



Review Article

Genetic Improvement of Swamp Buffalo through Cross Breeding and Backcrossing with Riverine Buffalo

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Abstract

A review of performance of the swamp buffalo concerning reproduction traits, growth and carcass, milk production, and work capacities was presented. The potential for breeding improvement of certain traits was discussed. Breeding objectives and goals to suit the need of buffalo farmers was emphasised. Breeding of swamp buffalo for draft and for multi-purpose was discussed. Genetic selection and crossbreeding of the swamp buffalo were reviewed. It was proposed that genetic selection for weight and gain should improve meat production. Crossing swamp buffaloes and riverine buffaloes, despite differences between karyotypes of the river and swamp buffaloes, resulting F1 crossbreds were found which are grown significantly faster than the swamp buffalo and produce milk three to four times more than the native swamp parents. It was also demonstrated that males and females of F1 crossbreds (2n = 49) were fertile. Based on the analyses of breeding and performance records, it is not recommended to pursue F1 x F1 mating and recommended backcrossing with the riverine type. From the above point of view, the future will see sustained and more intensive efforts to pursue the goal of transforming genetically the traditionally draft animal to producer of milk and meat and eventually establishment of viable and progressive buffalo-based enterprises.

Key words: Backcrossing, Cross Breeding, Genetic Improvement , Swamp Buffalo, Riverine Buffalo

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Introduction

The Bovidae family (Mammalia, Artiodactyla) comprises almost 140 species, usually divided phylogenetically into ten sub-families. The domestic buffaloes and cattle are included in the Bovini tribe, in the sub-tribe Bubalina and Bovina, respectively. Molecular studies have inferred that these two sub-tribes had a monophyletic origin with cattle having a more recent origin. Nevertheless, many Bovini species have



uncertain interspecific phylogenetic relationships due to their short period of evolutionary divergence (MacEachern *et al.*, 2009).

According to cytogenetic studies, the karyotype of the cattle, $2n = 60$ (FN = 58), is measured as the ancestral model for evolutionary comparisons with other Bovidae species due to its predominance of telocentric chromosomes, a karyotypic formula observed in different species (Wurster and Benirschke, 1968). The evolutionary history of Bovidae family is characterized by few rearrangements, comprising mainly centric fusions between uniarmed chromosomes resulting in derived biarmed chromosomes, such as observed in the domestic buffaloes (Degrandi *et al.*, 2014). There are two major types of water buffaloes in the world, the riverine type, generally are dairy breed and are found in Indian continent covering India, Pakistan, Bangladesh, Nepal, Sri Lanka, and in European countries, and the swamp type, found in China and Southeast Asia which are distinguished primarily based on their distinct morphology and karyotypes (Iannuzzi 2007; Michelizzi *et al.*, 2010; Degrandi *et al.*, 2014). The water buffalo (*Bubalus bubalis*) is a domesticated ruminant of the bovine family descended from the wild buffalo (*Bubalus arnee*) of the Indian Subcontinent (Marai and Haeeb, 2010). Two subspecies of domestic buffaloes (*Bubalus bubalis*) are recognized based on cytogenetic differences and adaptation to different habitats: The first group is denominated “river buffaloes” (*B. bubalis bubalis*) and presents $2n = 50$ (FN = 58), and the second group, the “swamp buffaloes” (*B. bubalis carabanensis*), have $2n = 48$ (FN = 56) and is represented by the Carabao breed (Bongso and Hilmi 1982; Iannuzzi *et al.*, 1996). There is also the wild Tamaraw buffalo (*B. mindorensis*) that has $2n = 46$ (FN = 56) (Tanaka *et al.*, 2000).

Although the morphology of the X and Y chromosomes is different in water buffalo and cattle (the X and Y are acrocentric in the buffaloes, but sub-metacentric in *Bos taurus*), the Synaptonemal Complex (SC) morphology and pairing behaviour of their XY pairs are very similar (Dai *et al.*, 1994). Comparisons between the GTG and RBG-banded karyotypes of river buffaloes and cattle indicated that the five larger biarmed chromosome pairs of the river buffalo were homologous to cattle chromosomes 1/25, 2/23, 8/19, 5/28 and 16/29, from which they originated by centric fusions (Iannuzzi *et al.*, 1990) and river buffalo BBU 9 is homologous to cattle chromosome 7 (CSK BB, 1994). On the other hand, swamp buffaloes diverged from river buffaloes by a tandem mixture between chromosomes 4 and 9, which gave rise to the largest chromosome pair in the first subspecies (Di Berardino *et al.*, 1981; Bongso and Hilmi 1982; Iannuzzi and Di Berardino, 1985). During the tandem fusion event, the centromere of BBU 9 was apparently deleted or inactivated while the nucleolus organizer regions (NORs) present at the telomeres of BBU 4p (BTA 28) (Iannuzzi *et al.*, 1996) were lost (Di Berardino and Iannuzzi, 1981).

Evaluation of the nucleolus organizer regions (NORs) indicated that Bovidae species have ten highly conserved NOR chromosomal sites (Di Berardino *et al.*, 1985; Di Meo *et al.*, 1991, 1993). However, domestic buffaloes have differences in the number of NOR-bearing chromosomes. The river buffalo (*B.*



bubalis bubalis) showed NORs in six chromosome pairs (3p, 4p, 6, 21, 23 and 24), whereas the swamp buffalo (*B. bubalis carabensis*) had five NOR-bearing pairs (4p, 6, 20, 22 and 24) (Iannuzzi *et al.*, 1990, 1996; Tanaka *et al.*, 2000). The divergence in river and swamp buffaloes was explained by the tandem fusion 4p; 9 in river buffaloes, which involved the NOR region (Bongso and Hilmi, 1982). In addition, some pairs were re-ordered in the karyotype with the largest chromosome (pair 1) in the karyotype. Accordingly, the NOR-bearing chromosome 3 of the river buffalo corresponds to pair 4 of the swamp buffalo, and chromosomes 21, 23 and 24 of the river buffalo are equivalent to pairs 20, 22 and 23, respectively, in the swamp buffalo.

Despite differences between karyotypes of the river and swamp buffaloes, these animals are not reproductively isolated and the F_1 crossbreed, with $2n = 49$, is viable and fertile. The hybrids have 11 NOR-bearing chromosomes, which is the maximum number of NORs observed, corresponding to a member of each chromosome pair from their parents: (3p, 6, 20, 22 and 23) in addition to the NOR on the river buffalo 4p which was confirmed with the 18S ribosomal probe (Degrandi *et al.*, 2014). Water buffaloes are important livestock among rural farmers in the South-East Asia, providing milk, meat and draft power. The riverine type, generally are dairy breed and are found in Indian continent covering India, Pakistan, Bangladesh, Nepal, Sri Lanka, and in European countries, and the swamp type, found in China and Southeast Asia. The world population of buffalo is estimated to be 199.7 million as of 2013 (FAO, 2015), of which 97% are in Asia.

India has vast resource of livestock which play a vital role in improving the socio-economic conditions of rural masses. India stands first in the world buffalo population, and the genetic diversity of Indian buffaloes is represented by 13 well-recognized breeds besides many lesser known, hitherto unexplored populations of significant local importance. Most of these breeds/populations are distributed in the mainland of India covering all regions (north, south, east, west and central parts). Apart from these, there are several buffalo populations in north-east India with unique morphological and behavioural characteristics. Most of these buffaloes of north-east India resemble swamp type in their appearance although no karyological evidence is available except for some limited analysis of few samples (Yadav *et al.*, 1998). Karyological studies on buffalo populations in other parts of north-east India such as north Assam, Nagaland, Mizoram and Manipur were found to be swamp type, while significant proportion (44.4%) of buffaloes from lower Assam were found to be hybrids of river and swamp types (Mishra *et al.*, 2010; Prakash *et al.*, 2011).

The buffaloes can utilize poorer roughages, adapt to harsher environments and are more resistant to several bovine tropical diseases. Despite these merits, buffalo have a relatively poor reproductive efficiency irrespective of their location throughout the world. Buffalo exhibit many of the known reproductive disorders including delayed onset of puberty, poor oestrous expression, longer postpartum ovarian

quiescence, and most importantly lowered conception rates particularly when bred artificially. However, higher fertility could be achieved through better feeding and management (Dahiya and Singh, 2013).

Water buffaloes have lower fertility than cattle, demonstrated by poor semen quality and sperm variability (Koonjaenak, 2006). The low intensity of oestrus in buffaloes may be due to low circulating concentrations of 17- β oestradiol in comparison with dairy cattle (Mondal *et al.*, 2007). Buffaloes are significantly longer – lived when compared to cattle. In general, buffaloes are more resistant to most of the diseases than domestic cattle. This feature favours the buffalo to survive in the hot humid regions, disease and productivity is often less deleterious than on cattle (Deb *et al.*, 2016). Generally semen quality is better in the swamp type than in the river type (Ramakrishnan *et al.*, 1989). Jainudeen (1986) indicated that dystocia is higher in river than in swamp buffaloes.

Buffalo milk is richer in both proteins (4.65%) and fat (8.30%) than cattle milk (3.5% and 3.4%) (Zicarelli, 2004). Buffalo meat (cara beef) is about one third (1/3) lower in cholesterol than beef (Iannuzzi and Di Meo, 2009). Buffalo meat is highly regarded for the low intramuscular fat content (1 - 3% fat), high protein (17 - 21% protein) and low cholesterol levels as compared to cow meat. There are no religious taboos surrounding buffalo slaughter as there are with the cow and thus India produces in excess of 500,000 MT of meat/year which is primarily exported to Malaysia, Philippines, UAE and other gulf countries (Anjaneyulu and Kumar, 2010).

Contribution of Buffalo to the Economy

In India, there are about 300 million bovines including 190.9 million cattle and 108.7 million buffalo as per 19th Livestock Census. India continues to be the largest producer of milk in the world with production of 146.3 million tonnes in 2014-15 and 155.5 million tonnes in 2015-16 showing an annual growth of 6.27% (FAO, 2015). In 2013, world milk production from water buffalo was 97,147,135 MT, representing 13.4% of the world's milk production and buffalo meat production was 3,597,340 MT, representing 5.37% of total global beef supply (FAO, 2015). India's export of dairy products was 39,397.62MT to the world, worth Rs. 910.44 Crores / 136.06 USD Millions during the year 2016-17 (APEDA, 2017). As per NDDDB Annual Report, 2016-17, the per capita availability of milk is around 337 grams per day in 2015-16. Nearly 36% of the milk production is contributed by indigenous buffaloes followed by 26% by crossbred cattle. Non-descript cattle and non-descript buffaloes contribute 9% and 13% milk production respectively. Buffalo meat is the cheapest when compared to other meat producing species, and therefore a valuable source of protein for the “weaker part of society”. India is the largest exporter of buffalo meat together with Pakistan toward the Middle East, to Australia and Hong Kong (Uriyapongson, 2013). Meat production in India in the beginning of the 12th Five Year Plan (2012-2013) was 5.95 million tonnes which has been further increased to 7.0 million tonnes in 2015-16 (NDDDB Annual Report, 2016-2017). Poultry contributed the

highest share of meat production during 2015-16 of 46% (3264 Th. tonnes) followed by buffalo of 23% (1611 Th. tonnes), goat (13%), sheep (7%), pig (5.5%) and cattle (5%) (NDDB Annual Report, 2016-2017). 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 the riverine- type buffaloes becomes profound given the growing demand for ruminant-derived products in view of increasing urbanization, increased income and the associated changes in food preferences.

Crossbreeding in Buffaloes

The genetic diversity among the two types of buffaloes (river and swamp) exists to the extent of different number of diploid chromosomes $2n = 50$ and 48 respectively. However, crossbreeding of two types of buffaloes produce fertile hybrids (F_1) with diploid number of chromosomes $2n = 49$. The crossbreeding of buffalo has been done to improve milk and meat potential of swamp buffaloes with river buffaloes in countries like China, Philippines (Cruz, 2012 & 2015), Vietnam, Malaysia, Thailand, Indonesia and Brazil (Tomar, 2004). The introduction of riverine buffalo genetic materials into distinctly swamp buffalo populated countries of China and South East Asia started as early as 1917 in the form of both live animals and frozen semen as cited by Balaine (1988). Most of the breeds infused were Murrah buffalo from India and Nili Ravi from Pakistan (Cruz, 2012& 2015). The cross breeding between riverine breeds of buffaloes has also been carried out in India. As a result, new breeds of buffaloes (Mehsana and Godavari) have been developed by crossing of Surti and local buffaloes with Murrah bulls, respectively. The Murrah x Surti crossbreds at NDRI had shown increase in milk production and reproduction traits, the milk yield per day of calving interval was higher in crossbreds (3.4 kg) than Murrah (3.2kg).

In Bulgaria, the Murrah x Bulgarian crossbreds were found close to Murrah for body weight at different ages whereas the milk yield and fat in crossbreds were close to mid-parent value (Tomar, 2004).

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 small hold farmers to rear these animals to provide opportunities for millions of small holder farming families to earn additional income, and also to meet the growing domestic demand for milk and meat, against the background of increasing farm mechanization, are good reasons to transform the huge number of draft animals into producers of milk and meat. The UNDP/FAO assisted project in the Philippines carried from 1982 to 1998, and the subsequent expansion of upgrading program have clearly proven that crossing swamp buffalo and riverine buffaloes, despite the differences in chromosome numbers,

are producing crossbreds with high growth rate potentials and with milk production abilities several folds over the swamp buffalo parents (Cruz, 2009).

Technical Considerations in the Swamp Buffalo Transformation

Chromosomal Analysis of Water Buffaloes and their Crosses

When crossbreeding between the 2 buffalo (river and swamp) types occur, males and females of the F_1 generation are heterozygous for the fusion and are apparently fertile with chromosome $2n = 49$. It had been reported that the F_1 river x swamp crosses had chromosomes numbers $2n = 49$. Of the chromosomes, 3 chromosomes included one metacentric; one sub metacentric and one telocentric chromosome were not in pair. The G-band analysis revealed that the metacentric chromosome in the three unpaired chromosomes belonged to the chromosome 1 swamp buffalo, and the other two chromosomes were response to chromosome 1 and 9, respectively, from river buffaloes, which may be homologous as they had type of G-band (Huang, 2006).

Inter-se mating of F_1 produce the F_2 hybrid of different karyotype categories viz. $2n = 48$, $2n = 49$ and $2n = 50$. 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 $2n = 50$. Three-way crossbred hybrids were obtained by crossing native Chinese Swamp buffalo x Murrah x Nili Ravi or native Chinese Swamp buffalo x Nili Ravi x Murrah whose had two chromosome categories viz. $2n = 49$ and $2n = 50$, respectively. The two types of karyotype were not only existed in the progeny of three-way crosses, but also on the F_2 hybrids and F_3 hybrids of grading crosses. During the meiotic division, It could be observed that the F_1 hybrid with $2n = 49$ chromosomes produced 24 synaptonemal complexes (SC) which consisted of 22 bivalents, an autosome trivalent and a XX bivalents. During the synapsis, the chromosome 1 from swamp buffalo undergoes partial alignment with sub metacentric chromosome 1 and telocentric chromosome 9 from river buffaloes. The synapsis was 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 one pole to another pole, which resulted in production of two types of sperms viz. $n = 24$ and $n = 25$, respectively. As the male river buffalo ($2n = 50$) produced only one type of sperm ($n = 25$), so, the hybrids of three-way crossbred and F_1 and F_2 grading crossbred hybrids had two types of karyotypes viz. $2n = 49$ and $2n = 50$. The ratio of the types of karyotypes 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 Synaptonemal Complexes (SC) karyotypes of swamp, river and hybrid water buffaloes as follows-

- a) $2n = 50$ group (river and $\frac{3}{4}$ river) – Among the autosomal bivalents, there were 5 sub-metacentric and 19 acrocentric SCs. There were six NOR-bearing bivalents: two sub-metacentrics, one large and three small acrocentrics. The nucleoli were located at telomeres of these SCs. The mean absolute length of SC karyotype was $214.4\mu\text{m}$ ($n = 27$, $SD = 32.4$) for the river buffalo and 195.9 ($n = 333$, $SD = 31.4$) for the % hybrids.
- b) $2n = 48$ groups (F_2 , and $\frac{3}{4}$ swamp) – The autosomal bivalents included a metacentric (the longest SC), four sub-metacentric and 18 acrocentric SCs. Five nucleoli were terminally located on one sub-metacentric, one large and three small acrocentrics. The mean absolute lengths of SC karyotype were $171.8\mu\text{m}$ ($n=20$, $SD=27.1$) for the swamp buffalo and $186.9\mu\text{m}$ ($n = 48$, $SD = 30.1$) for the hybrids.
- c) $2n = 49$ group (F_1 , F_2 , and $\frac{3}{4}$ swamp) – There were four sub-metacentric and 18 acrocentric autosome bivalents, and a trivalent. The trivalent was composed of a metacentric, one NOR-bearing sub-metacentric, and one acrocentric. Six nucleoli were located on a sub-metacentric and four acrocentric bivalents, and the trivalent. The mean absolute length of the SC karyotype for all $2n = 49$ hybrids was $194.9\mu\text{m}$ ($n = 65$, $SD = +25.3$).

Comparison of Purebreds and Crossbreds/Hybrids

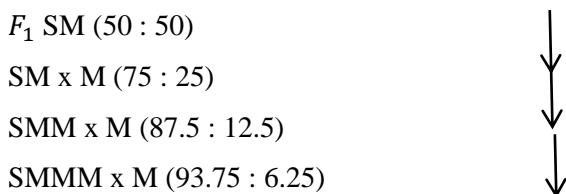
Among the F_2 and $\frac{3}{4}$ swamp bulls, those with a karyotype of $2n = 49$ has a higher abnormality frequency (63 – 68%) than those with $2n = 48$, not significantly different from the F_1 (73%). The frequency of abnormal configurations caused by interactions among non- trivalent SCs was lower 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 tend 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%) (Barker *et al.*, 1991). The abnormal sperm percentage was 52.4% in the F_1 (Situmorang and Sitepu, 1991), 30.7% in the $2n = 49$ $\frac{3}{4}$ swamp bull, and 53.4% in the $2n = 49$ $\frac{3}{4}$ river bulls (Barker *et al.*, 1991), while it was 9.78% in the $2n = 48$ $\frac{3}{4}$ swamp bulls and 19.8% in the $2n = 50$ $\frac{3}{4}$ river bulls (Barker *et al.*, 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 will be sub-normal (Harisah *et al.*, 1989). On crossing swamp buffalo ($2n = 48$) with the riverine type ($2n = 50$) the resulting F_1 crossbreds were found-



- a) To grow significantly faster than the swamp buffalo and to produce milk three to four times more than the native swamp parents.
- b) It was also demonstrated that males and females of F_1 crossbreds ($2n = 49$) were fertile. Based on the analyses of breeding and performance records, it is not recommended to pursue $F_1 \times F_1$ mating and recommended backcrossing with the riverine type.
- c) Moreover, backcrosses with increasing blood composition of the riverine breed registered linear increment in milk yield without detrimental to the reproductive and adoptive performances (Cruz, 2009).

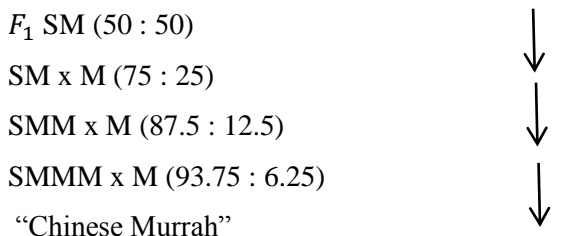
The backcrossing is aimed at producing backcrosses at least at 3rd and 4th generations and the breeding scheme followed in the Philippines is described below:

a) Swamp (S) x Murrah (M)

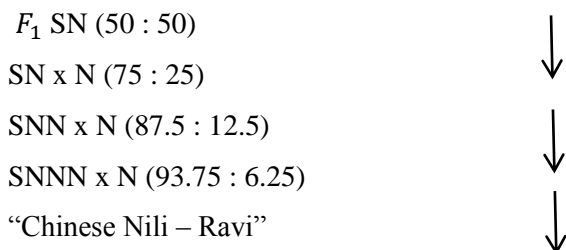


In China, organized crossbreeding of swamp and riverine buffaloes were initiated as early as 1957 with the importation of Murrah breed from India and followed by infusion of another riverine buffalo breed, Nili Ravi in 1974 through donation from Pakistan government. The scheme being implemented in China is described below:

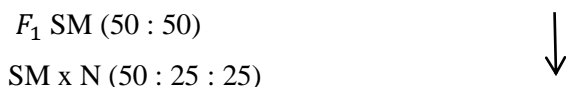
a) Swamp (S) x Murrah (M)



b) Swamp (S) x Nili-Ravi (N)



c) Swamp (S) x Murrah (M)



Milk Yield Performance of Crosses and Backcrosses

Chinese swamp buffalo has generally low milk production between 500 – 800 kg/production (Yang *et al.*, 2007), however, selected Chinese swamp buffaloes had higher milk yield as in the case of animals at government institutions were recorded, average milk production was 1,092.8 kg per lactation. Under similar conditions, purebred Murrah and Nili Ravi milk yield/lactation were reported to be 2,132.9 kg and 2,262.1 kg, respectively (Yang *et al.*, 2004). The F_1 crosses of swamp buffalo with Murrah breed had average of 1,233.3 kg milk/lactation, equivalent to 12.3% increase in milk production.

In Philippines, the recorded yield of swamp buffalo x Murrah crosses was 4.14 kg/day and represents 176.0% increase, mainly because the milk yield of Philippine swamp buffalo was only 1.5 kg/day on the average (Cruz, 2015). On the other hand, Chinese swamp buffalo (L) crossed with Nili Ravi (N) registered milk yield of 2,041.2 kg/lactation, an increase of 86.9% over the swamp buffalo parents. Backcrossing the (M x L) F_1 with Murrah or NL F_1 with Nili Ravi resulted in milk yield of 1,585.5kg and 2,267.6 kg/lactation, respectively. Clearly, the backcrosses with 75% riverine blood line have high 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 was demonstrated among Nili Ravi backcrosses, with reported increases of 107.5% compared to swamp parents and 11.0% compared to NL F_1 crossbreds (Yang *et al.*, 2004; Zhang, 2006).

Growth and Meat Production

Growth performance of swamp buffalo, and their crosses with Murrah breed are shown in Tables 1 and 2. There is no difference in birth weight between the two breeds groups, however, the growth rate of crossbreds started to move ahead than 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, F1 crossbred (50: 50) and backcrosses with 75% Murrah blood have registered weight advantage of 9.8 and to 21.4% over the swamp parents.

Table 1: Live weight (kg) from birth to 36 months of age of swamp buffalo and F_1 (50:50) cross with riverine type.

Age (months)	Swamp Buffalo (Live wt.)	F1 Cross (50 : 50) (Live wt.)	Difference (%)
Birth	31.4	31.3	1
6	100.3	110.4	10.1
12	132.5	170.9	29
18	196.6	244.5	24.4
24	213.9	255.9	19.6
30	225.9	296.3	31.2
36	260.9	33.6	27.9

Source: Faylon (1992)

Table 2: Live weight of Swamp buffalo and its crosses with Riverine breed (kg)

Breed/type	N (Total No. of Animals)	Age (Year)	Live Weight (kg)
Swamp			
Male	79	5-Apr	443
Female	92	5-Apr	398
SB x M (50:50)			
Male	11	5-Apr	531
Female	19	5-Apr	476
SB x M (25: 75)			
Male	8	5-Apr	530
Female	7	5-Apr	479
SB x Nili (50:50)			
Male	15	4	538
Female	18	4	482

Source: Faylon (1992)

Crossbreeding Swamp with Riverine Breed for Quality Beef

Growth rates in the crossbred from 3/8 (37.5%) and above riverine bloodline are outstandingly greater than the purebred swamp. Some 40% improvement in growth rates has been recorded in comparisons. With more crossbred carcasses processed, better the production data were obtained (Lempke, 2004). An example from 2003-2004 Tender Beef slaughtering in the Table below (Table 3).

Table 3: Tender Beef slaughtering data

Parameter	Swamp	Swamp x River	% 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	22.60%
Mean pH	5.54	5.51	-1%
Mean carcass length (cm)	104	108.6	4.40%
Mean grid \$/kg	\$3.05	\$2.96	-3%
Mean p8 fat (mm)	7.1	10	41%
Mean dressing %	51.2	51.7	1%
Mean price \$	\$686.07	\$768.68	12%

Source: Lempke (2004)

Reproductive Performance of F_1 Crossbreds

a) Reproductive Performance of Female Crossbreds

The reproductive performance of F_1 females produced out of crossing Murrah buffalo and Philippine carabao are not different (Huang, 2006). It has been demonstrated that no difference was observed on the first calving age, gestation length and first estrus between the three-way crossbred hybrids with $2n = 50$ chromosomes and those with $2n = 49$ chromosomes. However, significant difference on the calving interval was detected between these types of hybrids. Compared to hybrids with $2n = 50$ chromosomes, the calving rate of hybrids with $2n = 49$ chromosomes decreased. The reduced reproductive performance in hybrid ($2n$

= 49) may be resulted from the unbalanced gametes and cannot survive and produce conceptus (Lempke, 2004).

b) Assessment of Semen Quality of Murrah Buffaloes, Crossbreds and Native Swamp Buffaloes

In terms of total sperm output and total quality, Murrah bulls had advantage over the crossbred (Cruz, 2009). However, in a separate study, the crossbreds appeared to be better than the Philippine carabaos (swamp) in semen volume, initial motility and sperm concentrations (Nabheeron *et al.*, 1991). The semen freezing process appeared to have inflicted fewer tolls on the motility of the crossbreds than that of Murrah. No significant differences were seen between the pregnancy rate of the Phillipine carabaos inseminated with semen of either Murrah buffalo or F_1 crossbred (Cruz, 2009).

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 can yield concrete results, as follows:

1. Gene Pools for Selected Native Swamp Buffalo

While exotic germplasm is introduced for the specific purpose of improving milk and meat, the government should also ensure 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. An example of the Philippine carabao maintenance of gene pool is that 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 and reproduction ability (Cruz, 2012).

2. Gene Pool for Riverine Buffalo For Meat Improvement

Riverine buffaloes are to be selected for growth and reproduction abilities, with consideration for meat quality characteristics. Bulls and semen of outstanding genetics should be readily available to farmers interested in raising buffalo for meat.

3. Gene Pool for Improvement for Milk Production

Elite herds of riverine milch buffalo eg: Murrah are to be maintained in the country. Animals with outstanding performance at the farmer cooperatives can enroll as a part of the gene pool. With organized selection and testing system in place, there will be sustained sources of genetic materials for improvement for milk production. Among the good quality bulls produced per year, the top ranking bulls may be

subjected to organized progeny testing and then assigned as semen donors for use in the nationwide AI program, while the above average bulls can be used in the wide-scale bull loan for crossbreeding in the villages.

4. Embryo Biotechnology Laboratory

The development of facilities and reproductive biotechniques can be used as important tools in some specific areas not normally achieved through the traditional breeding techniques to hasten the genetic improvement. The use of sexed semen in combination with OPU and IVF is a hot topic in the buffalo world for the prospect of rapid production of high quality female embryos for the dairy industry (Buffalo Doctor, 2010). To date, the facilities established at the PCC (Philippine Carabao Center) Central Research Station and in the satellite laboratory in Maharashtra, India have developed technologies to produce high genetics embryos through the in vitro system. These efforts are complemented with the 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) (Cruz, 2012& 2015).

5. Genetic Improvement Efforts

i) A system of ranking and selecting the best animals with an aim to improve the milk production potential of the riverine buffalo population in the country can be carried out by animal model like BLUP for determining the genetic merit of individual animals with milk production record. Top ranking selected bulls are to be included for semen collection and processing and then into progeny testing. Progeny tested bulls become sires of future generation animals.

ii) Intensified use of DNA-based biotechniques as a tool for genetic improvement.

Marker-assisted-selection (MAS) has provided adequate opportunities to enhance selection and thus genetic improvement.

iii) Cryobanking of Animal Genetic Resources. Genetic resources in the form of frozen semen, embryos, DNA and tissues are to be collected from outstanding animals in the gene pools and cryopreserve and store in the gene bank.

6. Expanding Usage of Superior Germplasm

The utility of superior genetics obtained from the sustained selection and testing efforts can be expanded by using females as dams of future sires while proven sires for AI.

Component activities/strategies on how to expand usage of superior genetics are as follows:

i) Semen Processing Laboratory

Frozen semen from progeny tested bulls are to produce quantity more than sufficient to meet the national requirements, including the needs of the technicians of all local government units (LGUs), non-government associations (NGAs) and private AI technicians.

ii) Intensified Artificial Insemination and Bull Loan Program

To improve the genetic potentials for milk in cooperation with the LGUs, crossbreeding of native buffaloes with the dairy breed should be carried out nationwide. A system of incentive can be offered to farmers tending breeding bulls in the village to promote and intensify elite gene pools.

In the Philippine, as a way of government subsidy to the genetic improvement program, frozen buffalo semen were provided free of charge up until now. However, as the scheme to privatize the AI services is gaining acceptance, frozen semen were provided to private AI technicians at cost (Cruz , 2012). However, since the animal ownership is small but widely spread geographically, a system of consolidation, fattening, slaughtering, carcass fabrication, handling and distribution has to be organized in strategic locations.

7. Support to Establishment of Buffalo- Based Enterprises

As a way of shortcutting the relatively slow process of producing crossbreds through AI owing to the long gestation of buffaloes and their late maturity, incubator modules composing of purebred buffaloes are to be introduced into the impact zones. Impact zones are considered 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. Purebred riverine bulls in impact areas can be produced, with the corresponding program, to castrate non-purebred males. This will guarantee sustainable back crossings generation after generation.

Community organization, training and support to entrepreneurship are to be intensified in order to harness their potentials.

Conclusion

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. The possibility to improve buffalo production as a whole is linked to the exploitation and implementation of scientific advances, nutrition and related technologies in some fundamental fields. Single Nucleotide Polymorphism (SNP) chips, epigenetic studies and micro RNAs expression profiling in buffaloes, are helping understand the impact of gene diversity on economically significant traits and breeding strategies. The financial strength, input and cultural approaches to the improvement of living conditions will lead to such path for overall improvement of buffalo production. 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 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. Despite the significantly lower number of buffalo heads around the world, in comparison to cattle, they are still going to significantly have an impact on Asian countries and their zoo- economy, against the continuous mechanization and introduction of dairy cattle heads. We are witnessing improved living conditions and health standards, leading to increased life expectancy, together with a gradual increase in Asian and world human population. Such inevitable facts urge us to ensure that efforts are properly addressed in various fields of scientific enquiry, in order to enhance buffalo production in a sustainable and holistic manner.

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