements relative to the breed percentage of the cross. For example, F_{2S} (25% Murrah) showed a ratio of 2.6:1 for 2n=48:49 which was not significant from the expected ratio of 3:1

Table 1. Number of animals possessing different karyotypes in crossbred buffaloes

Types (M=Murrah x swamp)	nos of obs. n=160	Nos of buffaloes classified by chromosome complements[a]			ratio of chrom. Complements	λ-test significant for ratio
		2n=48	2n=49	2n=50	48 : 49 : 50	
F_1 50%M	35	none	35	none	0 : 1 : 0	
F_{2S} 25%M	11	8	3	none	2.6 : 1 : 0	ns
F_{2M} 75%M	78	none	34	44	0 : 1 : 1.3	*
F_2 (F_1 x F_1)	22	6	11	5	1.2 : 2.2 : 1	ns
F_3 87.5%	14	none	3	11	0 : 1 : 3.7	ns

[a] Swamp 2n=48, Murrah 2n=50

ns = non statistical difference
* significant difference (P<0.05)

The F_2 from F_1 *inter se* mating were found to have 2n=48:49:50 in the ratio of 1: 2 : 1 as expected. The forms of chromosomes of any crossbred buffaloes with their chromosome complements of 2n=48 or 50 were found to be similar to those exhibited by the pure breed of that chromosome complement. The Chi-square test of significance showed no difference between 2n=48 and 49 in F_{2S} (25%Murrah) and between 2n=49 and 50 in F_{2M} (75%Murrah and 87.5%Murrah). It is noticeable that the purebred chromosome structure (2n=48 or 50) in the F_2 (25%Murrah, 75%Murrah and 87.5%Murrah) occurs more than the abnormal one. It is of interest that the F_2 from *inter se* mating possess the chromosome complements of 2n= 48:49:50 in the ratio of 1:2:1. This study shows that F_1 with 2n=49 unbalanced chromosomal complements can reproduce offspring from both back crossing to the normal karyotype animals as well as *inter se* mating. Therefore, crossbreeding programs between swamp and river buffaloes can be used to potentially improve production characteristics using appropriate selection regimes.

Results of growth and milk performances

Environmental effect on growth traits. Test of significance on 1237 records for growth factors showed that season and year effects were highly significant ($p<0.01$) for yearling weight but there was no effect of season, year or sex for birth weight and weaning weight on F_1 (50%Murrah) with 2n=49, F_{2M} (75%Murrah) and F_{3M} (87.5%Murrah) with 2n=49 and 50. This is expected as the cows are fed supplements while they rear their calf, but once the calf is weaned, the calf is grazed in the pasture without supplements. Calves born during October-January were with healthy and well fed dams and weaned in a good wet season during June-September. Calves weaned outside of these times may encounter less optimal feed in the pasture. Also some years were in drought and feed for the animals was inadequate thus affecting the animal's growth.

Growth performance of F_1, F_2 and F_3 with different chromosome complements. The results of birth weight, weaning weight and yearling weight of different chromosome complements are summarized in Table 2. Weaning weight and yearling weight of F_{3M} crossbred buffaloes with chromosome complement of 2n=49 had significantly less weight ($p<0.05$) than the others, and a highly significant difference at birth weight ($p<0.01$) compared with any F_2 with 2n=50. However, there were only 3 animals in the F_{3M} category. So further studies would be required to validate these results. F_{2M} crossbred buffalo with chromosome complements 2n=50 had the heaviest weight in all traits. These findings were slightly different from previous work done by Azmi *et al.* (1989) that indicated crossbred F_{2M} (75%Murrah-25%swamp) buffaloes with 49 chromosomes performed better than 2n=50 animal in terms of growth rate before and after weaning. Results suggest that the F_{2M} calves have higher weaning weights that F_1 or F_{3M} perhaps due to the benefit of an F_1 dam. Previous research (Na-chiangmai *et al.,* 1990) has shown that crossbred animals perform better than the purebred swamp type.

Table 2. Least square means $\pm$ SE of growth of different genotype crossbred buffaloes

Trait /	F_1 (50%M)	F_{2M} (75%M-25%S)		F_{3M} (87.5%M-12.5%S)	
Genotype	2n=49	2n=49	2n=50	2n=49	2n=50
Animals	35	34	44	3	11
Birth weight	$33.2\pm1.5^{a*}$	31.1 ± 1.1^{a}	$32.6\pm1.6^{a*}$	$25.2\pm2.6^{bc*}$	30.6 ± 1.7^{ab}
Weaning wt	181.9 ± 8.1^{ab}	196.4 ± 8.9^{ab}	206.0 ± 10.1^{a}	164.6 ± 18.5^{b}	192.5 ± 11.4^{ab}
Yearling wt	213.1 ± 9.8^{ab}	228.5 ± 8.7^{ab}	236.1 ± 9.0^{a}	187.6 ± 17.9^{b}	221.4 ± 12.0^{ab}

^{abc}Values on the same row with different superscripts differ ($p<0.05$). * ($p<0.01$)

Fattening potential from crossbred buffaloes. The result from fattening different genotypes of crossbred buffaloes revealed that while not statistical different, the F_1 (50% Murrah) tended to show higher potential to produce meat than the F_{2M} (75% Murrah) (Table 3). This result supports previous studies on the growth of crossbred buffaloes. Sanghuayprai *et al.* (1994) reported that the growth of F_1 Murrah-swamp buffaloes raised under pasture condition was better than the F_{2M}. Na-Chiangmai *et al.* (1990) found that the average daily gain of the 50% Murrah-swamp crossbred was better than the purebred swamp buffaloes when fed a supplement. Bunyavejchiwin *et al.* (1987-1988) measured the production and physiological traits of the purebreds and various crossbreds. At birth, weaning and yearling they reported that the crossbred performance were better than either of the pure Murrah or swamp buffaloes.

Table 3. Growth of buffaloes with various genotypes from fattening

Description	F_1 (50%Murrah)	F_{2M} (75%Murrah)[†]
Number of animals	16	16
Average fattening period to 450 kg (day)	158	165
Initial weight (kg)	339.7±9.7[††]	342.5±7.1
Finishing weight (kg)	457.0±6.2	453.2±2.5
ADG (gm/day)	751.7±134.9	658.0±140.0
FCR (only feed supplement)	8.0±1.4	8.8±1.8
Age at first calving (female) (month)	34.1	33.5

[†] unknown chromosome conplements [††] ± SD

Milk production. The results for milk yield and milk composition of the Murrah-swamp crossbreds (Table 4) reveals that the percentage of fat and protein of milk from the F_1 crossbred is significantly higher ($P<0.05$) than the F_{2M} (75% Murrah) which is in line with the difference between the parent breeds. The F_{2M} has a higher total milk yield due to both increased lactation period and daily yield. This supports the study by Tawinprawat and Rattanapun (1985) which showed that the F_1 had better milk production than the pure bred swamp animals. The F_{2M} results are similar to those reported elsewhere for Murrah (Xiao, 1989; Shrestha and Parker, 1992). The F_{2M} crossbred shows higher milk yield and longer lactation than the F_1 which appears to reflect the percentage level of the different bufalo types.

Table 4. Milk production of F_1 and F_{2M} (back-crossing to Murrah)

Descriptions	F_1 (50% Murrah)	F_2 (75% Murrah)	F_2/F_1 %
numbers of animals	32	25	
Milk yield (kg)	647.98± 72.8[††]	898.07± 101.2*	+ 38%
Lactation period (day)	225.83± 65.9	264.33± 80.7	+ 17%
Average milk yield (kg)	2.87	3.40	+ 16%
Milk - Fat %	7.93*	3.99	- 51%
- Protein %	4.30*	3.90	- 9%
- Lactose %	5.02	5.34	+ 6%
- Total solid %	17.34	14.21	- 18%

* significant difference ($P<0.05$) [††] ± SD

CONCLUSION

There are approximately 2.7 million swamp buffaloes in Thailand which are used for meat and draught power. Crossbreeding swamp and river buffaloes to produce dual purpose meat and milk animals is highly desirable. From this study, it has been shown that the crossbreeding of river and swamp buffalo is feasible and that crossbreds and backcrosses with unbalanced chromosomes complements (2n=49) are fertile and are able to produce offspring. It is therefore feasible to use crossbreeding in buffaloes as a genetic improvement strategy.

The two buffalo types (river and swamp) have quite different milk compositions. There were significant ($P<0.05$) differences in yield, fat and protein percentages between the F_1 and F_{2M} (75% Murrah) crosses. These characteristics reflected the percent Murrah of the cross. The differences may be more significant if the cytogenetics of the crosses were determined as the chromosome complement may be part of the reason for the large SD in the F_{2M} compared with the F_1 . Crossbreeding with Murrah types significantly improved the milk yield of F_1 buffalo (+38%) and physical milking ability, but fat% and protein% declined (-51% and -9%, respectively) in this study.

It is of interest that this study found minimal differences between the growth characteristics of the crossbred types. There were only a small number of animals available for the F_{3M} (87.5% Murrah) cross which made significant conclusions difficult. However, the study indicates that similar meat production can be expected from the crosses. While it was not statistically significant, the balanced chromosome complement (2n=50) animals tended to exhibit heavier weights at all ages than their 2n=49 counterparts with similar Murrah blood percentage.

The study therefore indicates that significant improvement in milk yield characteristics and milking ability can be made without apparant loss of growth characteristics. Hence farmers in Thailand would get immediate benefit from crossbreeding.

The authors suggest that a long term national breeding strategy be developed for the genetic improvement of swamp buffalo in Thailand through both crossbreeding with river buffalo combined with appropriate selection strategies.

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