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TRANSFORMING SWAMP BUFFALOES TO PRODUCERS OF MILK AND MEAT THROUGH CROSSBREEDING AND BACKCROSSING

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INTRODUCTION

There are two major types of water buffaloes in the world: the **riverine type**, generally dairy breed and found in the Indian continent covering India, Pakistan, Bangladesh, Nepal, Sri Lanka, and in European countries, and the **swamp type**, found in China and Southeast Asia.

The total number of swamp buffaloes is about 37.6M and represents 21.8% of the world's buffalo population. The history of swamp buffalo is basically a history of small-hold land-based agriculture, since, for centuries, the swamp buffaloes have played a major role in draft animal-dependent farming system, mainly in the production of major agricultural crops, such as rice, corn, sugarcane and coconut. In recent years, however, developments in land-based agriculture, such as the expansion of irrigation facilities, have had significant impact on the use of draft buffaloes. Intensified rice production became more pronounced in irrigated areas and this has led to increased utilization of small farm machineries, significantly displacing the draft buffaloes for land tillage. To some extent, the introduction of tractors for land preparation and transport of produce in corn, sugarcane and other crops production areas has had similar effect.

In view of the intensification in land use and increasing farm mechanization, the interest in developing swamp buffaloes beyond being a draft animal, to improving the genetic potentials for meat and milk through crossing with riverine-type buffaloes becomes profound. This lends more meaning in the light of the need to address the growing issue of low income among smallholder farming families amidst the rising cost of farm inputs, creating significant impact on the net income derived from the traditional crop-dominant farming system. The technical aspect of such crossbreeding has been a subject of research interest for several years in view of the known differences in their chromosome numbers, swamp buffalo has 2n=48 while the riverine type has 2n=50.

II. Social and Economic Considerations in Swamp Buffalo Crossbreeding

Human population and swamp buffaloes: Human population in China and SEA countries with substantial swamp buffalo population is about 1.92 billion, representing 28.33% of the world's population. What is of interest is the fact that about 1.1 billion of these people depend on agriculture (Table 1), and that among people in land-based agriculture, the land holding size has become smaller over the years. Additionally, the rise in oil price in recent years had significant impact on cost of farm inputs, essentially the oil-based products, such as fuel and fertilizer, which led to a considerable increase in the cost of production. In grain-producing countries, this caused a shift in the use of grains for the production of biofuel.

Collectively, these array of developments have resulted in upward movement of food prices, with rural farming families caught in a tight situation of declining net income and reduced purchasing power. Undeniably, measures to generate sources of additional income for the millions of farming families are a priority consideration.

One prominent resource common among the smallholder farming families is their swamp buffaloes. In China and Southeast Asian countries, there are about 37.9M of these animals (Table 1), each family having 1-3 hd on the average. For hundreds of years, these swamp buffaloes have been used mainly for draft, and perhaps, in the many years ahead, a good percentage of the farming families will still depend on this animal resource for the same purpose.

It is also true that there are substantial land areas now under intensive use, owing largely to the development of major irrigation systems, coupled with the advent of small-sized farm machineries. In these areas, significant substitution has been taken place, with increasing number of farm tractors and declining usage of draft buffaloes. In fact, the intensive farm mechanization in Taiwan, Malaysia and Thailand is the single major reason for the decline in swamp buffalo population in those countries in the early 1970s and 1980s. Water buffalo in Taiwan is now considered an endangered species. In Malaysia, the role of buffaloes in the overall livestock sector is less than 4.0% (Abas et al. 2006).

Increased Demand for Milk and Meat: Many Asian countries have registered sustained economic growth in recent years and this has also resulted in increased establishment of urbanized areas. The rise in income among urban population has also brought about a corresponding shift in food preferences as demonstrated in greater demand for beef and milk. With the reduced land area for grazing and forage production, the only immediate option to meet the growing requirements is increase in imports of milk and beef in recent years.

Table1. Human and Swamp Buffalo Population in China and Southeast Asia

Country	2009 Human Population (Million)	2007 Buffalo Population (Million)	Buffalo/ Human	Population in Agriculture (%)	No. of Population in Agriculture (Million)
China	1,331.4	22,722,010	0.017	63.15	840.1
Cambodia	14.8	774,000	0.052	67.72	10.0
Indonesia	243.3	2,085,780	0.008	40.15	97.5
Lao People's Democratic Republic	6.3	1,120,000	0.177	75.45	5.2
Malaysia	28.3	130,000	0.004	13.84	3.9
Myanmar	50.0	2,841,733	0.056	68.29	34.1
Philippines	92.2	3,383,620	0.036	35.76	33.0
Thailand	67.8	1,743,546	0.025	44.21	29.9
Timor-Leste	1.1	110,000	0.100	81.06	0.8
Vietnam	87.3	2,996,400	0.034	64.86	57.8
TOTAL	**1,922.5**	**37,904,000**			**1,112.3**

Source: FAO, Production Yearbook 2007; FAOSTAT, FAO Statistics Division 2009; 2009 World Population Data Sheet

Southeast Asia (SEA) is a net importer of milk and dairy products, with the Philippines and Malaysia importing almost 99% of their requirements while Indonesia and Thailand import 61.5% and 50.0% of their milk and dairy requirements, respectively. On the other hand, China's dairy industry is growing in concert with its extremely fast economic growth, thus with barely 3.2% dairy imports (Table 2).

It is easy to understand that sudden rise in meat demand in the fast-growing population of Asia can be met by intensive production of chicken and pork. This has taken place in China and Southeast Asian countries in significant magnitude, of course, with corresponding increases in imports of feedgrains. Requirements for beef in these countries are met by massive imports of buffalo meat from India, with Malaysia and the Philippines leading with 75.2% and 35.8% of their domestic requirements, respectively, coming from importation. Indonesia imports about 15.7% of its beef requirements whereas China is nearly self-sufficient with only 2.5% of its requirements coming from imports (Table 2).

Table 2. Milk and beef sufficiency level of China and selected SEA countries

Country	Milk (M Tons)			Beef (Thousand Tons)		
	Production	Import/ Export	% Sufficiency	Production	Import/ Export	% Sufficiency
China	41.9	1.4	96.8	6510	170	97.4
Indonesia	1.0	1.6	38.5	480	90	84.3
Malaysia	-	1.0	0.01	27	82	24.8
Philippines	-	1.1	0.01	250	140	64.2
Thailand	0.7	.07	50.0	-	-	-

Source: FAO Food Outlook, June 2009.

As a long-term development strategy, however, efforts in fast-growing economies in Asia have also included programs to enhance growth in their respective local dairy industry with massive infusion of stocks of "tropicalized" dairy cattle from Australia and New Zealand. This development approach is becoming more meaningful in most of the Asian countries that remain net importers of milk and dairy products as prices of milk in the international market have surged in view of the policy and regulatory measures in some exporting countries and also due to unfavorable climatic factors that resulted in reduced production and thus in traded milk in the international market. With the rising demand for same dairy animals for restocking farms in post-BSE Europe and Latin America, however, prices of dairy breeder stocks have also significantly increased lately. This is further compounded by depreciation of local currencies against the US dollar, making importation of dairy cattle stocks for commercial production less economically viable.

Transforming Swamp Buffaloes to Producers of Milk and Meat: Utilization of the existing population of swamp buffaloes in hot and humid tropics and harnessing the age-tested abilities of the smallhold farmers to rear these animals to provide opportunities for millions of smallholder farming families to earn additional income, and also to meet the growing domestic demand for milk and meat, against the backdrop of increasing farm mechanization, are good reasons to transform the huge number of draft animals into producers of milk and meat. The successful crossbreeding of the Chinese swamp buffaloes has given enough lessons on this approach. Moreover, the UNDP/FAO-assisted project in the Philippines carried from 1982 to 1998 and the subsequent expansion of the upgrading program have also clearly proven that crossing swamp buffaloes and riverine buffaloes, despite the differences in chromosome numbers, are producing fertile crossbreds with high growth rate potentials and with milk production abilities several folds more than the swamp buffalo parents.

Given the abundance of low-cost labor among farming families, the production cost for milk and meat from crossbred buffaloes becomes competitive. With the net income derived from crop-dominant farming system as reference, it has been demonstrated that net income from milk of one to two crossbreds is sufficient to double the income of the smallholder family tending a hectare of rice. The added advantage in dairying is the derivation of cash income on a daily basis from the sales of milk while on the long wait for harvests from crops.

III. Technical considerations in the swamp buffalo transformation

Chromosomal Analysis of Water Buffaloes and their Crosses: The interest among scientists in the past has been anchored on the known fact that swamp and riverine buffaloes have different chromosome numbers: the diploid chromosome number of the swamp buffalo is 48 while that of the river buffalo is 50. The reduction in chromosome number in swamp buffalo is seen as the tandem fusion (telomere - centromere) of chromosome pair 4 and 9 of riverine karyotype. The general apprehension was based on other animal species of different chromosomes crossbreeding data indicating fertility problems among resulting offspring.

When crossbreeding between the 2 buffalo types occurs, males and females of the F_1 generation are heterozygous for the fusion with chromosome 2n=49. Of these chromosomes, 3 chromosomes included one metacentric, one submetacentric and one telocentric chromosome were not in pair. Through the G-band analysis, it was demonstrated that the metacentric chromosome in the three unpaired chromosomes belonged to the chromosome 1 swamp buffalo, and the other two chromosomes correspond to chromosomes 1 and 9, respectively, from river buffaloes, which may be homologous as they had G-band type (Huang, 2006).

Inter-se mating of F_1 produces F_2 hybrids of three different karyotype categories ($2n$=48, $2n$= 49 and $2n$=50). Chi-square tests on pooled data indicated that the distribution 1:2:1 ratio is expected if only balanced gametes with 24 and 25 chromosomes are produced by the F_1 hybrids. Backcrosses (75:25) produced out of mating F_1 (50:50) with swamp buffalo karyotype categories are $2n$=48 and $2n$=49. On the other hand, if F_1 (50:50) is backcrossed with riverine buffalo the resulting F_2 (75:25) has karyotypes of $2n$=49 and $2n$=50. In the three-quarters swamp and three quarters river types, the respective karyotypic categories are in ratios approximating 1:1. The distribution of chromosome categories among the F_2 hybrids and backcrosses suggests that only genetically balanced gametes of the F_1 hybrids are capable of producing viable F_2 and backcross generations (Bongso et al. 1983).

In China, three-way crossbred hybrids were obtained by crossing swamp buffalo x Murrah x Nili Ravi or swamp buffalo x Nili Ravi x Murrah. They had two chromosome categories viz. 2n=49 and 2n=50, respectively. The two types of karotype exist not only in the progenies of three-way crosses, but also in the F_2 hybrids and F_3 hybrids of grading crosses. It could be observed that during the meiotic division, the F_1 hybrid with 2n=49 chromosomes produced 24 synaptonemal complexes (SC), which consisted of 22 divalents, an autosomes trivalent and a XX divalents. During the synapsis, the meme 1 from swamp buffalo undergoes partial alignment with submetacentric chromosome 1 and telecentric chromosome 9 from river buffaloes. The synapsis is kept up until metaphase 1. The disjunction occurred during anaphase 1 when it was observed that the metacentric chromosome 1 from swamp buffalo was pulled on one pole to another pole, which resulted in production of two types of sperms viz. n=24 and n=25, respectively. The male river buffalo (2n=50) produced only one type of sperm (n=25). Therefore, the hybrids of three-way crossbred and F_1 and F_2 grading crossbreed hybrids had two types of karotypes viz. 2n=49 and 2n=50. The ratio of the types of karotypes was near 1:1 in the hybrids of three-way crossbred and the F_1 grading crossbred hybrids (Huang, 2006).

Dai et al. 1994, described the Snynaptonemal Complexes (SC) karyotypes of swamp, river and hybrid water buffaloes as follows: **a)** *2n=50 group (river and ¾ river)* – Among the autosomal bivalents, there were five submetacentric and 19 acrocentric SCs. There were six NOR-bearing bivalents: two submetacentrics, one large and three small acrocentrics. The nucleoli were located at telomeres of these SCs. The mean absolute length of SC karyotype was 214.4µm (*n*=27, SD=32.4) for the river buffalo and 195.9 µm (*n*=333, SD=31.4) for the ¾ hybrids; **b)** *2n=48 groups (swamp, F_2, and ¾ swamp)* – The autosomal bivalents included a metacentric (the longest SC), four submetacentric and 18 acrocentric SCs. Five nucleoli were terminally located on one submetacentric, one large and three small acrocentrics. The mean absolute lengths of SC karyotype were 171.8 µm (*n*=20, SD=27.1) for the swamp buffalo and 186.9 µm (*n*=48, SD= 30.1) for the hybrids; **c)** *2n=49 group (F_1, F_2, and ¾ swamp)* – There were four submetacentric and 18 acrocentric autosome bivalents, and a trivalent. The trivalent was composed of a metacentric, one NOR-bearing submetacentric, and one acrocentric. Six nucleoli were located on a submetacentric and four acrocentric bivalents, and the trivalent. The mean absolute length of the SC karyotype for all *2n*=49 hybrids was 194.9 µm (*n*=65, SD + 25.3).

Among the F_2 and ¾ swamp bulls, those with a karyotype of *2n*=49 have a higher abnormality frequency (63-68%) than those with *2n*=48, not significantly different from the F_1 (73%). The frequency of abnormal configurations caused by interactions among nontrivalent SCs was lower in the *2n*=49 group than in the *2n*=48 and *2n*=50 hybrids, confirming that when a trivalent is present in the karyotype the asynaptic regions on the autosomes or the X and Y tended to interact with the trivalent (Dai et al. 1994).

The data from SC analysis are consistent with those from semen investigations which have shown that spermatozoa abnormality is lower in the swamp bulls (8.95-10.3%) than in the river bulls (12.28-20.8%; Hilmi 1991). The abnormal sperm percentage was 52.4% in the F_1 (Situmorand and Sitepu 1991), 30.7% in the *2n*=49 ¾ swamp bull, and 53.4% in the *2n*=49 ¾ river bulls (Hilmi 1991), while it was 9.78% in the *2n*=48 ¾ swamp bulls and 19.8% in the *2n*= 50 ¾ river bulls (Hilmi 1991). Histological observations showed spermatogenic arrest in half of the testis tubules from F_1 and *2n*=50 backcross bull, but in less than one-sixth of tubules from a purebred swamp bull. Reduced quantity and quality of sperm can be expected to follow high abnormality frequency in SC preparations.

Histological examination of the hybrid testis revealed a large proportion of degenerating spermatocytes and abnormal spermatids in the process of spermiogenesis, suggesting that the various synaptic associations leading to unbalanced gametes may be responsible for the degenerating germ cells in the hybrids. The unbalanced meiotic products will probably lead to selection against such spermatozoa or early embryos after fertilization. Due to a large percentage of germinal epithelial cells in F_1 hybrids being wasted, the fertility of backcross and F_2 generations may be subnormal (Harisah et al. 1989).

Implementation of Crossbreeding and Backcrossing: China and the Philippines are the only two countries in Asia that are pursuing large-scale crossbreeding and backcrossing of swamp buffaloes with the intent to produce critical population of animals with higher genetic potentials for milk and meat production.

Prior to 1974, a few years after the creation of the Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD), crossbreeding and backcrossing in the Philippines were limited. The situation changed when serious consideration on crossing swamp buffalo with the riverine type was initiated after the 10-Year United Nations Development Programme-Food and Agriculture Organization (UNDP-FAO) assistance on buffalo R&D was approved and implemented in 1982 to 1992. This 10-year research initiative clearly defined the benefits of crossing the riverine buffaloes (Murrah) (2N=50) with the Philippine swamp (2N=48). The resulting F_1 crossbreeds were found to grow significantly faster than the swamp buffalo and to produce milk three to four times more than the native parents. It was also demonstrated that males and females F_1 crossbreds (2N= 49) were fertile. Based on the analyses of breeding and performance records, it is not recommended to pursue an F_1 x F_1 mating, thus the route pursued and expanded towards the end of the project and onward is the continued backcrossing with the riverine type. Backcrosses with increasing blood composition of the riverine breed registered linear increment in milk yield without detriment to the reproductive and adoptive performances. With these production potentials, the social and economic benefits accruing to rural farmers from raising crossbred carabaos instead of the local breed became pronounced.

On the basis of these encouraging results, the government implemented a national artificial insemination program for swamp buffaloes utilizing frozen semen of selected Murrah sires. And thus, in view of the growing need for frozen buffalo semen for AI, the establishment of the country's first frozen buffalo semen laboratory was initiated in 1984. Estrus synchronization procedures were also developed to permit synchronized breeding and allowed the coverage of many breedable females, which are scattered quite thinly in the villages all over the country, utilizing the relatively few available AI technicians.

The backcrossing is aimed at producing backcrosses at least at 3rd and 4th generations and the breeding scheme followed in the Philippines is described below:

a) Swamp(S) x Murrah (M)
F_1 SM (50 : 50)
↓
SM x M (75 : 25)
↓
SMM x M (87.5 : 12.5)
↓
SMMM x M (93.75 : 6.25)

The introduction of the dairy breed of buffalo in the Philippines first occurred with the importation of 67 head of Murrah buffaloes from India in 1918. A few batches of dairy buffaloes were also imported in subsequent years. Due to the absence of an organized breeding program, however, the introduction of riverine bloodlines in the earlier years resulted in crossbreds of varying blood composition in the surrounding areas of the breeding stations and eventual loss of a large percentage of the purebred animals.

Fresh genetics were infused into the country in the early 1980s with the importation of frozen semen of the Murrah breed from India through the UNDP-FAO funded project. This was followed by importation of live riverine buffalo stocks between 1994 and 1998 (Table 3). In 1994, "American Murrah" buffaloes (150 female and 70 male) were bought from the USA to improve the meat production potentials of the local carabaos. The American breeder combined the growth potentials of the Jafarrabadi breed with that of the Indian Murrah and the swamp buffalo from Guam in developing the line. On the other hand, 3,183 head of "Bulgarian Murrah" stocks were imported in 1995-1999 for use in improving milk production potentials. The animals from Bulgaria have the advantage of having been selected for milk production for at least three generations. These importations were further augmented with the infusion into the country of about 13,000 doses of frozen semen from the top three progeny-tested bulls of Bulgaria.

Table 3. Recent Importations of Riverine Buffalo Breeder Stocks, Philippines

Year	Number	Source	Important Traits/Usage
1994	237	USA	Meat
1995	459	Bulgaria	Milk
1996	403	Bulgaria	Milk
1998	1656	Bulgaria	Milk
1999	605	Bulgaria	Milk
2010	2000	Brazil	Milk

In 2010 the government infused 2000 hd of dairy-type buffaloes from Brazil. These germplasm infusions are designed to hasten the envisaged wide-scale transformation of swamp buffaloes as producers of milk and meat in the immediate future.

Aggressive crossbreeding and backcrossing have been pursued much earlier in China, which has the largest swamp buffalo population in the world (FAO, 2007) numbering to about 22.7M. Organized crossbreeding of swamp and riverine buffaloes was initiated as early as 1957 with the importation of Murrah breed from India and followed by infusion of another riverine buffalo breed, Nili Ravi, in 1974 through donation from Pakistan government. The national buffalo improvement program was initiated in 1974, although as early as 1973, there was a massive AI program, which included the establishment of semen processing laboratories in 5 of 15 provinces (Guangxi, Shanxi, Guangzhou, Hubei and Guangdong) representing areas with high density of swamp buffaloes. By 1975, the program reported to have produced 16,930 hd of F_1 crossbreeds and by 1985, the crossbred population increased by about 8 folds (150,000 hd). The Chinese government saw it fit to create a National Buffalo Development Program Team in 1989 which grew into the development of Dairy Buffalo Industry in Southern China in 1991. Under this program, Guangxi Province became the national pilot area for Buffalo Dairying in 1994 and the host of the Buffalo Research Institute, in cooperation with the Chinese Academy of Agricultural Sciences in 1995. The institute was strengthened with the EU-China Buffalo Program in 1996. Chinese crossbreeding and backcrossing involve three breeds, namely, the Chinese native swamp buffalo, the Indian Murrah and the Nili-Ravi from Pakistan. The schemes are shown below:

a) Swamp(S) x Murrah (M)	b) Swamp (S) x Nili-Ravi (N)	c) Swamp (S) x Murrah (M)
F_1 SM (50 : 50) ↓	↓ F_1 SN (50 : 50) ↓	↓ F_1 SM (50 : 50) ↓
SM x M (25 : 75) ↓	SN x N (25 : 75) ↓	SM x N (25:25:50)
SMM x M (12.5 : 87.5) ↓	SNN x N (12.5 : 87.5) ↓	
SMMM x M (6.25 : 93.75)	SNNN x N (6.25 : 93.75)	

Scheme A is designed to produce "Chinese Murrah", B for the production of "Chinese Nili Ravi", and C for the production of triple cross involving 75% riverine blood and 25% swamp buffalo blood.

Milk Yield Performance of Crosses and Backcrosses: Chinese swamp buffalo has generally low milk production, between 500-800 kg/lactation (Yang et al. 2007). However, selected Chinese swamp buffaloes have higher milk yield, as in the case of animals at government institutions where recorded average milk production is 1,092.8kg/lactation. Under similar conditions, purebred Murrah and Nili Ravi milk yield/lactation were reported to be 2,132.9kg and 2,262.1kg, respectively (Yang et al. 2004).

The F_1 crosses of swamp buffalo with Murrah breed had an average of 1,233.3kg milk/lactation, equivalent to 12.3% increase in milk production. In the Philippines, the recorded yield of swamp buffalo x Murrah crosses is 4.14kg/day and represents 176.0% increase, mainly because the milk yield of Philippine swamp buffalo is only 1.5kg/day on the average.

On the other hand, Chinese swamp buffalo crossed with Nili Ravi registered milk yield of 2,041.2kg/lactation, an increase of 86.9% over the swamp buffalo parents.

Table 4. Milk production parameters of different buffalo pure breeds, crossbreds and backcrosses

Breed	Head	Lactation	Lactation length (day) X±S CV%	Milk yield X±S(kg) CV%	Average milk yield per day (kg)	Corrected 305-day milk yield X±S(kg) CV%	Highest daily milk yield (kg)
L	70	70	280.4±20.2 7.2	1092.8±207.44 19.0	3.79		6.60
M	65	237	324.7±73.6 22.7	2132.9±578.3 27.1	6.57	2117.1±430.0 20.3	17.40
N	58	164	316.8±76.1 27.2	2262.1±663.0 29.3	7.14	2366.4±51.6 23.7	18.40
MLF$_1$	73	241	280.1±76.1 27.2	1233.3±529.7 42.9	4.40		16.50
MLF$_2$	16	54	303.2±83.1 27.4	1585.5±620.6 39.1	5.22		13.00
NLF$_1$	6	45	326.7±96.4 29.5	2041.2±540.9 32.4	6.25	2060.7±386.2 18.7	16.65
NLF$_2$	9	20	325.8±93.2 28.6	2267.6±774.8 34.2	6.96	2298.4±6044.4 26.4	18.37
NML F$_2$	45	168	317.6±78.4 24.7	2294.6±772.1 33.7	7.22	2348.0±533.2 22.7	18.80

Yang et al. (2004), Zhang (2006)
L = Chinese Swamp buffalo (this represent selected animals)
M = Murrah
N = Nili Ravi
MLF$_1$ = F_1 cross Murrah x Swamp
MLF$_2$= F_1 Murrah (Backcross)
NLF$_1$ = cross Nili Ravi x Swamp
NLF$_2$ = F_1 x Nili Ravi (Backcross)
NMLF$_2$ = (M x L) crossbred x Nili (triple cross)

Backcrossing the (MxL) F_1 with Murrah or NL F_1 with Nili Ravi resulted in milk yield of 1,585.5kg and 2,267.6kg/lactation, respectively. Clearly, the backcrosses with 75% riverine bloodline have higher milk yield than F_1 crosses. Among the Murrah backcrosses, the increase over the swamp parents is 45% and 28.5% over the F_1 cross. Similar trend is demonstrated among Nili Ravi backcrosses, with reported increases of 107.5% compared to swamp parents and 11.0% compared to NLF_1 crossbreds.

Growth and Meat Production: Growth performance of swamp buffalo and their crosses with Murrah breed are shown in Tables 5 and 6. There is no difference in birth weight between the two breed groups, but the growth rate of crossbreds started to move ahead than that of the swamp buffaloes, starting at age of 6 months up until 36 months, with growth advantage that ranged from 10 – 31.1%. At four years of age, F_1 crossbred (50:50) and backcrosses with 75% Murrah blood registered weight advantage of 9.8% to 21.4% over the swamp parents.

Table 5. Live weight (kg) from birth to 36 months of age of swamp buffalo and F_1 (50:50) cross with riverine type

Age	Swamp Buffalo	F_1 Cross (50:50)	% Difference
Birth	31.4	31.1	0
6 mos	100.3	110.4	10.0
12 mos	132.5	170.9	28.9
18 mos	196.6	244.5	24.3
24 mos	213.9	255.9	19.6
30 mos	225.9	296.3	31.1
36 mos	260.9	333.6	27.8

Parker, 1992

TenderBuff refers to buffalo meat with desired carcass quality specifications produced in Australia. Rates of weight gain should be from 0.800-1.2kg/head/day. Depending on starting weights, the required finish and temperament can be achieved over 60-120 day period of feeding, depending on feed quality. In the "wet" season, growth rates of 1kg/head/day can be expected from pastures, provided there is ample material available to buffalo (not overstocked) and a maintenance fertilizer program is adopted, which supplies at least P and S, and also K, if needed. Most of the NT-produced TenderBuff are farm-bred or purchased from other suppliers as swamp buffalo yearlings and grown out for a further 8-16 months to achieve target weights in pastures. The stocks are regularly weighted at 3-month intervals, then more often as target weights are approached. Batch groups are assembled when 380kg is achieved and then these groups are weighted virtually on a fortnightly basis until turned off. Ultrasounds P8 fat measurements are also taken to ensure fat specifications are met. By this stage, most of the buffaloes are sufficiently yard-trained to go through the QA system without any stress-induced pH problems. The duration of the feeding period is a vital part of the quartering process to reduce the likelihood of stress at slaughter.

Table 6. Live weight of Swamp buffalo and its crosses with Riverine breed, kg

Breed/Type	N	Age, Yr	Live weight	Δ% [a]
Swamp				
Male	79	4-5	443	-
Female	92	4-5	398	-
SBxM (50:50)				
Male	11	4-5	531	19.8%
Female	19	4-5	476	14.5%
SBxM (25:75)				
Male	8	4-5	530	19.6%
Female	7	4-5	479	20.3%
SBxNili (50:50)				
Male	15	4	538	21.4%
Female	18	4	482	21.1%

Faylon, 1992, [a] Δ % increase over the swamp buffalo parents

Crossbreeding Swamp with Riverine Breed for quality beef: In Australia, Lempke (2007) reported that the introduction of Riverine blood from the USA in 1994-1997 radically altered the productivity of Tender Buff. Growth rates in the crossbred from 3/8 and above are outstandingly greater than the purebred swamp available in the NT. Some 40% improvement in growth rates has been recorded in comparisons. Results from the NT Government Beatrice Hill Farm regularly confirm this trend. With more crossbred carcasses processed, better the production data are obtained. An example from 2003-2004 Tender Buff slaughtering is (tabled below).

Table 7. Tender Buff slaughtering 2003-2004

Parameter	Swamp	River Crosses	% Difference over Swamp
No. of Animals	52	24	
Mean HSCM (kg)	224.6	258.9	15.3%
Eye muscle area (cm^2)	57.1	70	22.6%
Mean pH	5.54	5.51	-1%
Mean carcass length (cm)	104.0	108.6	4.4%
Mean grid $/kg	$3.05	$2.96	-3%
Mean p8 fat (mm)	7.1	10.0	41%
Mean dressing %	51.2	51.7	1%
Mean price $	$686.07	$768.68	12.0%

Reproductive Performance of F_1 Crossbreds

Reproductive Performance of Females Crossbreds: The reproductive performance of F_1 and backcross females produced out of crossing Murrah buffalo and Philippine swamp buffalo are not different.

In China, Huang (2006) demonstrated that no difference was observed on the first calving age, gestation length and first estrus between the three-way crossbreed hybrids with 2n=50 chromosomes and those with 2n=49 chromosomes. However, significant difference in the calving interval was detected between these types of hybrids.

There was no significant difference in annual conception rate between the two types of hybrids (P>0.05). However, a significant difference in conception rate was observed (P<0.05). Compared to hybrids with 2n=50 chromosomes, the calving rate of hybrids with 2n=49 chromosomes decreased by 17.77% to 17.89% and the total calves reduced by 1.33 to 1.54 head from the first calving to age of 11 years. The reduced reproductive performance in hybrids (2n=49) may have resulted from the unbalanced gametes that can not survive and produce conceptus, Lempke, 2004.

Assessment of Semen Quality of Murrah Buffaloes, Crossbreds and Philippine Carabaos: Assessment of semen parameters obtained in one year from Murrah buffalo and crossbred bulls indicated that the crossbreds would tend to have significantly lower initial sperm motility and semen volume than the Murrah.

Incidence of ejaculates with less than 50% initial motility was also significantly higher among crossbreds. These parameters indicated that in terms of total sperm output and total quality, Murrah bulls had an advantage over the crossbreds. However, in a separate study, the crossbreds appeared to be better than the Philippine carabaos in those parameters.

A closer look at the semen quality of Murrah buffalo and crossbred bulls of practically the same age (three to four years old), common nutrition and management, and with the same semen collection frequencies, confirmed that Murrah bulls were better than crossbreds in semen volume and initial motility, and sperm concentrations (Table 8).

Table 8. Mean±SE semen characteristics of Murrah buffalo and F_1 crosses, PCRDC-CLSU, January-December 1990

Parameter	MB	CB
Volume, ml	2.8±0.09^{a} (735)	2.08±0.2^{b} (444)
Initial motility (%)	61.8±4.60^{a} (735)	45.5±11.4^{b} (444)
Sperm concentration (10^7/ml)	88.4±8.60^{a} (710)	91.0±9.7^{a} (300)
Pre-freezing motility (%)	64.8±9.10^{a} (581)	60.6±2.6^{a} (151)
Post-freezing motility (%)	26.3±3.20^{a} (636)	27.2±4.4^{a} (260)
Incidence of ejaculation with initial motility Less than 50 (%)	6.7^{b}	32.0^{a}
Total sperm output (Vol x conc.)	253.0± 23.3^{a}	191.8± 28.0^{b}
Total quality semen (Vol. x conc. X mot.)	156.9± 23.0^{a}	98.1±17.5^{b}

^{a,b} Means in the same row with different superscripts differ (P<0.05); Number in parenthesis represents number of ejaculates.

Table 9. Mean ± SE semen characteristics of Murrah buffalo and crossbred bulls of the same semen age group (three to four years old), nutrition, management, and semen collection frequencies.

Breed	Ejaculate	N	Volume (ml)	pH	Initial Motility (%)	Sperm Concentrate 10^7m/ml	Motility After Dilution (%)	Post freezing Motility (%)
MB	I	12	2.55	6.79	64.58	93.17	64.58	31.25
	II	4	2.42	6.72	61.25	64.00	62.50	30.00
		16	2.48	6.75	62.92	78.58	63.54	30.62
CB	I	14	2.33	6.74	60.71	72.57	61.43	28.67
	II	10	1.79	6.74	61.50	73.50	61.50	31.86
		24	2.06	6.74	61.10	73.04	61.46	30.72

The semen freezing process appeared to have inflicted less toll on the motility of the crossbreds than that of Murrah (Table 9). The semen trend holds true on the percent change on the incidence of abnormal sperm associated with storage of frozen semen (Table 10).

Assessment of the fertility of F_1 bulls was further evaluated on the basis of the pregnancy rate obtained from AI on Philippine swamp buffaloes using frozen semen from proven Murrah sire as reference for pregnancy rate test. The data indicated that there were no significant differences between the pregnancy rate of the Philippine carabaos inseminated with semen of either Murrah buffalo or F_1 crossbred (Table 11).

IV. Institutionalization of the National Buffalo Development Program: The program of transforming the Philippine swamp buffaloes to producers of milk and meat was institutionalized after the establishment of the Philippine Carabao Center and in China, after the establishment of the Guangzi Buffalo Research Institute. The components of the national program in the case of the Philippine experience are herein discussed.

Table 10. Effect of freezing procedure on percent change in motility, abnormal sperm, and dead sperm of Philippine carabaos, crossbreds, and Murrah buffaloes during the first 30 days of storage at -196ºC

Parameter	Breed		
	PC	**CB**	**MB**
Motility	0	-6.0	-5.5
Abnormal sperm	0.21	0.40	4.3
Dead sperm	0.07	0.20	2.0

Table 11. First service pregnancy rate of Philippine carabaos artificially inseminated with frozen semen of Murrah buffalo or crossbred (F_1).

Breed	N	Pregnant	% Pregnancy
Murrah	647	233	36.0
F_1 (MB x PC)	122	43	36.2

Establishing the Ground for Genetic Improvement: The fundamental initiative that is most consistent with the envisaged improvement in the productivity of the swamp buffalo is the establishment of germplasm pools from where superior materials can be obtained on a sustainable basis. Efforts along this line have yielded concrete results, as follows:

Gene Pools for Selected Native Philippine Carabao (PC): While exotic germplasm were introduced for the specific purpose of improving milk and meat, the government also ensured that the existing swamp buffalo germplasm are conserved for long-term genetic improvement program. The general premise is that through the years, domestic stocks of swamp buffaloes have adapted to the local conditions and therefore there are certain genes that can be very useful for future breeding and genetic improvement. Gene pools for the Philippine carabao were established in the three main islands of Luzon, Visayas and Mindanao. The animals are kept as Open Nucleus Herds (ONH), and selection of better stocks from the surrounding communities is done on a continuing basis. Selected animals outside of institution herd are taken in and shall form part of the ONH for the Philippine carabao. These animals have been chosen primarily for size, growth rate and reproduction ability.

There is also a swamp buffalo sanctuary in a separate island that is so well protected from the introduction of any exotic germplasm of buffalo of any form. Farmers are utilizing the indigenous buffaloes for their farming activities and this will certainly be carried through for many generations to come. Monitoring of the animals is regular.

Gene Pool for Riverine Buffalo for Meat Improvement: American Murrah" buffaloes, imported into the country and raised as a herd in a facility located at PCC at Ubay Stock Farm, Ubay, Bohol (Central Philippines), ensure availability of superior germplasm for improvement of the carabao's meat production abilities. In this facility, riverine buffaloes are selected for growth and reproduction abilities, with consideration for meat quality characteristics. Bulls and semen of outstanding genetics are readily available to farmers with swamp buffaloes and are interested in raising buffalo for meat.

Gene Pool for Improvement for Milk Production: Elite herds of "Bulgarian Murrah" are reared at the National Riverine Buffalo Gene Pool and at two separate institutions in the southern islands. Animals with outstanding performance at farmer-cooperatives are also enrolled as part of the gene pool. With organized selection and testing system in place, the country is now assured of sustained sources of genetic materials for improvement for milk production. The system can produce about 400 bulls of good quality per year, with the top-ranking bulls subjected to organized progeny testing and then assigned as semen donors for use in the nationwide AI program, while the above-average bulls are used in the wide-scale bull loan for crossbreeding in the villages.

Embryo Biotechnology Laboratory: Attempts to hasten the envisaged genetic improvement have also led to the development of facilities and reproductive biotechniques that can be used as important tools in some specific areas not normally achieved through the traditional breeding techniques. To date, the facilities established at the PCC Central Research Station and in the PCC satellite laboratory in Maharastra, India have developed technologies to produce high genetics embryos through the in-vitro system. These efforts are complemented with the newly refined ovum pick-up procedures, obtaining oocytes from superior donors for IVM/IVF as an alternative option to superovulation scheme that proved to be less predictable and more expensive. Likewise, the facility has just embarked on attempts to propagate superior genetics dairy buffaloes through the use of somatic cell nuclear transfer technique (SCNT).

Genetic Evaluation System: Breeding research that aims to improve the milk production potential of the riverine buffalo population in the country is carried out by putting in place a system of ranking and selecting the best animals. This is done by developing a BLUP animal model for determining the genetic merit of individual animals with milk production record. Initially, evaluation of cows was based solely on milk volume, but starting in 2005, milk fat and protein percentages were included as additional traits in the evaluation. The model for genetic evaluation, including the software, was developed in collaboration with the geneticists of the Animal Genetics and Breeding Unit (AGBU) and the Agri-Business Research Institute (ABRI) of the University of New England in Armidale, Australia funded by ACIAR. The model is expected to produce as much as five estimated breeding values (EBVs). The first lactation is divided into first, second and third trimester with corresponding EBV. This gives a total for five EBVs combined to a single index to rank the cows and bulls. The same also holds true for milk fat and protein percentages as these are considered separate traits.

Junior bulls are also ranked accordingly and selected animals are subjected to growth performance, after which selected bulls are sent to semen laboratory for testing of semen quality. Top ranking selected bulls are then included for semen collection and processing and then into progeny testing. Progeny tested bulls become sires of future generation animals

Intensified use of DNA-based biotechniques as a tool for genetic improvement: As can be gleamed from the latest studies in bovine, the use of recently developed biotechnologies, such as marker-assisted-selection (MAS), has provided adequate opportunities to enhance selection and thus genetic improvement. This area will therefore receive considerable attention in the future.

Cryobanking of Animal Genetic Resources: Genetic materials in the form of frozen semen, embryos , DNA and tissues are also collected from distinctly different breed groups and lines as well as from outstanding animals in the gene pools and are cyropreserved and stored in the gene bank.

Included in the gene bank are samples collected from livestock species such as indigenous cattle, goat, sheep and the Tamaraw (2n=46), an animal within the buffalo family that is classified as an endangered specie and is found only in the Philippines.

Expanding Usage of Superior Germplasm: The utility of superior genetics obtained from the sustained selection and testing efforts is expanded by using females as dams of future sires while proven sires are used for AI. Outstanding sires tested and selected from the gene pool have been fully harnessed as semen donors in order to cover as many native swamp buffaloes as possible to effect the desired genetic improvement. Component activities/strategies on how to expand usage of superior genetics are as follows:

Semen Processing Laboratory: In support of genetic improvement efforts, the country established semen processing facilities as early as 1984, and to date, such facility houses 40 semen donors and 15 junior bulls for testing. Frozen semen from progeny-tested bulls are produced at a quantity more than sufficient to meet the national requirements, including the needs of the technicians of all local government units (LGUs) and non-government associations (NGOs) as well as private AI technicians.

Intensified Artificial Insemination and Bull Loan Program: In cooperation with the Local Government Units (LGUs), crossbreeding of swamp buffaloes with the dairy breed to improve the genetic potentials for milk is carried out nationwide. This system has current annual AI service coverage of about 55,000 head. As a way of government subsidy to the genetic improvement program, frozen buffalo semen are provided free of charge up until now. However, as the scheme to privatize the AI services is gaining acceptance, frozen semen are provided to private AI technicians at cost. Provision of liquid nitrogen to preserve quality of frozen semen is at the shared account of national and local governments, but will likely be provided at a later stage at cost to private technicians who, in turn, charge for their services at a reasonable rate.

In the past two years, efforts were directed at privatizing the AI services to develop village-based private technicians in order to augment the limited number technicians of the national government agencies and the local government units. Based on the data so far, these private AI technicians are more cost-efficient and more responsive in many respects compared to LGU technicians. Their main advantage is their constant presence right in the village service area and their “pay-per-service” system that releases the government from costly subsidies in the form of salaries and allowances. Under a condition where animal ownership per farmer is only 1 to 3, and households are scattered widely in the rural communities, it appears that harnessing village-based AI technicians offers many advantages. In communities where advanced stage of privatization has been achieved, AI technicians are also trained as para-vets and they are also organized. As a group they source their AI supplies, including liquid nitrogen (LN_2) and frozen semen at cost.

The AI is augmented with the bull loan program, which is undertaken in villages where AI services are not available. In fact, even in some areas where AI services are accessible, many farmers have high preference for natural mating, owing to very good success rate in this method compared to AI. On the average, the national AI system has registered a success rate of only 30.0% first service. Subsequent services, of course, can result in higher percentage of calves on the ground, but such higher rate is obtained in single service under natural mating. A system of incentive is offered to farmers tending breeding bulls in the village wherein full ownership of the bull is awarded once the bull has sired at least 50 calves. Many farmers get their bull ownership in just a period of two years.

Support to Establishment of Buffalo-Based Enterprises: Two approaches are being introduced in areas considered to be impact zones for the project. These areas are considered as such in view of the density of breeder stocks in the community and their potential for the establishment of buffalo-based enterprises, proximity to market likewise being a major consideration. In these communities, massive AI services are carried out with the intent of producing critical number of crossbreds, which are potential dairy animals. While this activity covers many animal raisers, the process is relatively slow owing to the long gestation of buffaloes and their late maturity. As a way of "shortcutting" the process, incubator modules composed of purebred buffaloes are introduced in the impact areas. These modules serve as show window for the farmers to see and appreciate the benefits of rearing the correct animal and adopting the proper management and breeding practices. In the impact areas, carabao raisers have been organized into cooperatives to collect, process and market milk and milk products in a systematic manner. Support to these cooperatives takes the form of organizational as well as technical trainings and the provision of post-production equipment, mostly to preserve the quality of the milk.

In the National Impact Zone (Nueva Ecija), primary cooperatives involving thousands of families have formed into the Nueva Ecija Federation of Dairy Carabao Cooperatives (NEFEDCCO) to supply milk and dairy products to major urban markets. Throughout the country there are 13 regional impact zones.

There are also thousands of crossbreeds in the AI target villages and can likewise become potential sources of milk and meat products. Community organization, training and support to entrepreneurship are to be intensified in order to harness their potentials.

V. Concluding Statement: There are compelling social and economic reasons for the decision to pursue wide-scale crossbreeding and continuous backcrossing of swamp buffaloes with the riverine buffaloes in countries such as China and the Philippines. While there were apprehensions about the technical feasibility of carrying out such wide-scale efforts, first because of the differences in the chromosome numbers of these two buffalo types, and second, by the initial data about chromosomal behavior suggesting some potential reproductive abnormalities, performance of both male and female crossbreds and their backcrosses obtained in the field have shown otherwise. What has been avoided so far is the inter-se mating of F_1 ($2n$ = 49) as there are resulting F_2 offspring with undesirable phenotypic performance, more practically noted on F_2s with $2n$ = 48. As a measure in the Philippines, crossbreds and backcrosses males are readily castrated and are destined for draft or for meat purposes.

The assignment of purebred riverine bulls in impact areas, with the corresponding program to castrate the non-purebred males, has guaranteed sustained backcrossing generation after generation. Of course, the program of purebred riverine bull assignment in service area is to seen for a maximum of 4-5 years only, either replaced or rotated to another service area, to avoid potential father to daughter matings.

For wide-scale crossbreeding and backcrossing program to succeed, the mechanism needed for its implementation has to be institutionalized primarily because of the length of the required period, at least 15 to 20 years to achieve results of 3 to 4 generations of backcrossing. The establishment of Guangzi Buffalo Research Institute in China and the Philippine Carabao Center in the Philippines are instrumental for the needed efforts to be sustained throughout.

In the final analysis, the result of this genetic transformation of swamp buffaloes to producers of milk and meat is defined by the farming communities, increasing demand and utilization of the "new animal" on the basis of over-all profitability. Therefore for the genetic transformation to truly harvest the goals, the system should also recognize the requisites for "businessizing" the smallholders, raising them from subsistence husbandry to the level of entrepreneurship.

REFERENCES

Bongso, T. A. M. Hilmi, and P.K. Basrur (1983). Testicular cells in hybrid water buffaloes (Bubalus bubalis). Res Vet. Sci. 35:3, pp 253-258

Cruz, L. C. (1992). Carabao Reproduction. In Carabao Production in the Philippines, FAO/UNDP PHI/86/005 Doc. # 23 pp 65-130

Cruz, L. C. (2006). Buffalo Development in the Philippine Current Situation and Future Trends. Proc. 5th Asian Buffalo Congress. Nanning, China. Pp. 28-36

Cruz, L. C. (2007). Trends in Buffalo Production in Asia. Italian J. Anim. Sci. 6: Suppl. 2, part 1, pp 9-29

Dai, K., C. B. Gillies, A. E. Dollin and M. Hilmi (1994). Synaptonemal complex analysis of hybrid and prubred water buffaloes (*Bubalus bubalis*). Hereditas. 121, pp 171-184

Faylon, P. S. (1992). Carabao Development in the Philippines. In Carabao Production in the Philippines, FAO/UNDP PHI/86/005. Field Doc. # 13. pp 1-23

Guimareas, S. E., L. El Pinheiro and J. D. Guimareas. (1995). Meiotic peculiarities in hybrid buffalo. Therio.. 43 pp 579-583

Harisah, M., T. I. Azmi, M. Hilmi, M. K. Vidyadaran, T. A. Bongso, Z. M. Nava (1989). Identification of crossbred buffalo genotypes and their chromosome segregation patterns. Genome 32:6, pp 999-1002

Huang, Y. (2006). The Chromosome Polymorphism in Crosses from Riverine x Swamp Buffalo. Proc. 5th Asian Buffalo Congress, Nanning, China, pp 70-75

Lemcke, B. (2004). Production if Specialized Quality Meat Products from Water Buffalo: Tenderbuff. Proc. 7th World Buffalo Congress, Manila, Philippines, pp. 1: pp 49-54

Majid, M., Z. M. Nava, B. A. Parker, M. N. Faridi and V. G. Momongan (1991). Crhomosomal analysis of water buffalos (Bubalus bubalis) and their crosses in the Philippine. Phil. J. Vet and Ani. Sci. 17:3, p 58

Yang, B., X. Liang, X. Zhang, C. Zou and F. Huang. (2004). Status of Buffalo Production in China. Proc. 7th World Buffalo Congress. II: Pp 513-518

Yang, Bingzhuang and Chunxi Zhang. (2006). Buffalo Crossbreeding in China: Past and Current Situation with Future Prospects. Asian Buffalo Magazine pp 4-10

Yang, B., Y. L. Q. Zeng, J. Quin and C. Yang (2007). Dairy Buffalo Breeding in the Countryside of China. Italian J. Anim. Sci. 6: supp 2, part, pp 25-29

Zhang, Chuxi (2006). The Model of Chinese Buffalo Breeding. Proc. 5th Asian Buffalo Congress, Nanning, China, pp 166-185

Zhang, Chunxi, Baiyun Yang, Jing Qin and Ying Zhuge (2006). Development of Dairy Buffalo. Asian Buffalo Magazine 3:1 pp 12-17.