Vol. 38 No. 1 (Jan-Mar 2019)

eISSN: 2539-5696

Buffalo Bulletin

International Buffalo Information Center
Office of the University Library
Kasetsart University

International Buffalo Information Center (IBIC)

Buffalo Bulletin
ISSN: 0125-6726 (Print), 2539-5696 (Online)

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Publisher

International Buffalo Information Center, Office of the University Library, Kasetsart University

Online available

http://kuojs.lib.ku.ac.th/index.php/bufbu

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MORPHOLOGICAL STUDY IN AZERI AND KHUZESTANI BUFFALOES OF IRAN

Javad Rahmaninia[1,*], Mahdi Mokhber[2] and Hossein Moradi Shahrbabak[3]

ABSTRACT

In order to evaluate and compare the properties of type traits in Khuzestani and Azeri river buffaloes, 148 and 336 Khuzestani and Azeri buffaloes were used, respectively. Animals were evaluated for traits such as Height at Withers, Body depth, Body length, Heart girth, Hip width, Pin width and Hip to Pin length. The averages for these traits in Khuzestani breed were 145.2±6.63, 78.27±5.43, 140.5±10.05, 208.87±13.75, 57±4.44, 25.29±3.03 and 44±2.97 cm and in Azeri breed were 138.93±6.39, 76.4±5.61, 136.22±10.05, 184±13.66, 54.96±4.85, 26.43±3.94 and 43.8±3.44 cm, respectively. The effect of breed on height at withers, heart girth and hip width was quite significant ($P<0.001$) and values for these traits in Khuzestani breed was higher than Azeri. The effect of parity number for all studied traits was significant ($P<0.001$), except for the heart girth. Heifers had the lowest values among different age groups and differences in this group increased with increasing age and parity. Also, the effects of province as a factor for climate and culture circumstances on studied traits was quite significant ($P<0.001$), except for the heart girth and hip to Pin length. Buffaloes in Guilan province had lowest and buffaloes in Khuzestan and Kermanshah provinces had the highest values.

Keywords: *Bubalus bubalis*, buffalo, Azeri buffalo, Khuzestani buffalo, type traits, Karaj, Iran

INTRODUCTION

Buffalo population is scattered in 129 countries all over the world while they generally founded in Asia and only very few of them are in other continents (Iamartino *et al*., 2017). About 194 million or 97% of buffaloes are located in Asia. India (57%) and Pakistan (43%) with 149 million buffaloes have the biggest buffalo population and their people's life are strongly depended on water buffalo more than any other domesticated animals (Moaeen-ud-Din, 2014). Buffalo milk and meat plays a vital role in the economy and the health of people in different countries and according to the latest FAO report, 97 million tones of milk (12.9%) and 3.6 million tones of meat (4.5%) which produced all over the world is belonged to buffaloes (FAO, 2013).

In 2500 BC, Indo-Aryan civilization was one of the advanced civilizations. Evidence

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from this civilization has been indicative of the existence of domestic buffaloes and the extent of their distribution from the Silk Road in the East to the Mesopotamia in the West has been identified. Domesticated buffaloes were probably raised in Iran Before Christ (BC), and it has even been mentioned that about 538 BC, the breeding of buffaloes was common in Persia. A bunch of royal cane for 900 to 750 BC is available at the British Museum, which archaeologists believe that it's origin was Lorestan province. The image of three calves and three buffaloes is carved on this stick. According to this document, the experts admitted that buffaloes lived in Iran from the seventh century BC and completely adapted to the conditions of Iran's climate (Manzoor, 1994).

Unfortunately, trends belong to buffalo population in Iran in recent years have shown a significant reduction from ~523 thousand in 2002 (Borghese and Mazzi, 2005) to ~199 thousand in 2012 (Anonymous, 2012). This reduction in Iran, like some other countries in the world is because of high producing dairy cows replacement instead of buffaloes, mechanization of agriculture and the reduction of market demand for buffalo products (Borghese and Mazzi, 2005). Iran's buffalo population consists of three breeds known as Khuzestani, Mazandarani and Azeri. Khuzestani breed is located in Khuzestan, Lorestan, Kermanshah, Ilam, Fars and some other neighboring provinces. Azeri breed is scattered in different cities of East Azerbaijan, West Azerbaijan, Ardebil and Guilan and also is known as Caucasus breed. Mazandarani (or Caspian) breed is located in different cities of the Caspian Sea and it's population is in third place after Azeri and Khuzestani breeds. In some references, this breed is considered as a part of Azeri breed.

Each Azeri, Khuzestani and Mazandarani breed's population are about 145 thousand (73%), 50 thousand (25%) and four thousand (2%) from the total population of Iranian buffaloes (Anonymous, 2012), respectively. Buffalo production in Iran is mainly for its milk (293 thousand tons, which is 2.8% of all milk produced in Iran) and its meat (24.7 thousand tons, which is 2.5% of all meat produced in Iran) is in second concern (Anonymous, 2010).

Buffalo breeding in Iran is based on smallholder farming systems with an average number of five animals in most herds (99%) and also a small number of herds with between 20 to 50 and some of them are up to 300 buffaloes. Smallholder's management is according to environmental opportunities, such as pasture, straw, shrubs and fodder and also is based on environmental conditions in which they hold them. Buffaloes in Khuzestan are kept outdoors throughout the year, while in the north west of Iran, buffaloes are kept indoors in autumn and winter (Broghese and Mazzi, 2005).

Type traits are about the shape and form of some animal body components which are physical and visible and are used in the apparent judgment of the livestock to be placed in the appropriate classification (Trim-Berger *et al*., 1992). These traits are important for selection and decision-making about reproduction in dairy cows (del Schneider *et al*., 2003). These traits are also effective, directly and indirectly, in decision-making for animal removal, longevity and milk production (del Schneider *et al.*, 2003) because of their relation with production traits (Kavandi *et al*., 2011; Misztal *et al*., 1992). A buffalo breeder should be able to evaluate and judge his animals and categorize them according to the type for better nutrition and breeding and to overcome management problems (Trim-Berger *et al*., 1992).

Many environmental factors affect the

body size and body measurements and these effects can mask the true genetic value of these animals. Obtaining more accurate values for these traits can help to formulate selection strategies to improve the yield of milk production under field conditions (Javed *et al.*, 2013).

Norman and Van Vleck (1972) announced that the effect of year on type traits was low and contribution of the year to type changes was reported to be 2 to 3% (Norman and Van-Vleck, 1972). Short *et al.* (1992) reported that the effect of month was significant for some type traits (Short *et al*., 1992). Thompson *et al.* (1981) reported that the effect of age on 11 traits from 14 type traits was significant (Tompson *et al*., 1981). Norman *et al.* (1978) also reported that the effect of age was significant for all type traits in the Jersey breed (Norman *et al*., 1978). Given that the conditions in the herds such as raising systems, management, feeding systems and etc. are different, therefore, herd effect should be considered as a specific environmental factor in our analysis. This effect cannot be separated from other environmental effects (Swalve, 1995). Norman *et al*. (1978) declared that the importance of the herd effect is more than the year effect and they reported that the effect of herd on type traits were 9 to 13% (Norman *et al*., 1978). Also, the herd effect on all type traits announced significant by Thompson *et al.* (1981); (Tompson *et al*., 1981).

It is most likely to have error in evaluation of type traits, because this evaluation is often theoretical. Therefore, the evaluator's effect on evaluating these traits is significant (Short *et al*., 1992). Thompson *et al.* (1981) announced that the effect of the evaluator on all studied type traits was significant (Thompson *et al*., 1981). Vinston *et al.* (1982) reported that the contribution of the evaluator to the variance of different traits was 0.7 to 0.5 (Vinson *et al*., 1982). The final score for type traits at the beginning and the end of the lactation period have been reported higher than mid-lactation. At the end of the lactation period, the lactation has a lower score and the body capacity gets more points (Warwick, 1979). Norman *et al.* (1978) described that the effect of lactation stage on type traits in Jersey breed (excluding body capacity and dairy character) was insignificant (Norman *et al*., 1978). Thompson *et al.* (1981) also stated that type characteristics associated with body weight (strength and dairy character, fore udder attachment, Udder depth) are more likely to be affected by the stage of lactation (Thompson *et al*., 1981). Other factors such as breed, region (Shankar and Mandal, 2010; Moradi-Shahrbabak, 1997; Vander Warf and Schaeffer, 1997), sex (Kayastha *et al*., 2011) and parity (as a measure of age) (Javed *et al*., 2013; Shankar and Mandal, 2010) have been statistically significant effect on type traits, body measurements and body weight. Of course, due to the very high impact of sex effect on physical measurements, information about different sexes is reported separately (Borghese and Mazzi, 2005). The aim of this study is to evaluate and compare some type characteristics in Azeri and Khuzestani buffaloes and also to investigate the effects of genotype (breed), parity and calving age on these traits.

MATERIALS AND METHODS

In this study, 484 buffaloes (148 Khuzestani and 336 Azeri buffaloes) from 161 herds with pedigree and under recording system of Iranian Animal Breeding Center (ABC) were used. Azeri buffalo samples were prepared from East Azerbaijan, West Azerbaijan, Ardebil and Guilan

provinces and Khuzestanies samples were gathered from Khuzestan and Kermanshah provinces. We prefer to determine samples with the lowest relationship and highest variety for our data set. The animals were evaluated for height at withers, body depth, body length, heart girth, hip width, pin width and hip to pin length (Figure 1).

Prior to statistical analysis, descriptive statistics for each trait were determined and the normality test was performed to the data for each trait and for different provinces separately using Xlstat software. Each of the traits in the study for at least one of the normality tests (Shapiro-Wilk, Anderson-Darling, Lilliefors and Jarque-Bera) were non-significant and the data were normal or near normal. The statistical model to analyze the data was

$$y_{ijlk} = \mu + G_i + A_j + Sh_l + e_{ijlk}$$

$$y_{ijlk} = \mu + G_i + A_j + Sh_l + e_{ijlk}$$

where,

$y_{ijlk}y_{ijlk}$ = The value of kth individual under ith breed group, jth age, lth province.

μ = Total mean.

G_i = effect of ith breed (I = 1, 2).

A_j = effect of jth age (j = 3-20).

Sh_l = effect of lth province (l = 1-6).

e_{ij} = Residual effect or the random error.

All effects included in the model are considered as fixed effects. Since a series of factors such as sex, evaluator and evaluation time effects were similar for all animals, therefore they were not included in the model. Statistical analysis was performed using the GLM procedure by SAS 9.1.

RESULTS AND DISCUSSION

Descriptive statistics for height at withers, body depth, body length, heart girth, hip width, pin width and hip to pin length in Khuzestani and Azeri breeds are in Table 1. The average of height at withers for Khuzestani and Azeri breeds was 145.25 and 138.93 cm, respectively. Borghese and Mazzi had previously reported these values at 141 and 133 cm, respectively (Borghese and Mazzi, 2005). The obtained values for height at withers in Azeri and Khuzestani breeds were higher than the average mature Chilika females with 124 cm (Patro *et al*., 2003), Nili-Ravi with 125 cm (Borghese and Mazzi, 2005) and 132 cm (Javed *et al*., 2013), Murrah with 133 cm (Borghese and Mazzi, 2005), Banni with 136.7 cm (Mishra *et al*., 2009) and Anatolian breed whose area was adjacent to the Azeri breed with a height of 134 cm (Borghese and Mazzi, 2005). This value was 144 cm for mature Egyptian buffaloes, which was higher than the average for Azeri breed and close to the Khuzestan breed average (Borghese and Mazzi, 2005). Khuzestani or Iraqi breed is probably the largest breed of buffalo (Borghese and Mazzi, 2005) and the results obtained for height at withers as an indicator of the size of the animal confirms this. The average body depth for mature Khuzestani and Azeri breeds was 78.27 and 76.4 cm, respectively.

The average body length for Khuzestani and Azeri breeds was 160.5 and 136.22, respectively. These values are higher than those for the Chilika breed with 122 cm (Patro *et al*., 2003) and similar to the values found for mature Anatolian breed with 138.56 cm (***) and Nili-Ravi breed in different parities with 139.55 cm (Javed *et al*., 2013).

The average heart girth for Khuzestani and Azeri breeds was 208.87 and 184, respectively. This value for mature Nili-Ravi buffaloes in different

parities was 194.45 cm (Javed *et al*., 2013) and in other studies, this value was 170 cm for the Chilika buffaloes (Patro *et al*., 2003) and 205.5 cm for the Bannie breed (Mishra *et al*., 2009).

The average for hip width in Khuzestani and Azeri breeds was 57 and 54.96 cm, respectively. This value for mature Chilika buffaloes and Banni breed was 48 (Patro *et al*., 2003) and 55.4 (Mishra *et al*., 2009) cm, respectively.

The average for pin width in Khuzestani and Azeri breeds was 25.29 and 26.43 cm, respectively. This value for mature Banni buffaloes was 27.9 cm (Mishra *et al*., 2009).

Also, The average for hip to pin length in Khuzestani and Azeri breeds was 44 and 43.8 cm, respectively. The characteristics of pelvic tract are important for reproduction.

Descriptive statistics of the studied traits in Azeri and Khuzestani breeds indicate that Khuzestani breed is relatively larger than Azeri (Table 1). However, analysis of variance is necessary to ensure that the differences are significant. The results for analysis of variance and least squares means are presented in Table 2 and 3, respectively.

Considering that it is necessary to provide a suitable model for variance analysis, therefore, environmental factors affecting traits should be included in the statistical model as much as possible. In this study, all animals were female, so the sex effect as a factor was not included in the model. Also, due to the evaluation of all animals in a limited time period, and by an evaluator and also the singleton of all studied animals, there was no need to estimate the effects of the evaluator, the time of evaluation, and the type of birth in the model.

The herd effect as an essential factor in statistical analyzes should be placed in the model but due to the large number of herds (161 herds) and the small number of animals used per herd, estimating the effect of this factor on the model was not possible. Instead, the province factor was considered as a factor indicating different climates and different breeding methods. It has been reported that the use of zoning in the country in analytical models as an effective factor for type traits, has been significant (Vander Warf and Schaeffer, 1997). Also, in the analysis model, parity was introduced as a measure of age at the time of sampling. Heifers used in this study were sexually mature and more than 2.5 years old.

According to tables 2 and 3, the effect of genotype on height at withers, heart girth and Hip width was completely significant ($P<0.001$) and on body depth, body length, pin width and hip to pin length was non-significant. Table 3 shows that the least squares means of all studied traits in Khuzestani breed is greater than Azeri, except for pin width, which was the same for both breeds and equal to 26 cm. However, except for height at withers, heart girth and Hip width, these differences were non-significant.

The effect of parity as an indicator for animal age was significant on all traits ($P<0.001$) except heart girth. Significant effect of animal age on type traits was consistent with the results of Norman et al. on Jersey (1978); Javed *et al*. (2013); Thompson *et al*. (1981) and also Shankar and Mandal (2010). The effect of different parity on the estimated buffalo weight, which was calculated from physical measurements, was significant in this study ($P<0.001$) Non-significant effect of parity on heart girth was inconsistent with Javed *et al*. (2013). The age range of buffaloes used in this study was from heifers with 2.5 years old to buffaloes with 16 parity. Due to the large number of age groups and also considering that buffaloes

reach the maturity in their third parity (Shankar and Mandal, 2010), and fewer animals in older age groups, therefore, buffaloes were classified into 6 age groups including Pregnant heifers and buffaloes which are in their first to fifth parities. The results show that the significant differences in the studied traits are mainly related to low age groups such as heifers and first parity buffaloes with older ones. In all studied traits, where the parity effect was significant, heifers were the lowest and the animals with more than two parities had the highest values (Table 3). Low values in lower age groups are probably due to the lack of physical maturity and the presence of the animal in the growth phase. There was no significant differences in the studied traits between higher age groups. This can be due to physical growth stop or animal's skeletal growth Stop due to physical maturity (Shankar and Mandal, 2010).

Because these two studied breeds were not present together in any provinces and we can not estimate this effect alone in the model, therefore, it was placed in the model as an interbreed effect as a factor of different climates and different breeding methods. As shown in table 3, Azeri breed is scattered in West Azerbaijan, East Azerbaijan, Ardebil and Gilan provinces and Khuzestani breed is in Khuzestan and Kermanshah provinces. According to the results of tables 2 and 3, it is seen that the inter-breed effect of province on the studied traits is significant ($P<0.001$) except for heart girth and hip to pin length. The significance of province effect or region is compatible with Moradi Shahrbabak (1997); Shankar and Mandal (2010); Vander Warf and Schaeffer (1997). Least squares Means related to effect of provinces on all traits were investigated. It was observed that the average performance in the provinces where the Khuzestani breed is scattered is lower than the provinces where the Azeri breed is. Within the provinces with Azeri breed, the highest values are for buffaloes in western Azerbaijan and the lowest values for buffaloes in Guilan province. Further studies indicates a decrease in the quantities from

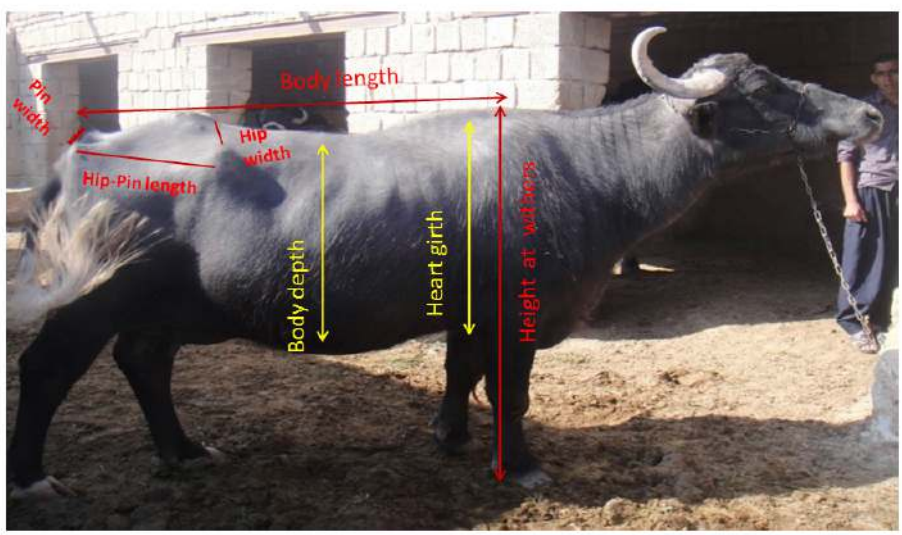

Figure 1. A Khuzestani buffalo.

Table 1. Descriptive statistics for type traits in Azeri and Khuzestani buffaloes.

Breed	Trait	Number of observation	Average (cm)	Standard deviation (cm)	Min (cm)	Max (cm)	Range (cm)
Khuzestani	height at withers	148	145.25	6.63	133	162	29
	body depth	133	78.27	5.43	66	104	38
	body length	110	140.5	8.39	115	159	44
	heart girth	85	208.87	13.75	180	242	62
	Hip width	131	57	4.44	42	68	26
	pin width	131	25.29	3.03	18	34	16
	hip to pin length	129	44	2.97	36.5	50	13.5
Azeri	height at withers	336	138.93	6.39	104	150	46
	body depth	336	76.4	5.61	50	93	43
	body length	335	136.22	10.05	106	170	64
	heart girth	221	184	13.66	140	230	90
	Hip width	336	54.96	4.85	27	68	40
	pin width	336	26.43	3.94	15	42	27
	hip to pin length	335	43.8	3.44	35	54	19

Table 2. Variance analysis for some factors affecting some type traits in Azeri and Khuzestani buffaloes.

Sources of variance	df	height at withers	body depth	body length	heart girth	Hip width	pin width	hip to pin length
		478	460	425	164	457	458	459
Genotype	1	693.32	64.81^{ns}	302.40^{ns}	5576.52***	250.48***	0.53^{ns}	96.11^{ns}
Province	4	613.20***	196.36***	2897.63***	620.40^{ns}	157.34***	397.40***	95.50^{ns}
Parity	5	184.91***	303.89***	735.5***	844.54^{ns}	348.22***	141.14***	168.02**
Residual		35.77	24.63	91.40	455.14	17.35	8.91	54.10

* = P<05, ** = P<01, *** = P<0001, NS = non significant

Table 3. Least-square means and standard errors for some type traits in Azeri and Khuzestani buffaloes.

Sources of variance	Categories	Number of animals	Height at withers	Body depth	Body length	Heart girth	Hip width	Pin width	Hip to pin length
Khuzestani		148	$143.63^{b}\pm1.03$	77.30 ± 0.85	138.45 ± 1.73	$195.37^{b}\pm2.88$	$57.29^{b}\pm0.72$	26.03 ± 0.51	45.41 ± 1.27
Azeri		336	138.73 ± 0.34	75.76 ± 0.29	135.04 ± 0.57	183.89 ± 1.95	54.30 ± 0.24	26.07 ± 0.18	43.56 ± 0.56
Parity	Heifers	40	$139.01^{a}\pm0.96$	$72.67^{a}\pm0.81$	$129.86^{a}\pm1.60$	$179.98^{a}\pm6.33$	$49.59^{a}\pm0.69$	$22.95^{a}\pm0.49$	$41.88^{a}\pm1.21$
	the first	62	$139.22^{a}\pm0.94$	$75.10^{b}\pm0.80$	$134.3^{ab}\pm1.59$	$186.68^{ab}\pm4.01$	$55.32^{b}\pm0.67$	$24.98^{b}\pm0.48$	$43.34^{ab}\pm1.17$
	Second	78	$140.73^{ab}\pm0.87$	$75.60^{b}\pm0.73$	$136.05^{b}\pm1.47$	$188.08^{ab}\pm3.13$	$56.47^{b}\pm0.61$	$26.41^{c}\pm0.44$	$43.93^{ab}\pm1.07$
	Third	83	$143.16^{c}\pm0.85$	$78.41^{c}\pm0.71$	$139.63^{c}\pm1.42$	$193.05^{ab}\pm3.18$	$57.72^{bc}\pm0.59$	$26.34^{cd}\pm0.43$	$45.66^{bc}\pm1.05$
	the fourth	73	$142.61^{bc}\pm0.88$	$78.88^{c}\pm0.74$	$140.77^{c}\pm1.47$	$196.09^{b}\pm3.60$	$57.99^{c}\pm0.63$	$26.90^{cd}\pm0.45$	$45.55^{bc}\pm1.11$
	the fifth	148	$142.35^{bc}\pm0.73$	$78.59^{c}\pm0.62$	$139.87^{c}\pm1.24$	$193.91^{b}\pm2.51$	$57.68^{c}\pm0.52$	$27.72^{d}\pm0.37$	$46.53^{bc}\pm0.91$
Province	Khuzestan	136	$144.82^{d}\pm0.58$	$77.27^{b}\pm0.50$	$137.13^{c}\pm1.05$	$195.37^{b}\pm2.88$	$56.01^{c}\pm0.42$	$24.24^{ab}\pm0.30$	44.67 ± 0.75
	Kermanshah	11	$142.44^{c}\pm2.05$	$77.32^{ab}\pm1.71$	$139.8^{bcd}\pm3.4$	-	$58.57^{c}\pm1.43$	$27.83^{c}\pm1.03$	46.15 ± 2.53
	West Azerbaijan	82	$142.45^{cd}\pm0.69$	$78.51^{b}\pm0.58$	$144.59^{d}\pm1.11$	$180.84^{a}\pm2.63$	$55.88^{c}\pm0.48$	$29.05^{cd}\pm0.34$	44.70 ± 0.85
	Guilan	102	$135.47^{a}\pm0.61$	$74.90^{a}\pm0.51$	$128.45^{a}\pm1.01$	$181.50^{a}\pm2.65$	$52.52^{a}\pm0.42$	$23.50^{a}\pm0.30$	42.42 ± 0.45
	East Azerbaijan	76	$139.74^{bc}\pm0.70$	$74.62^{a}\pm0.58$	$133.80^{b}\pm1.15$	$191.93^{ab}\pm5.16$	$55.05^{bc}\pm0.49$	$26.69^{c}\pm0.35$	44.48 ± 0.86
	Ardebil	77	$137.24^{a}\pm0.69$	$75.11^{a}\pm0.57$	$133.32^{b}\pm1.11$	$181.29^{a}\pm3.81$	$53.75^{ab}\pm0.48$	$25.06^{b}\pm0.35$	42.63 ± 0.86

Means with different superscripts differ significantly ($P<0.05$).

the west to the east of the Azeri Distribution Zone, respectively. In other words, the animal's size decreases in this direction. These differences were significant for height at withers, body depth, body length, hip width and pin width ($P<0.001$). Due to the large difference in climate in different provinces and their different breeding styles, the existence of these differences are predictable and the results confirm them.

CONCLUSION

Given the challenges ahead of gathering record in buffaloes, this study is broadly a part of the rare studies that have been carried out on this species. In this research, the characteristics of Azeri and Khuzestani buffaloes were determined and compared. In addition to the characterization of each breed for desired traits, average performance of the studied traits particularly the height at withers and heart girth, which have a very high correlation with animal weight and Chuck, are higher for the Khuzestani breed than the Azeri. Using this information along with production information can be useful in designing corrective strategies.

ACKNOWLEDGEMENTS

The authors would like to thanks "Animal Breeding Center (ABC)" and Ministery of Agriculture-Jihad branches in West Azerbaijan, East Azerbaijan, Ardebil, Guilan, Kermanshah and Khuzestan provinces and the "TAKDANA" company for their financial supports and data gathering.

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STUDY OF GENETIC AND NON GENETIC FACTORS AFFECTING AGE AT FIRST CALVING AND WET AVERAGE IN MEHSANA BUFFALOES

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ABSTRACT

Mehsana buffalo is one of the best dairy breed of buffalo in Gujarat state. The aim of this study was to analyse the effects of non-genetic factors on Age at first calving (AFC) and Wet average (WA). The data set comprised 12560 records up to three lactations of 7870 Mehsana buffaloes, sired by 200 bulls from 1989 to 2013 under field progeny testing programme of Dudhsagar Research and Development Association (DURDA), Dudhsagar Dairy, Mehsana. The least squares maximum likelihood (LSML) program was used for estimation of various non-genetic factors on AFC and WA. The least squares mean with standard error of AFC and WA were 1383.30±3.50 days and 7.00±0.22 lit. Highly significant (P<0.01) effect of season and period of birth was recorded on AFC however, effect of clusters on AFC was observed to be non-significant (P<0.05). Further, highly significant (P≤0.01) effect of all the non-genetic factors (parities, season of calving, clusters, period of calving and age at first calving group) under study was observed on wet average in Mehsana buffaloes. The heritability of AFC and WA were estimated as0.11±0.02 and 0.34±0.03 respectively. Heritability of traits are useful for prediction of genetic response to selection and measure for accuracy in selection. Very high and significant estimate of heritability of WA. It was concluded that selection of Mehsana buffaloes may be based on this production trait in the herd.

Keywords: *Bubalus bubalis*, buffalo, Mehsana buffalo, age at first calving, wet average, genetic, non-genetic

INTRODUCTION

India is leading milk producing country in the world. Gujarat possesses rich biodiversity of buffalo population, inhabits four well-established buffalo breeds *viz.*, Mehsana, Surti, Jaffarabadi and Banni. As per 19th Livestock Census total number of buffalo in the country is 108.7 million. The buffalo constitute around 21.23% of total livestock population of India (Annual Report, 2016-2017). Mehsana is one of the best dairy breed of buffaloes and considered to be regular breeder. Although the breed has contributed significantly in the milk production and had greater role in 'Operation flood' programme initiated to augment milk production in India, but the genetic potential of this breed has not been utilised to the fullest.

Age at first calving and wet average are the economic traits which are of important monetary value in the production of livestock. Age at first

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calving is one of essential traits for bringing improvement in milk production. It considerably affects the productive life of an animal and the number of calves obtained during the life time of that animal. Reduction in AFC is desirable for reducing rearing cost of heifer and for economizing milk production cost. Further, association of wet average with total milk yield and many of the reproduction rate necessitates the study the non-genetic and genetic factor affecting it. The genetic improvement in Mehsana buffaloes can be brought about by understanding the factors that affect these two traits. In the present study, an attempt has been made to estimate the genetic parameters of these traits as well as the non-genetic factors affecting them in Mehsana buffaloes.

MATERIALS AND METHODS

The data for the present study was collected from Dudhsagar Research and Development Association (DURDA), Dudhsagar Dairy, Mehsana for the period from 1986 to 2013. Dudhsagar Research and Development Association (DURDA) is actively engaged with the genetic improvement of buffalos and cows. The Mehsana district Co-operative Milk Producers' Union Limited, with the help of National Dairy Development Board, Anand, is carrying out field progeny testing programme through DURDA in Mehsana buffaloes since 1985. Geographically, the farms are located in Mehsana district (area of over 4500 km^2) in Gujarat, which is situated at the cross point of 23.40ºN latitude and 72.30ºE longitude, at an altitude of 265 meters above mean sea level. The climate of region is tropical and semi-arid maximum temperature recorded is 45ºC and minimum is 15ºC. Average rainfall in the district is around 668 mm and rainy season lasts for approximately 45 days. A total of 12560 lactation records belonging to 7825 buffaloes were utilised for the study. The data were classified according to parity, clusters, periods, seasons and age at first calving group. A total of 74 villages were clustered into three groups based on geographical location. Generally, the production and reproduction performance of Mehsana buffaloes in the present study scattered over 25 years, beginning from 1989. However, considering the contiguous years to have more or less similar effect, the entire data was grouped into the five periods according to year of calving for wet average and year of birth for AFC. Each year was divided into two seasons *viz* breedingseason - 1 (January to June) and breedingseason - 2 (July to December), season of birth and season of calving for both AFC and WA respectively. Classification in to AFC groups were done by taking the mean and standard deviation (SD) of age at first calving and three groups were defined, *viz*. < X-1 SD = <1100 days, X±1 SD = 1101-1678 days, > X+1 SD = 1679 days.

The least squares analysis of variance for unequal sub-class numbers (Harvey, 1990) was used to estimate the effect of non-genetic factors. The statistical model for AFC is $Y_{ijkl} = \mu+A_i+B_j+C_k+e_{ijkl}$ Where; Y_{ijkl} is l^{th} record of buffalo born in i^{th} cluster, j^{th} period and k^{th} season; μ is population mean; A_i is fixed effect of i^{th} cluster where, i is 1, 2 and 3; B_j is fixed effect of j^{th} period of birth where, j varies between 1, 2, 3, 4 and 5; C_k is fixed effect of k^{th} season of birth where, k is 1 and 2; e_{ijkl} is random error assumed to be normally and independently distributed with zero mean and constant variance (NID, 0, σ^2). The statistical model for WA is $Y_{ijklmn} = \mu+A_i+B_j+C_k+D_l+E_m+e_{ijklmn.}$ Where all notations have their usual meaning as mentioned above except, instead of birth, calving is to be considered;

D_l is fixed effect of l^{th} parity where, l is 1, 2 and 3; E_m is fixed effect of m^{th} age at first calving group where, m is 1, 2 and 3. The differences of means between subclasses of cluster, period, season, parity and age group were tested for significance using modified Duncan's Multiple Range Test (Kramer, 1957).

Genetic parameters (heritability and their genetic correlations) were estimated using paternal half sib correlation method (Becker, 1975). The data adjusted for significant effects of non-genetic factors were used for estimation of heritability. The model used to estimate the heritability was, $Y_{ij} = \mu+S_i+e_{ij}$ Where, Y_{ij} is j^{th} observation on a trait from progeny of i^{th} sire and S_i is effect of i^{th} sire. The standard error of heritability was estimated as per Swiger *et al.* (1964). The repeatability was estimated for WA with maximum three parities using intraclass correlation among repeated records of the same animal. The model was similar to that used for estimation of heritability except that between animal variance was used instead of between sire variance.

RESULTS AND DISCUSSION

The least squares mean for AFC in the present study was obtained as 1383.30±3.50 days in Mehsana buffaloes. The present estimate of AFC in Mehsana buffaloes was close to those reported previously by Prajapati *et al.* (2017); Chaudhary (2016) in Mehsana buffaloes and Jamal *et al*. (2017) in Murrah buffaloes. Whereas, it was higher than the values reported by Gupta *et al*. (2012); Jamuna *et al*. (2015b) in Murrah. On other hand, the LSM for AFC in the current study was lower than those reported by Galsar *et al*. (2016) in Mehsana and Chaudhary (2015) in Murrah buffaloes (Table 1).

The results of analysis of variance showed that season of birth had highly significant ($P\leq0.01$) effect on AFC in the present investigation. The buffaloes born during first season was having significantly higher AFC than those born during the second season. The significant effect of season on AFC is in line with those reported by Galsar *et al.* (2016); Chaudhari (2016) in Mehsana; Gupta *et al.* (2012); Jamal *et al*. (2017) in Murrah buffaloes. Season may influence the expression of performance traits in buffaloes because of wide variation of climatic condition throughout the year.

The cluster of distribution of buffaloes had non-significant effect on AFC however, lowest AFC was observed in first cluster (1377.91±6.64) and highest in second cluster (1393.37±4.52). The variation observed in the AFC in different clusters accounted for the variation in managemental practices and resources available within the village receiving random sires. On the contrary, highly significant ($P\leq0.01$) effect of period of birth on AFC was observed, which was highest in second period and lowest in first period However, there was no significant difference in AFC between 4^{th} and 5^{th} period, which differed significantly from rest of the periods. This finding is analogy with those reported by Galsar *et al.* (2016); Chaudhari (2016) in Mehsana; Gupta *et al*. (2012); Jamal *et al.* (2017) in Murrah buffaloes. The variation observed in different period is the reflection of differences in the feeding and fodder availability during different periods influencing the growth rate and age at first calving in the heifers.

The least squares mean of wet average for Mehsana buffaloes across progeny testing area was estimated to be7.00±0.22 lit. This was close to those reported previously by SDAU (2015) in Banni (simple mean), Jamuna *et al*. (2015a); NDRI, (2017) in Murrah buffaloes.

The parity had highly significant effect on WA and it was observed to be lowest in 1st parity and highest in 3rd parity. This was in equivalence with those reported by Jamuna *et al.* (2015[a]) in Murrah buffaloes. The significant effect of parity may be due to the fact that animals are in the growing stage during their first pregnancy and first lactation and the physiological development in the body of an individual animal in the early ages, which have stabilized in the subsequent lactations. Season of calving also had a highly significant (P≤0.01) effect on WA in the present study. It was significantly lower in buffalo calves born in second season. The present finding of highly significant effect of season of calving on WA was in contradiction with those reported by Jamuna *et al.* (2015[a]) in Murrah buffaloes and Tripathy *et al.* (2017) in Karan-Fries cattle.

Different clusters had highly significant (P≤0.01) influence and WA obtained was highest in cluster-3. Similarly, period of calvingwas also having highly significant (P≤0.01) effect on wet average and it was in equivalence with those reported by Jamuna *et al.* (2015[a]); Tripathy (2015) in Murrah buffaloes and Sahiwal cattle, respectively. Effect of age at first calving group was again found to have highly significant (P≤0.01). It was significantly lower in group-3 (A_3) as compared to those in A_1 and A_2. Jamuna *et al.* (2015[a]) reported similarly significant effect of AFC group on WA in Murrah buffaloes.

Among genetic parameters, the estimate of heritability for AFC was calculated to be 0.11±0.02, which was low but significantly differ from zero. The present finding of low estimate of heritability for AFC was in conformity with those reported by Chaudhary, (2016) in Mehsana and Barros *et al.* (2016) in Murrah. The heritability estimate for wet average (WA) was found to be 0.34±0.03, which was significantly different from zero (P≤0.01). Jamuna *et al.* (2015[b]) reported lower heritability for the same trait in Murrah buffaloes. Moreover, comparatively higher estimates of heritability for WA than the present estimate was reported by Tripathy *et al.* (2017) in Karan Fries cattle (Table 2).

Repeatability tells about the probability at which the repetition of the trait in an individual is expected in future. WA being the repeatable trait, as the repeatability was also estimated for this and it was found to be0.44±0.06, which is high and significantly different from zero. High repeatability estimate implies that early selection of the buffaloes will be reliable on basis of wet average. The phenotypic correlation ($r_{g)}$ between WA and AFC was estimated as 0.44±0.11whichis positive, high and highly significant. This implicate that these two traits are positively correlated which is undesirable hence, care must be taken while using both these traits simultaneously as selection criteria.

CONCLUSION

The effect of period and season of birth on age at first calving and season, period, parity, age at first calving group on the wet average suggested that traits should be adjusted for non-genetic factors. The high heritability of WA showed the importance of including this trait in the future selection programme when genetic improvement is sought. The genetic and phenotypic correlations between WA and AFC were on higher side but undesirable, suggesting that care should be taken while using wet average as selection criteria, as it may increase AFC too. The high repeatability (0.44±0.06) estimates of WA suggest that buffaloes could be selected for this trait based on early

Table 1. Least squares means with their standard errors and coefficient of variations for age at first calving and wet average in Mehsana buffaloes.

Traits	AFC(days)	WA(lit)
μ	1383.30±3.50 (21.8,7870)	7.00±0.22 (22.3,12524)
Parity		**
1	-	6.26±0.21 (20.8,7870)
2	-	7.20±0.30 (21.3,3153)
3	-	7.56±0.39 (20.5,1501)
Season	**	**
1	1396.12±5.05 (22.6,1386)	7.14±0.33 (22.5,2140)
2	1370.47±4.43 (21.6,6484)	6.87±0.20 (22.2,10384)
Cluster	NS	**
1	1377.91±6.64 (21.9,1835)	7.02±0.31 (21.9,2825)
2	1393.37±4.52 (21.7,4068)	6.85±0.24 (22.1,6501)
3	1378.61±6.43 (22.0,1967)	7.14±0.30 (22.7,3198)
Period	**	**
1	1267.54±8.29 (20.9,1159)	6.93±0.40[a](20.2,1502)
2	1462.96±7.49 (24.5,1498)	6.95±0.31[a] (22.0,2572)
3	1352.81±6.52 (21.9,1916)	6.96±0.28[a] (23.0,3515)
4	1426.46±6.27[a] (21.0,2104)	6.91±0.27[a] (23.0,3694)
5	1406.71±8.28[a] (19.7,1193)	7.26±0.44[b] (20.4,1241)
AFC group		**
1	-	7.05±0.33 (21.9,2166)
2	-	7.05±0.22 (22.3,8543)
3	-	6.91±0.36 (22.7,1815)

Figures within parentheses are the coefficient of variation and number of observations; ** = P≤0.01; * = P≤0.05; NS: non-significant. Superscripts may be read column wise for each effect for mean comparison. Similar superscript shows that the means do not differ significantly.

Table 2. Heritability and repeatability of traits in Mehsana buffaloes.

	AFC	WA
AFC	**0.11±0.02****	0.43±0.91
WA	0.44±0.11**	**0.34±0.03**** 0.44±0.06**

** = P≤0.01; Above diagonal = genetic correlations;
below diagonal = phenotypic correlations;
diagonal= heritability (bold) and repeatability.

lactation records.

ACKNOWLEDGEMENTS

Authors are thankful to Director of Research, Sardarkrushinagar Dantiwada Agricultural University (SDAU), Dean, College of Veterinary Science and Animal Husbandry, SDAU for providing all the necessary facility to carry out this work. The authors are also thankful to Dudhsagar Research and Development Association (DURDA), Dudhsagar Dairy, Mehsana for providing data to carry out this work.

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HISTOENZYMIC STUDIES ON THE OVARY OF INDIAN BUFFALO DURING DIFFERENT REPRODUCTIVE STAGES

Devendra Pathak* and Neelam Bansal

ABSTRACT

Distribution pattern of phosphatases, oxidoreductases and non-specific esterases were studied in ovary of prepubertal, follicular phase, luteal phase and in pregnant animals. In the ovary of prepubertal buffaloes, distribution of phosphatases (AKPase and G-6-Pase) was negligible in the surface epithelium, tunica albuginea and stroma in the cortex region while it was weak during the follicular, luteal and pregnant animals. Its activity was strong in the primordial and primary follicles in all the phases of the reproduction. Strong to intense reaction was observed in the theca cells and granulosa cells. The luteal cells and blood vessels in corpus luteum of luteal phase showed moderate reactions while strong reaction was observed in the pregnant buffaloes. The enzyme reaction of dehydrogenases revealed that the steroidogenic cells were more active during follicular phase, luteal phase and pregnancy while weak activity was observed in the surface epithelium and tunica albuginea. Reactivity of diaphorases were weak to moderate in surface epithelium and tunica albuginea, moderate activity in theca cells and oocyte and intense reaction in granulosa cells of tertiary follicles and weak activity in connective tissue septa and intense in luteal cells in parenchyma of corpus luteum of buffalo. Activity of NSE was strong in the granulosa cells and corona radiate cells of secondary and tertiary follicles while it was moderate in the theca cells. Most of the enzymes were more active in pubertal and pregnant buffaloes compared with prepubertal buffaloes and could be correlated with the steroid synthesis. The difference in the intensity of enzyme in different compartments of ovary also correlated with the cells participating in the hormone synthesis.

Keywords: *Bubalus bubalis*, buffalo, histoenzymic study, ovary, reproductive stages

INTRODUCTION

Buffalo is one of the important livestock resources of India and it plays a vital role in improving the socio-economic status of rural masses. Buffalo can be considered as a pillar for the development of dairy industry in India as the species is a major contributor in country's milk production (56.00% of total milk), despite the fact that they constitute only 34.60% of total bovine population, and this can be attributed to steady increase in buffalo population in India during last two decades, which is about 1.93% (FAO, 2012). Delayed puberty, poor expression of estrus, high incidence of early embryonic mortality, long inter-calving period and seasonal variation in fertility compromise the productivity of buffalo (*Bubalus*

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bubalis). A coordinated relationship exists between hypothalamus, pituitary and ovary of animals and ovary undergoes changes in its activity of cell types based on influences from hypothalamus and pituitary at different stages of reproduction. The location and the reactivity of various enzymes vary during different stages of reproduction and they play important role at each stage of reproductive physiology. The complete study of various enzymes during different stages of reproduction is required to understand the follicular development and steroidogenic activity of ovaries in buffaloes. Thus the present investigation was designed to elucidate phosphatases, oxidoreductases and non-specific esterases in ovary during prepubertal, follicular phase, luteal phase and pregnant buffaloes.

MATERIALS AND METHODS

The ovaries of buffaloes (n = 52) were collected from New Delhi Municipal Corporation Abattoir, Gajipur; M.K. Overseas Pvt. Ltd., Dera Basi, local abattoir at Bareilly and Teaching Veterinary Clinical Complex, GADVASU, Ludhiana. They were classified into four groups, prepubertal (based on the history and age of the animal), follicular phase (presence of a dominant follicle on the surface and absence of corpus luteum), Luteal phase (presence of a fully developed corpus luteum), pregnant (presence of foetus in the uterus).

Fresh unfixed tissues from ovary of Indian buffaloes were collected from slaughter houses immediately after the slaughter of the animals and placed in tissue freezing medium (Leica) and frozen in liquid nitrogen. Cryostat sections of 6 to 7 µm thicknesses at -20°C were obtained on glass slides with cryostat microtome and incubated with different substrates for the demonstration of phosphatases, oxidoreductases, esterases as described in Table 1. The positive and negative controls were carried out wherever possible.

RESULTS AND DISCUSSION

Distribution of phosphatases, oxidoreductases and non-specific esterases were studied in ovary of prepubertal (Table 2), follicular phase (Table 3), luteal phase (Table 4) and in pregnant animals (Table. 5).

Phosphatases

Alkaline phosphatases (AKPase)

In the ovary of prepubertal buffaloes, distribution of AKPase was negligible in the surface epithelium, tunica albuginea and stroma in the cortex region while it was weak during the follicular, luteal and pregnant animals. Its activity was strong in the primordial and primary follicles in all the phases of the reproduction (Figure 1(a)). AKPase is known for synthesis and transport of substances across the cell membrane and thus it might be required for growth and differentiation of ovarian follicles. Roy and Uppal (2005) discussed that this enzymes occurred in cells specialized for endocytosis and pinocytosis. This enzyme has been found to be involved in energy transfer reactions (Freeland and Szepesi 1971). Strong to intense reaction was observed in the theca cells and granulosa cells (Figure 1(b)). Weak reaction was observed in the oocyte and zona pellucida while intense reactions were noted in the corona radiate cells in all the stages of reproductive cycle (Figure 1(c)). The distribution of this enzyme was moderate in the ovarian medulla during prepubertal period while weak to moderate during all pubertal stages.

Table 1. Histoenzymic techniques used on cryostat sections of ovary.

Sr. No	Enzyme	Substrate	Method	Incubation Time (minute)	Reference
A. Phosphatases					
i.	Alkaline phosphatise (AKPase)	Naphthol AS-MX phosphate disodium salt in combination with Fast Blue RR	Simultaneous coupling azo dye method using substituted naphthol	30	Barka and Anderson (1963)
ii.	Glucose-6- phosphatase (G-6-Pase)	Glucose-6-phosphate and lead nitrate	Lead nitrate method of Wachstein and Meisel (1956)	20	Barka and Anderson (1963)
B. Oxidoreductases					
iii.	Succinic Dehydrogenase (SDH)	Di-NA-succinate	Standard method of Bound enzyme by Nitro BT method	15	Pearse (1972)
iv.	Lactate dehydrogenase (LDH)	Na-DL-lactate	Standard method of Bound enzyme by Nitro BT method	30	Pearse (1972)
v.	Glutamic dehydrogenase (GLD)	Na-L-glutamate	Standard method of bound enzyme by Nitro BT method	15	Pearse (1972)
vi.	Glucose-6-phosphate dehydrogenase (G-6-PD)	Di-Na-Glucose-6-Phosphate	Standard method of bound enzyme by Nitro BT method	30	Pearse (1972)
vii.	Reduced nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-diaphorase)	Co-enzyme (NADPH)	Standard method of Bound enzyme by Nitro BT method	35	Pearse (1972)
vi.	Reduced nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase)	Co-enzyme (NADH)	Standard method of Bound enzyme by Nitro BT method	35	Pearse (1972)
vii.	Monoamine oxidase (MAO)	Tryptamine hydrochloride	Standard method of Bound enzyme by Nitro BT method	60	Pearse (1972)

Table 1. Histoenzymic techniques used on cryostat sections of ovary. (Continue)

Sr. No	Enzyme	Substrate	Method	Incubation Time (minute)	Reference
A. Phosphatases					
C. Esterases					
viii.	Non-specific esterase (NSE)	Alpha naphthol Acetate	Naphthol acetate method	10	Barka and Anderson (1963)

Table 2. Enzyme histochemistry on the ovary of prepubertal buffalo.

Enzyme/Ovarian components	Cortex												Medulla				
					Follicle												
					Secondary				Graafian/Tertian								
	SE	TA	CS	P	TH	MG	ZP	O	TH	MG	ZP	O	IGC	MS	BV	RO	HG
AKPase	O	O	O	+++	+++/+++	++	-	+	+++/++++	++	-	+	++/+++	O	+++	+/++	-
G-6-Pase	+	+	+	+/++	+	+	-	-	+	+	-	-	+	+	+	+	-
G-6-PD	O/+	O/+	O/+	+	+/++	+	-	-	+/++	+	-	-	+	O/+	+	+	-
SDH	+	+	O/+	+	O/+	O/+	-	-	O/+	O/+	-	-	+	O/+	+	+	-
GLD	+	+	O/+	+	+	+	-	-	+	+	-	-	++	O/+	+	+	-
LDH	++	+	+	++	+	++	-	-	O/+	+/++	-	-	+/++	+	+	O/+	-
NADH diaphorase	+	O/+	+	++++	++++	+++	O	+	+++/++++	++	O	+	++	+	+	++	-
NADPH diaphorase	+	O/+	O/+	+++	+++	+	O	+	+++	+	O	+	++	O/+	+	+	-
MAO	O/+	O/+	O/+	++	+	+	-	-	+	+	-	-	+	O/+	O/+	+	-
NSE	+	+	O/+	+	O/+	+	-	-	O/+	+	-	-	+	O/+	+	+	-

- Not Observed, O-Absent; + Weak; ++ Moderate; +++ Strong, ++++ Intense

SE- Surface epithelium, TA-Tunica albuginea, CS -cortical Stroma, P-Primordial and Primary follicle

TH- theca, MG-Membrana Granulosa, ZP- Zona Plellucida, O-Oocyte

IG-Interstial gland, MS- Medullary Stroma, HG-Hilar Gland, BV- Blood vessel

Table 3. Enzyme histochemistry on the ovary of buffalo during follicular phase.

Enzyme/ Ovarian components	Cortex												Medulla				Corpus Luteum
					Follicle												
					Secondary				Graafian/Tertian								
	SE	TA	CS	P	TH	MG	ZP	O	TH	MG	ZP	O	IG	MS	BV	HG	
AKPase	O	O	O/+	+++	+++/++++	++/+++	O	+	+++/++++	++/+++	-	+	++	O/+	+++	+	++
G-6-Pse	+	+	+	++	++	+	-	-	++	+	-	-	++	+	+	+	++
G-6-PD	O/+	O/+	O/+	+++	+++	++	O	+	+++	++	-	-	++	O/+	+/++	-	++++
SDH	+	+	+	+	++	++	-	+	+/++	+/++	-	+	+/++	+	O/+	-	+++/++++
GLD	+	+	+	++	++/+++	+	-	-	++/+++	++	-	-	++	+	++	-	+++
LDH	++	+	++	++	++	++	-	+/++	++	++	-	+/++	++/+++	++	++	-	+++
NADH diaphorase	+	O/+	+	++++	+++	++	O	+	+++/++++	++	O	+	++	+	++	++	+++
NADPH diaphorase	+	+	+	++++	+++	++	-	+	++++	++	-	+	+++	+	+	-	++/+++
MAO	+	+	+	++	++	++	-	-	+	+	-	-	+++	+	++	-	++
NSE	O/+	O/+	O/+	++	+/++	+++	-	-	+++	+++	-	-	++++	O/+	++	-	++++

- Not Observed, O-Absent; + Weak; ++ Moderate; +++ Strong, ++++ Intense

SE- Surface epithelium, TA-Tunica albuginea, CS -cortical Stroma, P-Primordial and Primary follicle

TH- theca, MG-Membrana Granulosa, ZP- Zona Plellucida, O-Oocyte

IG-Interstial gland, MS- Medullary Stroma, HG-Hilar Gland, BV- Blood vessel

Table. 4. Enzyme histochemistry on the ovary of Buffalo during luteal phase.

Enzyme/ Ovarian components	Cortex												Medulla				Corpus luteum
					Follicle												
					Secondary				Graafian/Tertian								
	SE	TA	CS	P	TH	MG	ZP	O	TH	MG	ZP	O	IGC	MS	BV	HG	
AKPase	+	+	O/+	+++/++++	++/+++	++	-	+	++/+++	++	-	+	++	O/+	+++	+/++	+++/++++
G6Pase	+	+	O/+	+++/++++	++/+++	++	-	+	++/+++	++	-	+	++	O/+	+++	+/++	+++/++++
G-6-PD	+	+	+	++/+++	++/+++	++	-	-	++/+++	++	-	-	++	+	O/+	-	+++
SDH	O/+	O/+	O/+	+	+/++	+	-	-	+/++	+	-	-	+++	O/+	+/++	-	+++
GLD	+	O/+	+	++	+	++	-	-	+	+	-	-	+	+	O/+	-	+++
LDH	+	+	+	++	+	++	-	+/++	+	++	-	+/++	++	+	+	-	+++
NADHd	+	+	+	+	+++	++	-	-	+++	++	-	-	++	+	+/++	-	++/+++
NADPHd	+	+	+	+	+++	++	-	-	+++	++	-	-	++	+	+	-	+++/++++
MAO	+	+	+	+	+	+	-	-	+	+	-	-	+++	+	+/++	-	++++
NSE	O/+	O/+	O/+	+++	O	+++	-	-	+	+++	-	-	++++	O/+	+	-	++++

- Not Observed, O-Absent; + Weak; ++ Moderate; +++ Strong, ++++ Intense

SE- Surface epithelium, TA-Tunica albuginea, CS -cortical Stroma, P-Primordial and Primary follicle

TH- theca, MG-Membrana Granulosa, ZP- Zona Plellucida, O-Oocyte

IG-Interstial gland, MS- Medullary Stroma, HG-Hilar Gland, BV- Blood vessel

Table. 5. Enzyme histochemistry on the ovary of pregnant buffalo.

Enzyme / Ovarian components	Cortex												Medulla				Corpus Luteum
	SE	TA	CS	P	Follicle												
					Secondary				Graafian/ Tertian								
					TH	MG	ZP	O	TH	MG	ZP	O	IGC	MS	BV	HG	
AKPase	+	+	O/+	+++/++++	++/+++	++	-	+	++/+++	++	-	+	++	O/+	+++	+/++	+++/++++
G6Pase	+	+	O/+	+++/++++	++/+++	++	-	+	++/+++	++	-	+	++	O/+	+++	+/++	+++/++++
G-6-PD	+	+	+	++/+++	++/+++	++	-	-	++/+++	++	-	-	++	+	O/+	-	+++
SDH	O/+	O/+	O/+	+	+/++	+	-	-	+/++	+	-	-	+++	O/+	+/++	-	+++
GLD	+	O/+	+	++	+	++	-	-	+	+	-	-	+	+	O/+	-	+++
LDH	+	+	+	++	+	++	-	+/++	+	++	-	+/++	++	+	+	-	+++
NADH D	+	+	+	+	+++	++	-	-	+++	++	-	-	++	+	+/++	-	++/+++
NADPH D	+	+	+	+	+++	++	-	-	+++	++	-	-	++	+	+	-	+++/++++
MAO	+	+	+	+	+	+	-	-	+	+	-	-	+++	+	+/++	-	++++
NSE	O/+	O/+	O/+	+++	O	+++	-	-	+	+++	-	-	++++	O/+	+	-	++++

- Not Observed, O-Absent; + Weak; ++ Moderate; +++ Strong, ++++ Intense

SE-Surface epithelium, TA-Tunica albuginea, CS-cortical Stroma, P-Primordial and Primary follicle

TH-theca, MG-Membrana Granulosa, ZP-Zona Plellucida, O-Oocyte

IG-Interstial gland, MS-Medullary Stroma, HG-Hilar Gland, BV- Blood vessel

Similar observations were recorded by Bhardwaj and Roy (2001) in Indian buffalo at different ages.

The luteal cells and blood vessels in corpus luteum of luteal phase showed moderate reactions while strong reaction was observed in the pregnant buffaloes (Figure 1(d)). The higher activity in the pregnant buffaloes may be correlated with its secretory activity. Similar findings were reported by Boos *et al.* (1988) in cows and Singh and Roy (1999) in buffaloes.

Glucose-6- phosphatase (G-6-Pase)

The distribution of G-6-Pase in ovarian surface epithelium, tunica albuginea and cortical stroma of prepubertal and pubertal buffaloes was weak while it was moderate in the primordial and primary follicles. Follicular cells of primary follicle and granulosa cells of secondary follicles showed strong to intense reaction (Figure 1(e)). Granulosa cells showed strong activity while the theca cells exhibited weak activity (Figure 1(f)). The activity of this enzyme was weaker in the prepubertal buffaloes than that during follicular, luteal and pregnant animals. The activity of this enzyme was weak to moderate in the ovarian medulla cells. The findings of the present study were supported by the observations of Bhattacharya and Saigal (1990) in goats.

The luteal cells of corpus luteum exhibited moderate reaction both during luteal phase and in pregnant buffaloes. The moderate activity of this enzyme indicated better glucose metabolism in cells undergoing steroid synthesis. G-6-Pase enzyme is responsible for glucose metabolism and may be associated with steroid synthesis of the cells.

Oxidoreductases and dehydrogenases

Glucose-6-phosphatase dehydrogenase (G-6-PD)

The surface epithelium, tunica albuginea and cortical stroma exhibited weak activity of G-6-PD while moderate reaction was observed in the primordial and primary follicles during all the stages of buffaloes (Figure 2(a)). The membrana granulosa cells exhibited strong reaction of G-6-PD while theca cells showed weak to moderate reaction during follicular and luteal phases of the estrous cycle (Figure 2(b)). The enzyme is responsible for conversion of glucose to acetyl coenzyme-A, which is utilized for the active synthesis of fatty acids and steroids. Similar observations were recorded by Bhardwaj and Roy (2001a) in buffalo ovary at different age groups.

The capsule, stroma and blood vessels were devoid of G-6-PD activity. Micro granular reaction of moderate intensity was exhibited by the luteal cells during luteal phase and in pregnant animals (Figure 2(c)). The observations of the present study were in consonance with that of Roy and Saigal (1985) in pregnant sheep ovary.

Succinate dehydrogenase (SDH)

The activity of SDH enzyme was not appreciable in surface epithelium, tunica albuginea and cortical stroma of prepubertal buffalo ovary and weak reaction was observed during pubertal stage (Figure 2(d)). Reaction was moderate in the primordial and primary follicles. In the growing and tertiary follicles during follicular phase and luteal phase of estrous cycle, theca cells exhibited weak reaction during prepubertal period while it was moderate during the pubertal stages whereas granulosa cells exhibited strong granular reaction (Figure 2(e)). SDH plays important role in steroidogenesis as it being closely linked to

cytochrome system (Motta and Hafez, 1980).

Luteal cells of buffalo ovary exhibited moderate to strong reaction of SDH in the luteal phase of estrous cycle while it was strong in the pregnant animals (Figure 2(f)). The reaction was perinuclear and granular in nature. Similar findings were made by Singh and Roy (2001) in buffalo corpus luteum. The higher enzyme activity may correspond to the higher secretory activity of the luteal cells.

Glutamate dehydrogenase (GLD)

The activity of GLD was negligible in the surface epithelium, tunica albuginea and cortical stroma. A weak reaction was observed in the primordial and primary follicles during all the stages of reproduction. The theca cells and membrana granulosa cells showed moderate to strong reaction during follicular while luteal phase of pubertal buffaloes (Figure 2(g)). The ovarian medulla showed moderate GLD activity while the blood vessels revealed weak activity. The luteal cells showed weak activity of GLD both during luteal phase and in pregnant animals. Similar observations have been recorded by Bhardwaj and Roy (2001b) in buffaloes.

Lactic dehydrogenase (LDH)

The activity of LDH was weak in the surface epithelium, tunica albuginea and cortical stroma was weak to moderate reaction was observed in the primordial and primary follicles during all the stages of buffaloes (Figure 2(h)). The membrane granulosa cells and theca cells exhibited moderate reaction of LDH during follicular and while weak during luteal phase of the estrous cycle (Figure 2(i)). The enzyme is responsible for conversion of glucose to acetyl coenzyme-A, which is utilized for the active synthesis of fatty acids and steroids. The observations of the present study are in consonance with that of Roy and Saigal (1985) in sheep.

Luteal cells showed micro granular reaction of LDH both during luteal phase and in pregnant buffaloes. The capsule, stromal cells and blood vessels had negligible reaction. Similar observations were made by Singh and Roy (1996). The activity of this enzyme may be correlated with the synthesis of steroid hormone by luteal cells.

Diaphorases

NADH diaphorases

The activity of NADH-diaphorase was weak to moderate in the surface epithelium, tunica albuginea and cortical stroma in prepubertal buffaloes whereas moderate reaction in the pubertal animals (Figure 3(a)). Moderate reaction was observed in the primordial and primary follicles during the pubertal period while a strong activity was exhibited in the ovary during follicular phase.

Strong to intense activity was recorded in the theca cells and membrane granulosa cells of growing and tertiary follicles during follicular phase (Figure 3(b)). The medulla of ovary showed moderate reaction while the blood vessels showed weak reaction. Similar observations were made by Bhardwaj and Roy (2003). The enzyme is related with the steroidogenic activity. Guraya (1985) reported that the inner layer of granulosa cells of preantral follicles had more activity than the outer layer.

The luteal cells exhibited strong to intense reaction of NADH-diaphorase in pregnant animals while moderate to strong reaction was observed in the corpus luteum during luteal phase (Figure 3(c)). The weak to moderate activity was also observed in the connective tissue components and blood vessels. Similar observations were recorded by Singh and Roy (1996) in Indian buffaloes.

NADPH diaphorases

The activity of NADPH-diaphorase was negligible in the surface epithelium, tunica albuginea and cortical stroma in all stages of buffaloes. Moderate to strong reaction was observed in the primordial and primary follicles during the prepubertal, follicular phase and luteal phase but was weak in pregnant animals (Figure 3(d)).

Moderate activity was observed membrana granulosa cells of growing and tertiary follicles during follicular and luteal phase while strong activity was seen in the theca cells (Figure 3(e)). Similar observations were made by Bhardwaj and Roy (2001b). The ovarian medulla of ovary showed moderate reaction while the blood vessels showed weak reaction. Roy and Saigal (1985) also reported weak activity in blood vessels in ovarian medulla of sheep. The enzyme is related with the steroidogenic activity. The enzyme is responsible in steroidognesis by converting cholesterol to progesterone (Sorensen and Singh, 1973).

Moderate to strong reaction was observed in the luteal cells in corpus luteum during luteal phase and in pregnant animals (Figure 3(f)). As reported by Singh and Roy (1996) in buffaloes and Roy and Saigal (1985) in sheep. The increased activity of NADPH during luteal phase and prengnancy might be correlated to increased secretory activity of cells as the enzyme is responsible for conversion of cholesterol to progesterone (Sorensen and Singh, 1973) and fatty acid synthesis (Hoyer 1980).

Monoamine oxidase

The MAO activity was negligible in the surface epithelium, tunica albuginea and cortical stroma and weak in the primordial and primary follicles in prepubertal and whereas it was weak to moderate in pubertal buffaloes (Figure 4(a)). The granulosa cells and theca cells during follicular and luteal phase of estrous cycle exhibited weak to moderate activity of MAO. Strong MAO activity was observed in the interstitial gland present in the medulla of ovary during follicular and pregnant animals (Figure 4(b)). Yoshimoto *et al.* (1986) recorded highest activity in blood vessels of proestrous rat and postulated its role in ovulation.

MAO activity was negligible in the connective tissue capsule. Luteal cells exhibited weak activity during luteal phase while the reaction was moderate in pregnant animals (Figure 4(c)). MAO is involved in the process of vasodilation (Raekallio, 1970). Its role in the corpus luteum of pregnant animals may be related to efficient delivery of this hormone. It is also involved in the biological oxidation and reduction for the energy production which is required for steroidogenesis (Singh and Roy, 1996)

Non Specific Esterases (NSE)

The surface epithelium, tunica albuginea and cortical stroma exhibited negligible NSE activity and the primordial and primary follicles showed weak reaction in in prepubertal and pubertal buffaloes. The NSE activity was moderate in the theca cells of tertiary follicles during follicular phase, luteal phase and in pregnant buffaloes while it was weak in the prepubertal buffaloes (Figure 5(a) and 5(b)). The reactivity was micro granular in nature. The granulosa cells exhibited strong reactivity in the follicular and luteal phase of the estrous cycle. Bhatttacharya and Saigal (1990) also observed moderate to strong activity in the thecal cells of goat ovarian follicles. Ovarian medulla showed moderate to strong reaction while the blood vessels showed weak reaction.

Luteal cells during luteal phase and pregnant buffaloes exhibited strong reaction

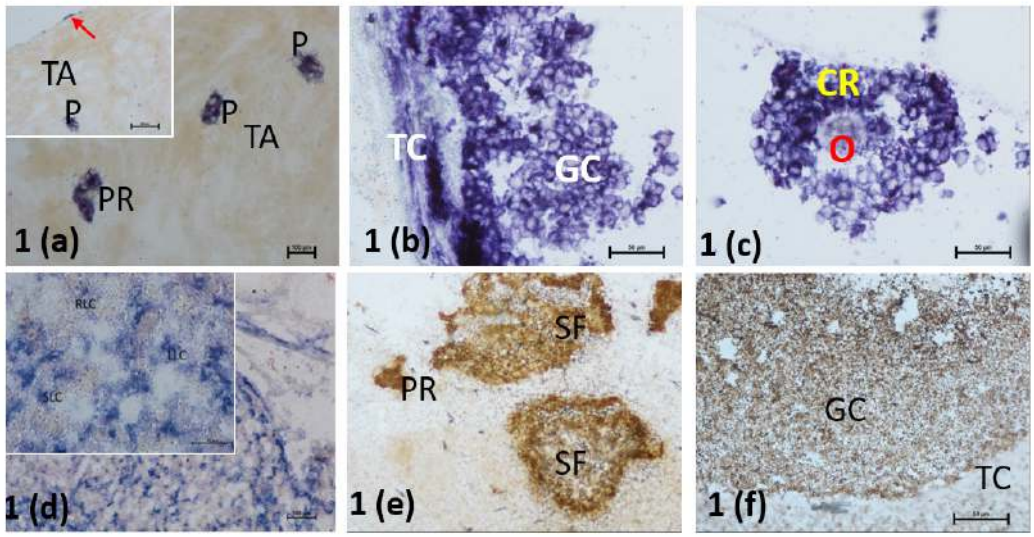

Figure 1. Representative cryostat sections showing phosphatase enzyme activity, Azo Dye method for AKPase and. (a) AKPase activity in surface epithelium (arrow), negligible activity in tunica albuginea (TA) and strong reaction in primordial (P) and primary follicles (Pr) of buffalo ovary during follicular phase. Original magnification. X 100; inset with AKPase activity in surface epithelium (arrow), tunica albuginea (TA) and strong reaction in primordial (P). Original magnification. X 400. (b) Strong to intense reaction of AKPase in granulosa cells (GC) and theca cells (TC). Original magnification x 400. (c) Strong to intense reaction of AKPase in oocyte (O) and corona radiata cells (RC). Original magnification x 400. (d) Strong reaction of AKPase in corpus luteum. Original magnification x 100. Inset showing strong reaction in large luteal cells (LLC) and small luteal cells (SLC) and weak reaction in regressing luteal cell (RLC). Original magnification x 400. (e) Glucose 6-Pase activity in primary follicle (PR) and secondary follicle (SF). Original magnification x 100. (f) Glucose 6-Pase activity in granulosa cells of tertiary follicles (GC) and weak reaction in theca cells (TC). Original magnification x 400.

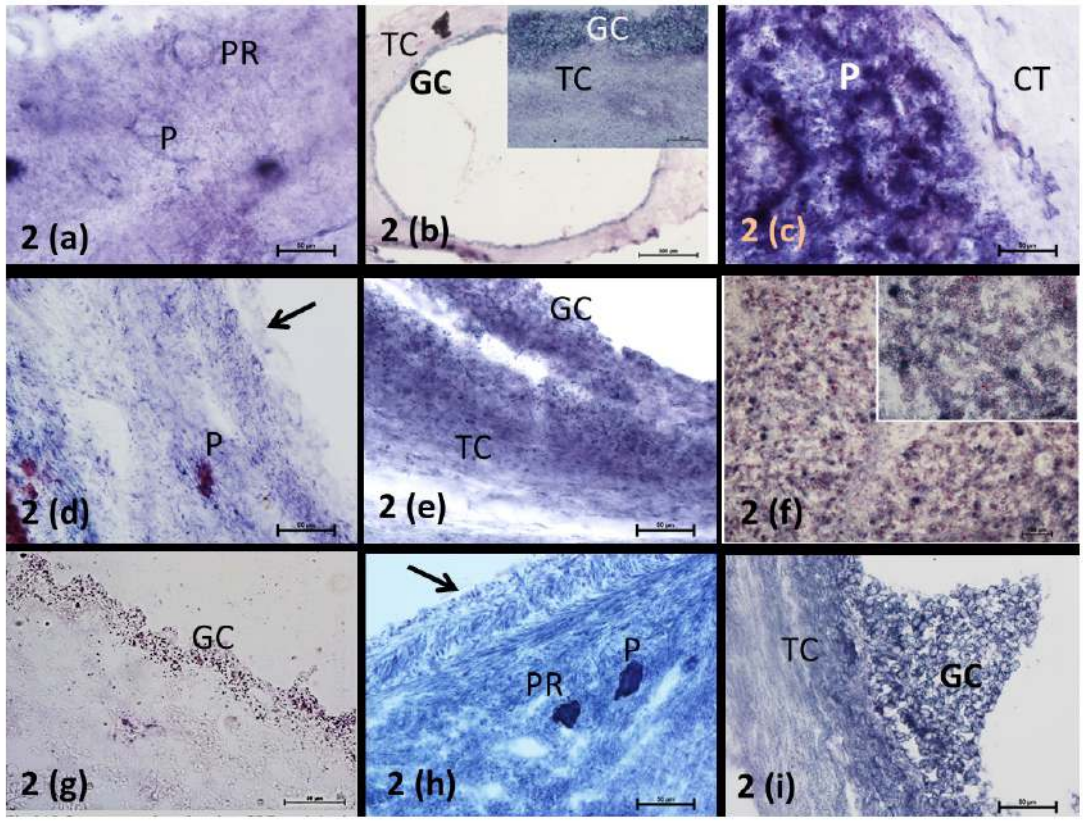

Figure 2. Representative cryostat sections showing activity of dehydrogenases, Nitro BT method. (a), (b) and (c) showing G-6-PDenzyme activity; moderate enzyme reaction in primordial (P) and primary follicles (PR), weak activity in theca cells (TC) and intense reaction in granulosa cells (GC) of tertiary follicles; and weak activity in connective tissue septa and intense in luteal cells in parenchyma of corpus luteum of buffalo. Original magnification (a) X 400; (b) x40, inset x 400 and (c) x400; (d), (e) and (f) showing SDH enzyme activity; Weak activity in surface epithelium (arrow), tunica albuginea and moderate to strong in primordial follicle (P) and primary follicles, strong to intense in the membrane granulosa cells (GC) and moderate in the theca cells (TC), intense reaction in the luteal cells in corpus luteum. Original magnification (d) X 400; (e) x400, inset x 400 and (f) x100 and inset x400. (g) Showing GLD enzyme activity in the membrane granulosa cells (GC). Original magnification x400. (h) and (i) showing LDH enzyme activity; Weak activity in surface epithelium (arrow), moderate to strong in primodial follicle (P) and primary follicles, strong to intense in the membrane granulosa cells (GC) and moderate in the theca cells (TC), intense reaction in the luteal cells in corpus luteum. Original magnification (d) X 400; (e) x400, inset x 400 and (f) x100 and inset x400.

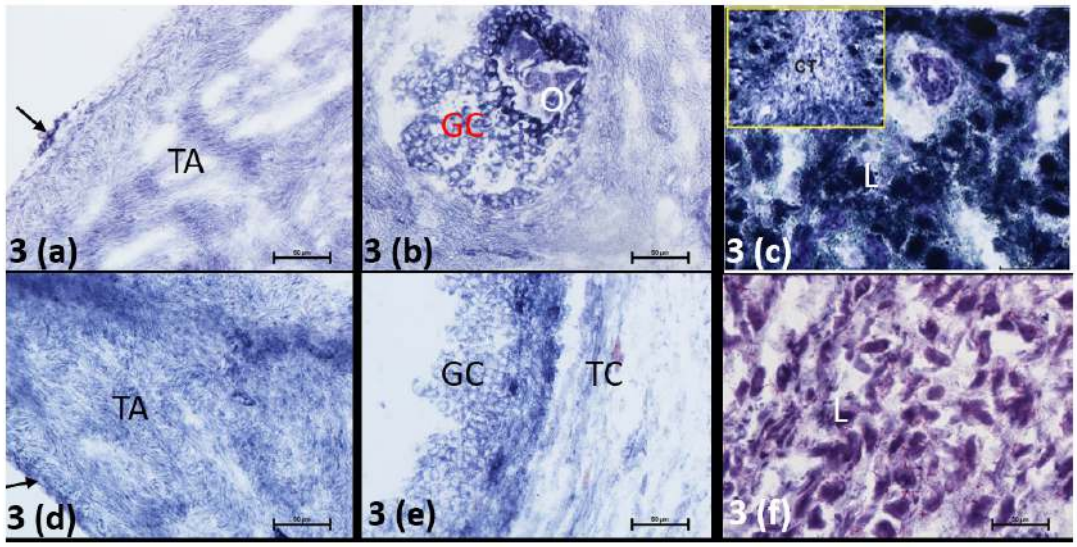

Figure 3. Representative cryostat sections showing activity of diaphorases, Nitro BT method. (a), (b) and (c) showing NADHd activity and (d), (e) and (f) showing NADPHd enzyme activity; weak to moderate enzyme reaction in surface epithelium (arrow) and tunica albuginea (TA), moderate activity in theca cells (TC) and oocyte (O) and intense reaction in granulosa cells (GC) of tertiary follicles; and weak activity in connective tissue septa (CT) and intense in luteal cells(L) in parenchyma of corpus luteum of buffalo. Original magnification (a) X 400; (b) x40, inset x 400 and (c), (d) and (f) x400.

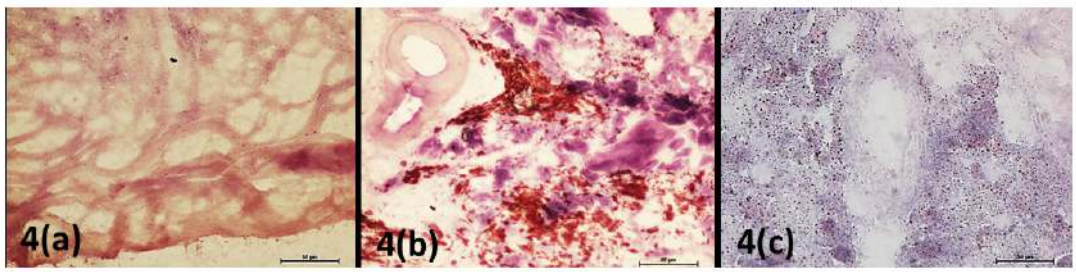

Figure 4. Representative cryostat section of buffalo ovary showing weak MAO activity in cortical zone (a) moderate to strong in medullary zone (b) strong activity in luteal cells in pregnant buffalo (c). Nitro BT method. Original magnification x400.

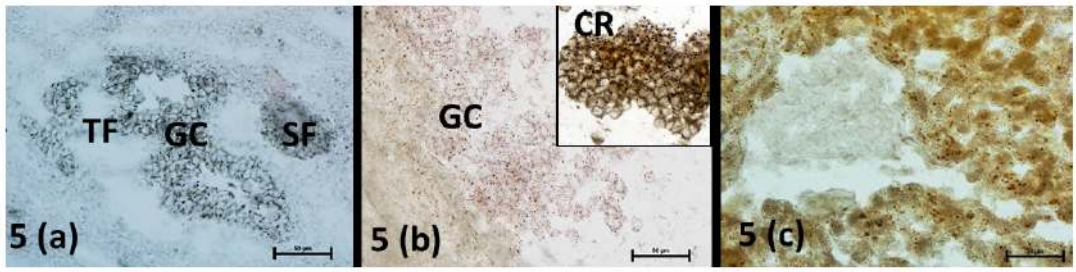

Figure 5. Representative cryostat sections showing activity of NSE activity in ovary of buffalo. Naphthol acetate method. (a) Strong reactions in granulosa cells (GC) of secondary (SF) and tertiary follicles (b) strong reaction in granulosa cells and intense reaction in corona radiate cells (CR); strong to intense reaction in the luteal cells (c). Original magnification (a), (b) and (c) x400.

for NSE while the connective tissue exhibited negligible or weak reactions. The reactivity was micro granular in nature in the cytoplasm of the luteal cells. Similar observations were reported by Bhattacharya and Saigal (1990) in sheep and Bhardwaj and Roy (2003a) in buffaloes.

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BIOCHEMICAL AND HORMONAL PROFILES IN BUFFALOES WITH RETAINED FETAL MEMBRANES

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ABSTRACT

The present study was designed to investigate the incidence of retention of fetal membranes (RFM) on 605 buffaloes at 2nd to 5th calving with normal calving, premature birth and abortion. The normally calved buffaloes with retention of fetal membranes (after 12 h) were randomly classified in treatment (n = 6) and control (n = 6) groups. The buffaloes in treatment group were administered Dinoprost ($PGF_2\alpha$ analogue, 25 mg); Meloxicam (anti-inflammatory drug 0.5 mg/kg body weight,); Oxytetracycline (Broadspectrum antibiotics, 5 mg/kg body weight) intramuscularly and intravenous calcium therapy (Calcium gluconate, 1.86% w/v; Magnesium hypophosphite, 5% w/v; Dextrose anhydrous, 20% w/v; Chlorocresol 0.1% w/v, 450 ml) after 12 after calving. The incidence of retention of fetal membranes in buffaloes was 9.98 %, 34.61% and 46.87% in normal calving, premature birth and abortion, respectively. The overall incidence of RFM was 14.04%. There was significant rise in the level of calcium ($P<0.01$), phosphorus ($P<0.05$) and magnesium ($P<0.05$) on day 30 and day 45 in treatment than control group. However, there was no significant difference observed in level of glucose, NEFA and progesterone between treatment and control group on day 0, 30 and 45. RFM is considered as one of the major problem with higher incidence in premature birth and abortions by ultimately affecting the reproductive efficiency in buffaloes.

Keywords: *Bubalus bubalis*, buffaloes, biochemical, fetal membrane retention, hormone, incidence

INTRODUCTION

Buffaloes are high producing animals (Khan *et al.*, 2004) and are considered "Black Diamond" as contributing more than half of the total milk production in India. Various reproductive disorders creating hindrance in the exploitation of its production potential and thus poor reproductive efficiency remained a major economic concern in terms of reduced fertility, low life time production, longer calving interval and

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increased medication costs in farm animals (Samad *et al.*, 1987). Retained fetal membranes (RFM) in buffaloes constitutes one of the major postpartum complications leading to severe endometritis, metritis, pyometra, perimetritis, ovaritis (Roberts, 1971), cystitis and peritonitis (Wetherill, 1965) and vaginal prolapse (Odegard, 1977) prone to drastic milk reduction and poor female fertility (Laven and Peters, 1996) characterized by prolonged calving intervals (Joosten *et al.*, 1988) and repeat breeding (Narsiinhan and Deopurkar, 1994). The role of certain biochemical constituent's glucose, serum total protein and macro-minerals in various reproductive processes have been well documented (Quayame *et al.*, 1985) in buffaloes. Disturbance of endocrine function, high progesterone and cortisol and low estradiol levels were traced in blood of animals with RFM (Michal *et al.*, 2006). Therefore, the present research work was designed to study the incidence of retention of fetal membranes in buffalo with normal parturition, premature birth and abortion and its association with biochemical and hormone profiles.

MATERIALS AND METHODS

The study was conducted on 605 buffaloes at 2nd to 5th calving in Durg and Rajnandgaon districts of Chhattisgarh state to calculate the incidence of retention of fetal membranes with normal parturition, premature birth and abortion. The normally calved buffaloes with retention of fetal membranes (after 12 h) were randomly classified in treatment (n = 6) and control (n = 6) groups. The buffaloes in treatment group were administered Dinoprost ($PGF_2\alpha$ analogue, 25 mg); Meloxicam (anti-inflammatory drug 0.5 mg/kg body weight,); Oxytetracycline (Broadspectrum antibiotics, 5 mg/kg body weight) intramuscularly and intravenous calcium therapy (Calcium gluconate, 1.86% w/v; Magnesium hypophosphite, 5% w/v; Dextrose anhydrous, 20% w/v; Chlorocresol 0.1% w/v, 450 ml) after 12 after calving. However, the buffaloes in control group were not given any treatment. Blood samples were collected randomly in vaccutainer tubes by jugular vein puncture aseptically after 12 h (day 0); 30 days and 45 days from both treatment and control group in normally calved buffaloes with retention of fetal membranes. Blood samples were allowed to clot at 4ºC for 24 h and then centrifuged at 3000 rpm for 20 minutes in refrigerated centrifuge machine. Serum was poured into sample tubes and stored at -20ºC until analysis. Blood glucose was estimated by the glucometer immediately after collection through strip method. Serum calcium and phosphorus were analyzed through diagnostic kit by semi auto-analyzer (Systronics India Ltd.). Serum concentration of magnesium was determined by Titan Yellow method (Neill and Neely, 1956), NEFA was determined by soap extraction method (chloroform: heptane: methanol, 49:49:2) as per Shipe *et al.* (1980) and progesterone by Radio Immune Assay (RIA; Kubasic *et al.*, 1984). Independent 't' test was carried out with the help of SPSS computer software.

RESULTS AND DISCUSSION

Normal calvings were recorded 86.11% (521/605) in buffaloes while the incidence of premature birth and abortion was 8.53% (52/605) and 5.28% (32/605), respectively. The incidence of retention of fetal membranes was 9.98% (52/521), 34.61% (18/52) and 46.87% (15/32) in normal calving, premature birth and abortion,

respectively. The overall incidence of retention of fetal membrane in buffaloes was 14.04% (85/605). The present finding approximates the findings of Kumar and Kumar (1995); Arthur (1979), While Salisbury and Van Denmark (1961); Mc Donald (1969) reported the incidence of retention of placenta as 5 to 15% and 10%, in normal calvings, respectively. Gautam (2000) reported 6.09% (24/394) incidence of retention of fetal membranes in buffaloes. The incidence of retention of fetal membranes in buffaloes is affected by number of factors *viz* parity, twins and premature births; herd to herd, season, nutritional status (Choudhury *et al*., 1993), stress and weaning practices (Azawi *et al*., 2008; Akar and Yeldiz, 2005).

Biochemical and progesterone profiles in normally calved buffaloes with retained fetal membranes are presented in Table 1. The mean level of glucose (mg/dl) on 0 day (start of treatment i.e. 12 h after calving), 30 and 45 days (58.66±4.41 vs 56.83±5.69; 71.16±4.52 vs 69.66±5.43 and 67.66±4.38 vs 65.0±4.66); calcium (8.7±0.27 vs 7.88±0.44; 10.35±0.80 vs 8.06±0.59 and 11.46±0.69 vs 8.06±0.30 mg/dl); Phosphorus (3.22±0.38 vs 3.09±0.27; 3.52±0.32 vs 3.20±0.30 and 3.70±0.42 vs 3.26±0.21mg/dl); magnesium (1.95±0.02 vs 1.9±0.01; 2.31±0.01 vs 1.95±0.16 and 2.46±0.01 vs 2.07±0.01 mg/dl); NEFA (0.04±0.02 vs 0.08±0.02; 0.06±0.03 vs 0.08±0.04 and 0.05±0.01 vs 0.09±0.04 mM/ml) and progesterone on day 0, 30 and 45 days (0.57±0.15 vs 0.67±0.09; 0.59±0.19 vs 0.55±0.08 and 0.46±0.07 vs 0.54±0.10 ng/dl) was recorded in treatment and control group, respectively. There was significant rise in the level of calcium ($P<0.01$), phosphorus ($P<0.05$) and magnesium ($P<0.05$) on day 30 and day 45 in treatment than control group. However, there was no significant difference observed in

Table 1. Biochemical profile and progesterone level in serum of buffaloes with retention of fetal membranes (12 hr after calving).

Parameter	0 days		30 days		45 days	
	Control (n = 6)	Treatment (n = 6)	Control (n = 6)	Treatment (n = 6)	Control (n = 6)	Treatment (n = 6)
Glucose (mg/dl)	56.83±5.69	58.66±4.41	69.66±5.43	71.16±4.52	65.0±4.66	67.66±4.38
Calcium (mg/dl)	7.88±0.44	8.7±0.27	8.06±0.59	10.35±0.80**	8.06±0.30	11.46±0.69**
Phosphorus (mg/dl)	3.09±0.27	3.22±0.38	3.20±0.30	3.52±0.32*	3.26±0.21	3.70±0.42*
Magnesium (mg/dl)	1.9±0.01	1.95±0.02	1.95±0.16	2.31±0.01*	2.07±0.01	2.46±0.01**
NEFA (mM/ml)	0.08±0.02	0.04±0.02	0.08±0.04	0.06±0.03	0.09±0.04	0.05±0.01
Progesterone (ng/dl)	0.67±0.09	0.57±0.15	0.55±0.08	0.59±0.19	0.54±0.10	0.46±0.07

In row *values differ significantly ($P<0.05$) and **differ significantly ($p<0.01$).

level of glucose, progesterone and NEFA between treatment and control group on day 0, 30 and 45. Similar to our findings increase level of calcium (Dutta and Dugwekar, 1983; Mohanty *et al*., 1994; Mandali *et al*., 2002); Phosphorus (Ban *et al*., 1996; Akar and Yildiz, 2005; Perumal *et al*., 2013) and magnesium (Ban *et al*., 1996; Akar and Yildiz, 2005; Tillard *et al*., 2008; Perumal *et al*., 2013) was reported in treatment group on day 0, 30 and 45.

The present study indicates that the lower incidence of retention of fetal membranes in buffaloes with normal calving as compare to premature birth and abortion. Significant rise in the level of calcium, phosphorus and magnesium on day 30 and day 45 was observed in treated animals.

ACKNOWLEDGEMENTS

Authors are highly thankful to Dean, College of Veterinary Science to carry out the research and providing necessary facilities.

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INTERLEUKIN 1β (IL-1 β) AND PROTEIN PROFILE DURING DIFFERENT STAGES OF GESTATION IN BUFFALO

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ABSTRACT

Eighteen apparently healthy pregnant buffaloes divided into three groups according to gestational stages, i.e. early, mid and late gestation were selected and blood samples were collected. Serum total protein, albumin, globulin and A: G ratio were estimated along with IL-1β. The concentration of IL-1β increased from early gestation to mid gestation and declined towards late gestation. Concentration of serum total protein, albumin, globulin and A: G was highest during late gestation (8.23±0.26 g/dl), mid-gestation (3.95±0.10 g/dl/), late gestation (4.68±0.21 g/dl/) and early gestation (0.84±0.12). Concentration of IL-1β, serum total proteins, albumin, globulin and A: G was statistically non-significant during all three stages of gestation.

Keywords: *Bubalus bubalis*, buffalo, gestation, interleukin 1 β, protein profiles

INTRODUCTION

For establishment of pregnancy many hormones and factors play important role. But apart those hormones there are cytokines which affect reproduction. Cytokines are category of small proteins that are important in cell signalling. Cytokines are produced by a broad range of cells, including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells. A given cytokine may be produced by more than one type of cell. (Lackie, 2010; Ibelgaufts, 2013). Cytokines are expressed in a variety of cell types of the reproductive system, although in most instances their functions are not understood. There are, however, a few instances where a role in early pregnancy has been established. (Mathialagan and Roberts, 1994).

Cytokines regulate pregnancy by both autocrine and paracrine mechanisms. The inflammatory cytokines, interleukins-1β (IL-1β), IL-6 and IL-8, help to maintain the trophoblast in early pregnancy. Serum IL-1β, IL-6 and IL-8 appear to play a major role during pregnancy (Denison *et al*., 1997; Hebisch *et al*., 2001; Laham *et al*., 1999; Stallmach *et al*., 1995), towards term and at delivery (Bahar *et al*., 2003; Makhseed *et al*., 2000; Vassiliadis *et al*., 1998), notably with increased levels in labour (Laham *et al*., 1993; Laham *et al*., 1996; Hebisch *et al*., 2001). In humans and primates, IL-1β was found to be involved in embryonic implantation and establishment of pregnancy through acting as a physiological mediator of acute phase response

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during conceptus invasion and placental formation. (Geisert *et al*., 2012). Cheng *et al*. (2016) observed higher concentration of plasma IL-1β in pregnant cows on 21st day post insemination. To study the role of IL-1β during gestation in buffaloes, level of serum IL-1β was investigated in early, mid and late stage of pregnancy along with serum total protein, albumin, globulin and A: G.

MATERIALS AND METHODS

Animals for experiment

Eighteen apparently healthy pregnant buffaloes were selected from Instructional Livestock Farm Complex, Bombay Veterinary College and some other units in Aarey milk colony, Goregaon, Mumbai - 400065. The experimental animals were divided into three groups according to the stages of gestation, each consisting of six animals. The three groups were early gestation (0 to 100 days), mid gestation (101 to 200 days) and late gestation (201 to 310 days) stages, respectively. The pregnancy diagnosis was done by per rectal examination by veterinarian on the farm. Buffaloes were maintained under uniform and standard conditions of feeding and management. The animals were housed in animal shed with asbestos cement roof, under natural daylight and temperature. The buffaloes were given maintenance ration of 15 kg of paragrass and 2.5 concentrate mixture, during morning and evening hours, daily. Paddy straw was given ad-libitum. The buffaloes were provided with clean drinking water.

Sampling

Blood samples (10 to 12 mL) were collected in vacutainers aseptically from experimental buffaloes by jugular vein puncture. Serum was separated out after centrifugation at 2000 rpm and was stored at -20°C until used for analysis.

Blood analysis

Blood samples were analysed for serum total proteins and albumin using an autoanalyzer - Prietest Touch (Robonik, India). Concentrations of serum globulin and A: G ratio were obtained from the values collected after analysis of serum total proteins and albumin. Analysis was carried out in the Deparment of Veterinary Physiology, Bombay Veterinary College, Parel, Mumbai - 400012.

Interleukin 1 β assay

Concentration of the serum Interleukin 1β was estimated by using KinesisDx Bovine Interleukin 1β (IL-1β) ELISA kit, 1179, W 29th St., Apt. 9, Los Angeles, CA 90007, USA. (www.kinesisdx.com). Each sample was run in duplicate.

Statistical analysis

Data are presented as Mean±S.E. ANOVA was used to analyze the significance of differences. Correlations were calculated and $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Serum intrleukin 1 β

The mean concentrations of serum IL-1β during early, mid and late gestation were 195.10±49.43, 233.65±51.45 and 125.90±36.18 pg/ml respectively. The serum IL-1β concentration increases from early to mid-gestation and then declines in late gestation. No work on Interleukin 1β concentration in serum during early, mid and late gestation in buffaloes is reported in available literature. The serum IL-1β in cow was elevated

around day 21 post AI (Cheng *et al*., 2016) whereas, in HF cows it was found to be decreased after parturition (Trevisi *et al*., 2015). In pigs, serum IL-1β was found to be enhanced on days 12 to 13 in pregnant sows (Franczak *et al*., 2011). In mice, it was observed that the IL-1β was low and stable throughout pregnancy and increased markedly in late pregnancy (Orsi *et al*., 2006) while in women, the serum Il-1β levels did not differed significantly between beginning and the end of gestation (Hebisch *et al*., 2004).

Obsevations from various investigations indicate that the levels of IL-1β in different animals show different trends. Contrary to the belief that IL-1β is required for embryonic implantation and establishment of pregnancy through acting as a physiological mediator of acute phase response during conceptus invasion and placental formation (Geisert *et al*., 2012), the concentration of IL-1β has increased during mid-pregnancy. This could be the trend in buffalo that can be considered as species specific (Figure 1).

Serum total proteins

The average values of serum total protein concentration in buffaloes were 7.99±0.65, 7.48±0.15, 8.23±0.26 g/dL during early, mid and late stages of gestation respectively. The concentration of serum total protein decreased gradually from early to mid-gestation and increased again during late gestation. The concentration of serum total proteins was highest in last trimester of pregnancy. This increase in the serum total protein concentration during late gestation may be due to increase in amino acids from tissues and muscles into blood and increase in feed intake (Ghadhban, 2008). This increase can also be due to increase in the levels of oestrogen with advancement of pregnancy. The secretion of glucocorticoids and thyroxin also increases sex hormone secretion which intensifies metabolic events. Increase in beta and gamma contents of serum proteins during gestation could also be the reason of increase in total proteins and this increase may be due to increased demand of proteins by growing foetus (Patel, 2014). The lower total protein concentration during early gestation than late gestation may be due to its utilization in colostrum synthesis (Pathak and Janakiraman, 1983; Singh *et al*., 1988) also, there is drain of immune fractions in the formation of colostrum (Mehta *et al*., 1989). Further, the lower concentration of total proteins during early gestation may be due to decrease in globulin concentration and increased protein breakdown required for gluconeogenesis (Quayam *et al*., 1998) (Figure 2).

Serum Albumin

The mean concentration of serum albumin in buffaloes in were 3.47±0.15, 3.95±0.10 and 3.54±0.07g/dL during early, mid and late gestation respectively. The serum albumin level increased from early to mid-gestation and decreased again from mid to late gestation. Similarly, Ghadhban (2008) reported decrease in the concentration of serum albumin in Iraqi cows in late pregnancy, which can be due to increased level of serum globulins during same period. Al-Mujalli *et al*. (2008) observed that the serum albumin concentration was low during first week before parturition in cows. In contrast, Mir *et al*. (2008) in cattle and Patel (2014) in crossbred cows reported that the concentration of serum albumin increased throughout the gestation (Figure 3).

Similarly, according to Padodara *et al*. (2012), decrease in albumin from mid to late gestation may be due to increase in nutrient requirement of growing foetus whereas Yokus

and Cakir (2006) reported no changes in albumin concentration throughout pregnancy.

Serum globulin

The average values of serum globulin concentration recorded in present study are 4.52±0.88, 4.16±0.12 and 4.68±0.21 g/dL during early, mid and late gestation respectively. The serum globulin level is highest during late gestation and lowest in mid-gestation. The relative percentage concentration of serum globulin did not show significant variation during different stages of gestation in buffaloes (Seshagiri *et al.*, 1979). Observation of this study is in contrast with the findings of Mir *et al.* (2008); Patel (2014) in crossbred cows (Figure 4).

A: G

Concentration of Serum A: G ratio in buffaloes during early, mid and late gestation were 0.84±0.12, 0.79±0.02 and 0.75±0.02 g/dL respectively. The serum A: G ratio was highest during early gestation. Similar observations were recorded by Tekade (2009) in buffaloes and Gadhave (2000) in crossbred cows. Increased A: G ratio can be due to declined serum globulin concentration during the same period (Gadhave, 2000) (Figure 5).

Thus it can be concluded that serum IL-1β concentration increased from early to mid-gestation and declined sharply in late gestation suggesting to be a trend in buffaloes. Higher concentration of serum Interleukin 1 β during mid-gestation indicates that it may play some important role during pregnancy which needs further investigation. Similarly, the concentration of IL-1β in the buffalo conceptus also needs to be investigated to find out if it has some role during implantation. The high concentration of total proteins during late pregnancy could be for optimum secretion of gonadotropin releasing factors and number of other hormones needed for culmination of pregnancy.

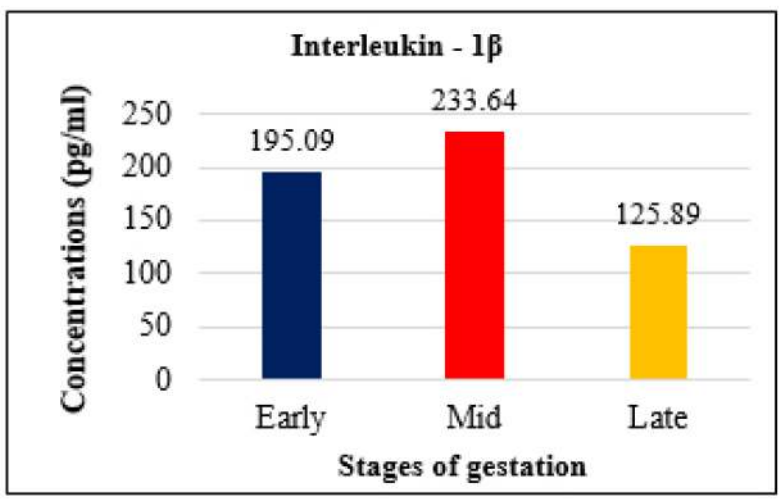

Figure 1. Serum Interleukin 1β (pg/ml) in early, mid and late gestation in buffaloes.

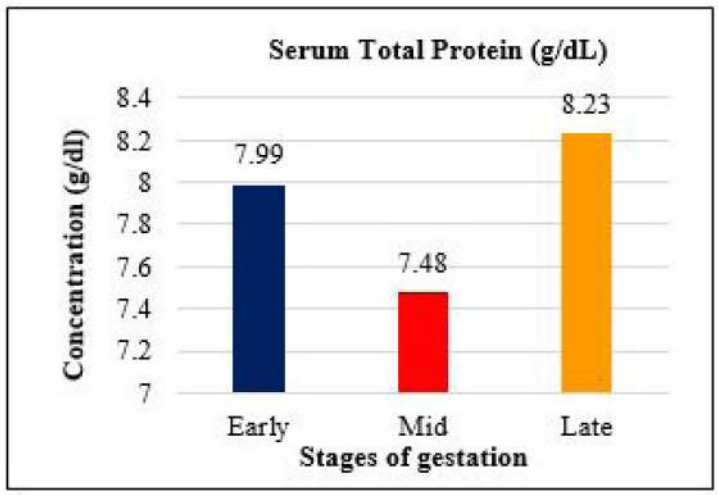

Figure 2. Serum total proteins (g/dL) in early, mid and late gestation in buffaloes.

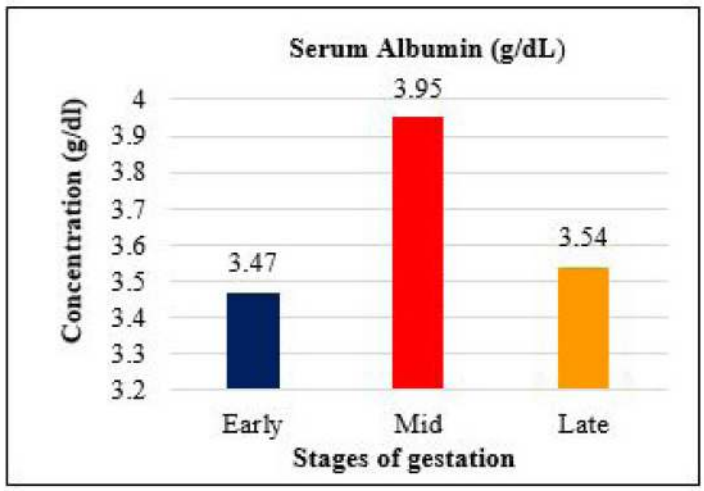

Figure 3. Serum albumin (g/dL) in early, mid and late gestation in buffaloes.

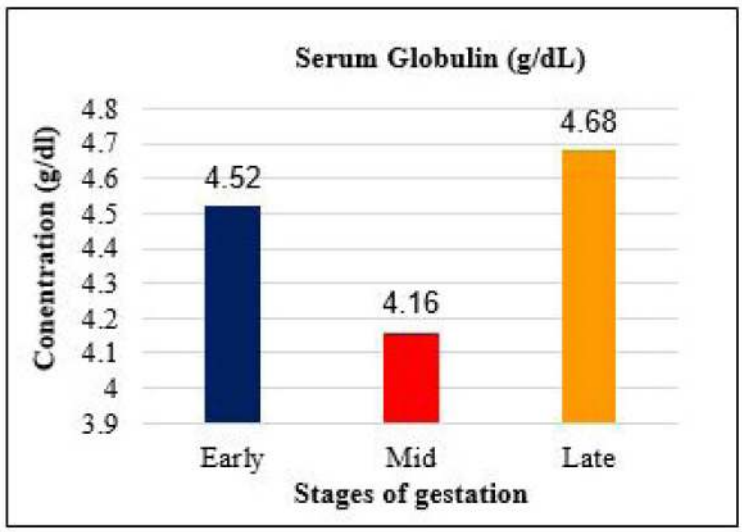

Figure 4. Serum globulin (g/dL) in early, mid and late gestation in buffaloes.

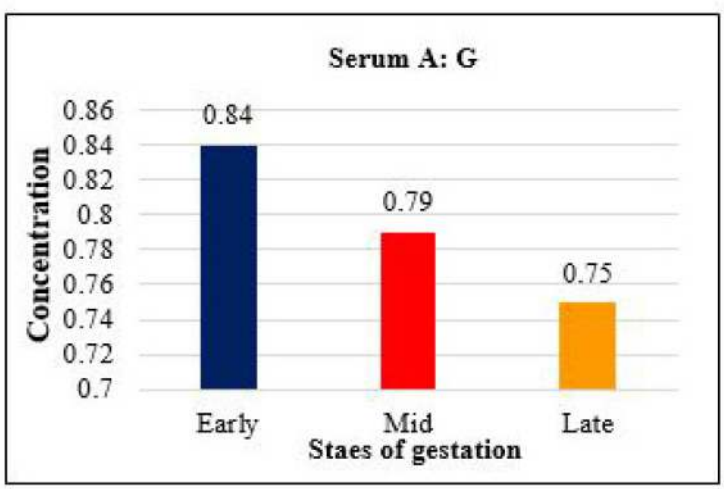

Figure 5. Serum albumin: globulin (A: G) in early, mid and late gestation in buffaloes.

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CONSEQUENCE OF PRILL FAT AND RUMEN PROTECTED CHOLINE SUPPLEMENTATION ON MILK YIELD AND ITS MAKEUP IN MURRAH BUFFALOES

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ABSTRACT

This study was conducted to analyze the consequence of prill fat and rumen protected choline (RPC) supplementation on performance of lactating Murrah buffaloes. Murrah buffaloes (n=18) were distributed into three groups (Control, T_1 and T_2) having six buffaloes in each, based on milk yield, fat per cent and stage of lactation. During the experimental period of 90 days, buffaloes in all the three groups were fed same basal diet, containing green fodder (Berseem), wheat straw and concentrate mixture to meet their requirements for maintenance and milk production. Buffaloes in Group T_1 were daily supplemented with prill fat 2.5% of total DMI whereas; buffaloes in Group T_2 were daily supplemented with 54 g rumen protected choline along with same quantity of prill fat fed as in Group T_1.

In contrast to the control group, overall milk yield (kg) of lactating Murrah buffaloes was significantly ($P<0.01$) increased by 0.99 and 1.78 kg in Group T_1 and T_2. The average 4% FCM (kg/d), ECM (kg/d) and fat yield (kg/d) was significantly ($P<0.01$) higher in Group T_2 followed by Group T_1 and Group control. There was no significant difference in milk composition of buffaloes except milk fat (%) and total solids (%) which were improved significantly ($P<0.01$) in supplemented groups. It was observed that prill fat supplementation in the ration helped to make better performance of early lactating Murrah buffaloes by enhanced milk yield and fat percent which can be further increased by supplementing the ration with rumen protected choline.

Keywords: *Bubalus bubalis*, buffaloes, prill fat, rumen protected choline, milk yield, milk fat

INTRODUCTION

Often the quantity of energy needed, in early lactating buffaloes, for maintenance of body tissues and milk productions higher than the amount of energy available from the diet (Goff and Horst, 1997), which forces mobilization of body fat reserves to satisfy energy requirement. At the same time, daily nutrient intake is not sufficient to match demands for milk production and energy balance become negative (Bell *et al.*, 1995). The period of negative energy balance often get moving

#This research paper is prepared from research findings of my Master of Veterinary Science (Animal Nutrition) thesis submitted to Nanaji Deshmukh Veterianry Science University, Jabalpur, India

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prior to calving due to depressed feed intake at the termination of gestation. The negative energy balance in early lactation impact on peak milk production and gross lactation performance apart from causing delayed post-partum ovarian activity (Garnsworthy and Webb, 1999). The degree of non-esterified fatty acids (NEFA) heightens in plasma as an effect of body fat mobilization and direct to hepatic lipidosis.

The balanced feeding during postpartum period affects the lactation performance of dairy animals. It has been found that Calcium salts of long-chain fatty acids are effective for lactating cows as ruminally inert fat supplements (Grummer, 1995). These salts improve productive performance as good source for increasing energy density of the diet. Prill fat remains inert in the rumen and resist hydrolysis and association with the bacterial cells of feed particles. Thus, total supplemented energy in diet of a lactating animal get available for the productive purposes (Singh *et al.*, 2014). Prill fat is prepared by liquefying mixture of fatty acid and spraying it under pressure into a cooled atmosphere. It is a non-hydrogenated vegetable oil and contains more than 85% palmitic acid with high melting point which by passes rumen degradation and is digested in small intestine by lipase enzyme.

Fat metabolism can be improved with the help of choline for better energy production and also helps in improving milk production. Choline, plays an important role in very low density lipoprotein synthesis and thereby contributes to fat export from the liver. It is reported that choline can be synthesized by the animals even though the dietary supply of choline in early lactating dairy animals may be inadequate, (Pires and Grummer, 2008). The choline supplemented in the protected form as it degraded rapidly in the rumen, (Elek *et al.*, 2008). The rumen protected form of choline has been developed to pass choline to the small intestine for absorption (Garg *et al.*, 2012).

Considering the efffect of prill fat as an energy source in diet of lactating animals and role of choline in fat metabolism the aim of the present experiment was to find out the consequence of prill fat and rumen protected choline supplementation on milk yield and its composition in Murrah buffaloes.

MATERIALS AND METHODS

Eighteen Murrah buffaloes (n=18) were distributed into three groups (Control, T_1 and T_2) having six buffaloes in each, based on level of milk production, fat %, and stage of lactation (2 to 3 weeks post-partum). During the experimental period of 90 days, buffaloes in all the three groups were fed a same total mixed basal diet, comprising green fodder (Berseem), wheat straw and concentrate mixture to match the requirements for maintenance and milk production (Kearl, 1982). Buffaloes in Group T_1 were enriched with prill fat 2.5% of total DMI per animal per day while, buffaloes in Group T_2 were enriched with 54 g rumen protected choline along with equal quantity of prill fat fed as in Group T_1 in their basal ration.

The buffaloes were dewormed before the beginning of the experiment and standard basic practices were followed in the shed. All the experimental buffaloes were kept in a well-ventilated shed having cemented floor with individual feeding and watering arrangement. Weighed quantity of feed (concentrate and roughage) was offered to the buffaloes in morning and evening. *Ad-libitum* clean and fresh water was provided to the buffaloes during the study. The buffaloes were let loose for about 1 to 2 h daily in

the surrounded paddock for exercise.

Milk samples were collected in 30 day interval from all the buffaloes in different treatments and were analyzed for fat (%), solids-not-fat (%), milk protein (%), density (g/cm^3) and lactose (%) content by using Lacto scan. Average daily milk yield of individual lactating buffaloes were documented and average milk production of individual animal was computed on fortnightly basis. Four percent fat corrected milk (4% FCM) was calculated by the following formula given by Gains (1928).

$$4\%\ FCM\ (kg) = [(0.4 \times total\ milk) + (15 \times total\ fat)]$$

Energy corrected milk (ECM) was calculated as per the formula given by Tyrrell and Reid (1965).

$$ECM\ (kg) = [(7.2 \times kg\ protein) + (12.95 \times kg\ fat) + (0.327 \times kg\ milk)]$$

The statistical analysis of the data was done using SPSS computer package (SPSS version 20.0, SPSS Inc., Chicago, USA) adopting standard statistical procedures (Snedecor and Cochran, 2004).

RESULTS

The data regarding fortnightly and overall milk yield in different groups of Murrah buffaloes is presented in Table 1. The fortnightly average milk yield (kg/d) in T_2 group was documented to be significantly ($P<0.01$) higher than T_1 and control group. The overall average milk yield (kg/d) of Group T_1 and T_2 was significantly ($P<0.01$) greater by 0.99 and 1.78 kg than that of Control group.

The mean monthly 4% FCM, ECM and fat yield in different groups of Murrah buffaloes is shown in Table 2. Mean 4% FCM (kg/d) was found to be significantly ($P<0.01$) higher in T_2 Group than T_1 and Control group. Within same treatment group highest mean 4% FCM was documented at 90th day followed by 60th and 30th day. Analysis of variance revealed significant ($P<0.01$) effect of period and treatment on ECM (kg/d) of buffaloes. The significantly ($P<0.01$) highest mean ECM (kg/d) was recorded in Group T_2 succeeded by Group T_1 and Control group. Mean fat yield (kg/d) was also significantly ($P<0.01$) highest in Group T_2 succeeded by Group T_1 and Control group. Mean fat yield (kg/d) in Control group at 60th and 90th day was undoubtedly ($P<0.01$) higher than that at 30th day. The milk composition of Murrah buffaloes in different groups is presented in Table 3. There was no significant change in milk composition of buffaloes in different groups except mean milk fat (%) and mean total solids (%) which were significantly ($P<0.01$) highest in Group T_2 followed by Group T_1 and Control group.

DISCUSSION

In this experiment treatment means of the average milk yield (kg/d) and fat corrected milk yield (kg/d) indicated that supplementation of basal diet with either prill fat alone or along with rumen protected choline in the diets of buffaloes undoubtedly ($P<0.01$) enhanced their milk yield and 4% fat corrected milk yield as compared to control group. Overall average milk yield (kg/d) was increased by 12.10 and 21.76% in prill fat alone and prill fat + rumen protected choline supplemented groups than the control group. The higher milk production in supplemented groups

was attributed to more TDN intake in conjunction with prill fat which increased the energy density of ration and reduced deleterious effect of negative energy balance as evident from lower blood NEFA levels.

The significant increase in milk production in prill fat supplemental group is well corroborated with findings of many researchers reporting an increased milk yield between 0.40 to 3.11 kg/d in experimental cows (Fahey *et al*., 2002; McNamara *et al*., 2003; Mishra *et al*., 2004; Salem and Bouraoui, 2008; Shelke *et al*., 2011). Similarly, Kumar and Thakur (2007); Garg *et al*. (2008); Barley and Baghel (2009); Sirohi *et al*. (2010); Rajesh *et al*. (2014) also reported significant improvement in milk yield in different species of ruminants fed bypass fat. However, no improvement in milk yield in bypass fat supplemented cows have also been reported by some researchers (Klusmeyer *et al*., 1991; Sklan *et al*., 1992; Elliott *et al*., 1996), which could be due to different degree of inertness and amount of dietary fat offered.

Results regarding significantly ($P<0.01$) higher milk yield in rumen protected choline supplemented group are in agreement with Elek *et al*. (2008); Lima *et al*. (2007), who observed significant improvement in milk yield of dairy cows after supplementing rumen protected choline (RPC) to basal diet which can be correlated to the role of RPC in elevating the export of triglycerides from the liver and sparing methionine as a methyl donor (Pinotti *et al*., 2002). In present study, improvement in milk production in response to RPC supplementation may be attributed to its methyl donor sparing effect, thus enhanced intestinal supply of choline might have further improved milk production in Murrah buffaloes.

In present study, milk fat (%) and total solid (%) was significantly higher ($P<0.01$) in supplemented groups than the control. Milk lactose (%), SNF (%), density (g/cm^3) and protein (%) content were not influenced by feeding prill fat either alone or along with rumen protected choline. Significant ($P<0.01$) increase in milk fat and total solid (TS) of prill fat supplemented group is supported by Mishra *et al*. (2004); Garg *et al*. (2008); Sirohi *et al*. (2010). Lima *et al*. (2007); Garg *et al*. (2012) supports our results regarding significant higher milk fat (%) in RPC supplemented group. Non significant change in milk protein (%) of supplemented groups is supported by Sirohi *et al*. (2010).

The increase in milk fat content in prill fat or prill fat + protected choline supplemented group may be due to availability of more fatty acid (SFA and USFA) to the mammary gland and their incorporation into milk fat (Gulati *et al*., 2003). Further, as choline is used for phospholipid synthesis its supplementation facilitates lipid absorption and transport, thereby favouring milk fat synthesis.

The study has made it amply clear that high producing lactating buffaloes do need the bypass fat supplement in their diet, in order to meet their energy requirements fully to express their milk production potential, which was demonstrated by the significant increase in milk yield, FCM yield, fat percentage and TS percentage in milk as a result of feeding prill fat alone or along with rumen protected choline.

CONCLUSION

Present investigation reveled that supplementing prill fat in the ration of Murrah buffaloes helped in improving milk yield and fat per cent, which can be further increase by

Table 1. Fortnightly and overall average milk yield (kg/d) in different groups of Murrah buffaloes.

Fortnights	Control	T_1	T_2	Period Mean±SE
Initial	$06.31^{D}\pm0.24$	$06.61^{C}\pm0.43$	$06.79^{C}\pm0.35$	$06.57^{E}\pm0.19$
1	$07.08^{Cc}\pm0.26$	$08.13^{Bb}\pm0.49$	$09.21^{Ba}\pm0.16$	$08.14^{D}\pm0.28$
2	$07.79^{Bc}\pm0.24$	$08.76^{Bb}\pm0.20$	$09.83^{Ba}\pm0.17$	$08.79^{C}\pm0.23$
3	$08.66^{Ac}\pm0.44$	$09.71^{Ab}\pm0.24$	$10.62^{Aa}\pm0.09$	$09.67^{B}\pm0.25$
4	$09.29^{Ac}\pm0.30$	$10.45^{Ab}\pm0.14$	$11.21^{Aa}\pm0.11$	$10.32^{A}\pm0.22$
5	$09.14^{Ac}\pm0.18$	$10.33^{Ab}\pm0.30$	$11.11^{Aa}\pm0.08$	$10.19^{A}\pm0.22$
6	$09.01^{Ac}\pm0.18$	$10.18^{Ab}\pm0.26$	$10.95^{Aa}\pm0.15$	$10.05^{AB}\pm0.22$
Overall	$08.18^{c}\pm0.21$	$09.17^{b}\pm0.27$	$09.96^{a}\pm0.05$	

^{a,b,c} Means with different superscripts in the same row are significantly different ($P<0.01$).

^{A,B,C,D,E} Means with different superscripts in the same column are significantly different ($P<0.01$).

Table 2. Monthly average 4% FCM, ECM and fat yield in different groups of Murrah buffaloes.

Attributes	Periods			Treatment Mean± SE
	30th Day	60th Day	90th Day	
4% FCM (kg/d)				
Control	$09.87^{Cb}\pm0.31$	$12.29^{Ca}\pm0.43$	$12.80^{Ca}\pm0.26$	$11.65^{C}\pm0.36$
T_1	$11.47^{Bc}\pm0.46$	$14.43^{Bb}\pm0.20$	$15.38^{Ba}\pm0.38$	$13.76^{B}\pm0.45$
T_2	$13.23^{Ac}\pm0.22$	$16.18^{Ab}\pm0.12$	$17.25^{Aa}\pm0.26$	$15.55^{A}\pm0.43$
ECM (kg/d)				
Control	$10.14^{Cb}\pm0.29$	$12.59^{Ca}\pm0.41$	$13.13^{Ca}\pm0.27$	$11.95^{C}\pm0.36$
T_1	$11.75^{Bb}\pm0.44$	$14.89^{Ba}\pm0.23$	$15.64^{Ba}\pm0.40$	$14.09^{B}\pm0.45$
T_2	$13.55^{Ab}\pm0.24$	$16.55^{Aa}\pm0.18$	$17.41^{Aa}\pm0.24$	$15.84^{A}\pm0.42$
Fat Yield (kg/d)				
Control	$0.46^{Cb}\pm0.02$	$0.58^{Ca}\pm0.02$	$0.61^{Ca}\pm0.01$	$0.55^{C}\pm0.02$
T_1	$0.54^{Bc}\pm0.02$	$0.69^{Bb}\pm0.01$	$0.75^{Ba}\pm0.02$	$0.66^{B}\pm0.02$
T_2	$0.63^{Ac}\pm0.01$	$0.79^{Ab}\pm0.01$	$0.86^{Aa}\pm0.01$	$0.76^{A}\pm0.02$

^{a,b,c} Means with different superscripts in the same row are significantly different ($P<0.01$).

^{A,B,C,} Means with different superscripts in the same column are significantly different ($P<0.01$).

Table 3. Milk composition in different groups of Murrah buffaloes.

Attributes	Periods			Treatment Mean± SE
	30th Day	60th Day	90th Day	
Fat (%)				
Control	$6.19^{Bc}\pm0.03$	$6.47^{Cb}\pm0.05$	$6.74^{Ca}\pm0.05$	$6.46^{C}\pm0.06$
T_1	$6.40^{ABc}\pm0.17$	$6.88^{Bb}\pm0.09$	$7.34^{Ba}\pm0.12$	$6.87^{B}\pm0.12$
T_2	$6.60^{Ac}\pm0.09$	$7.21^{Ab}\pm0.09$	$7.76^{Aa}\pm0.09$	$7.19^{A}\pm0.12$
SNF (%)				
Control	9.23±0.07	9.68±0.15	9.68±0.18	9.53±0.09
T_1	9.56±0.18	10.03±0.19	9.63±0.23	9.74±0.12
T_2	9.97±0.14	9.80±0.20	9.52±0.11	9.77±0.10
Total Solid (%)				
Control	$15.42^{Cb}\pm0.08$	$16.15^{Ca}\pm0.16$	$16.42^{Ca}\pm0.19$	$15.99^{C}\pm0.13$
T_1	$15.96^{Bb}\pm0.29$	$16.91^{Ba}\pm0.20$	$16.97^{Ba}\pm0.15$	$16.61^{B}\pm0.16$
T_2	$16.57^{Ab}\pm0.20$	$17.02^{Aa}\pm0.24$	$17.28^{Aa}\pm0.12$	$16.96^{A}\pm0.13$
Protein (%)				
Control	3.30±0.12	3.34±0.14	3.43±0.11	3.36±0.07
T_1	3.31±0.14	3.60±0.10	3.46±0.09	3.46±0.07
T_2	3.36±0.06	3.54±0.09	3.42±0.04	3.44±0.04
Lactose (%)				
Control	4.77±0.17	5.01±0.20	5.05±0.17	4.94±0.10
T_1	4.97±0.21	5.39±0.15	5.15±0.16	5.17±0.10
T_2	4.89±0.34	5.18±0.06	5.20±0.06	5.09±0.11
Density (g/cm^3)				
Control	1.033±0.001	1.033±0.001	1.032±0.001	1.033±0.001
T_1	1.033±0.001	1.032±0.001	1.031±0.001	1.032±0.001
T_2	1.033±0.001	1.031±0.001	1.031±0.002	1.032±0.001

^{a,b,c} Means with different superscripts in the same row are significantly different ($P<0.01$)

^{A,B,C,} Means with different superscripts in the same column are significantly different ($P<0.01$)

supplementing the ration with rumen protected choline chloride.

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ASSESSMENT OF CALF HEALTH CARE AND MANAGEMENT PRACTICES UNDER SMALLHOLDER PRODUCTION SYSTEM IN PUNJAB, PAKISTAN

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ABSTRACT

This study was conducted to evaluate health care and management practices for calves less than or equal to 12 months of age in smallholder production system. Data was collected using household survey technique in Lodhran district, Punjab, Pakistan. 14 villages were selected using stratified proportionate random sampling method and 10 calf keeping households from each village were interviewed using a semi-structured questionnaire. Calves were not allowed to free range therefore all farmers practiced stall feeding. Housing facilities were of poor quality in half of farms (50.7%). Farmers were not using modern technologies of milk replacer, urea treated wheat straw and urea molasses blocks. All farmers offered colostrum however timings of offering differed. 75.7% farmers performed navel cord cutting and disinfection. All farmers offered treatment but majority (87.1%) practiced self-medication first. Vaccination rate was good at 94.3%. Almost all farmers performed drenching and dipping on their calves but there were vast differences in when and on what conditions they will be performed. Calf mortality rate for the last 12 months was 18.78%. Weaning age was high from modern calf rearing perspective. These findings suggest that there have been marked improvements in some parameters but farmers are still following traditional methods and practices of calf rearing due to severe lack of training related to calf rearing. There is a need for improvement in various aspects related to calf rearing including feed, housing, weaning and training.

Keywords: *Bubalus bubalis*, buffalo, calf health care practices, calf management practices, livestock, smallholder production system, Pakistan

INTRODUCTION

Cattles and buffaloes are the most important of all livestock species reared in Pakistan from dairy farming perspective. The estimated livestock population of cattle and buffalo in Pakistan is 46.1 million and 38.8 million respectively. Pakistan is 4th largest milk producer country in the world (FAO, 2015). Pakistan's milk production from cows and buffaloes for the year 2017 to 2018 was 20,903 and 35,136 thousand tonnes respectively whereas beef production stood at 2,155 thousand tonnes (GOP, 2018).

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Calves are the most important by-product of dairy farming system from sustainability perspective. Future herd depend upon today's calves, therefore, proper care and management of young calves is needed to sustain and expand cattle (including buffaloes) production. Poor upbringing of young stock will lead to the degradation of cattle herd production performance and quality. Therefore, in order to improve the general situation of cattle farming priority has to be given to calf rearing.

Proper measures and specific interventions to improve and expand calf rearing cannot be planned and implemented without relevant, reliable and up to date data on aspects related to calf production and rearing. This research is aimed at describing current situation and analyzing calf and health care and management practices currently followed. This data and assessment will help policy makers in developing holistic strategy aimed at improving and expanding calf production in rural smallholder farms. An improvement in rural calf production performance will bring improvement in livelihood of majority of poor rural population and will help in meeting ever increasing demand for meat and dairy products. This will also help in achieving true cattle production potential.

MATERIALS AND METHODS

Lodhran is a district of Punjab province, Pakistan covering an area of 2778 square km. It is situated between 29°41'12.67"N latitude and 71°40'2.41"E longitude. It lies at an altitude of 116 meters from sea level. The district is well noted in Pakistan for its fertile land, irrigation network, crop production and livestock. Lodhran has a total population of 1,700,620 out of which 84.3% reside in rural areas (PBS, 2018). District Lodhran is composed of three tehsils i.e. Tehsil Lodhran, Tehsil Kahror Pacca and Tehsil Dunyapur. These tehsils are further divided into 73 union councils. Tehsil Lodhran, Tehsil Kahror Pacca and Tehsil Dunyapur are composed of 28, 23 and 22 union councils respectively. We employed stratified proportionate random sampling and selected 7 union councils out of a total of 73. Three union councils were selected randomly from Tehsil Lodhran and two union councils each from Tehsil Kahror Pacca and Tehsil Dunyapur respectively. From each union council 2 villages were selected and from each village 10 farmers were selected randomly based on the criteria of possession of at least 1 calf less than or equal to 12 months of age. Thus a total of 140 farmers were selected from 14 villages. 60 farmers in total were selected from Tehsil Lodhran and 40 each from Tehsil Kahror Pacca and Tehsil Dunyapur. The sample size was determined by the available financial, human and material means. For the purpose of our study we define smallholder production system as the one having a maximum of 30 large ruminants including, cattles, buffaloes and their calves. A semi-structured questionnaire was developed and pretested in two villages in order to further refine it. Data was collected through individual face to face interviews by the principal author himself from January 2018 to April 2018. Verbal consent was obtained prior to commencing the interview. Questionnaire was prepared in English but administered in Urdu, Saraiki and Punjabi languages. The author is fluent in all four languages. Data entry and statistical analysis were performed using IBM's Statistical Package for Social Sciences (SPSS) Version 23.

RESULTS AND DISCUSSION

Socioeconomic characteristics

Farmer's socioeconomic characteristics are presented in Table 1. There is severe lack of formal training related to calf rearing with only 2.9% farmers reported getting formal training. Due to lack of training, farmers are using traditional calf rearing methods and practices resulting in lower productivity. Regarding education, 32.1% of farmers were found illiterate. Low education level of farmers involved in calf rearing is hindering the adoption of modern methods and practices of calf rearing. Our findings related to farmer's education levels are better than those reported by Mustafa *et al.* (2010) but worse than the findings reported by Ashraf *et al.* (2013).

Calf housing management

None of the farmers provided separate calf housing. This finding is in line with Ahmad *et al.* (2009); Mustafa *et al.* (2010). Normally, calves are tied in the corner of adult animal housing. Permanent pens were provided by 49.3% of the farmers while rest provided semi-permanent stall shed or pens (Table 2). Regarding permanent pens proper ventilation was not cared for in at least half of them. There were a wide variety of combinations of different varieties of shelter sidewalls and shelter roof construction in semi-permanent stall shed or pens. Most primitive of them was shelter construction with no sidewalls in 22.9% farms. Shelter construction with no sidewalls expose animals to cold winter, rain and high winds and may cause pneumonia. Semi-permanent shelter roof type in different combinations (51.4%) is also not suitable for different extreme weather conditions. Tiwari *et al.* (2007) reported similar findings in his study. One plausible explanation for poor housing structures in at least 40% farms is farmer's poor economic condition. Poor housing management of livestock seriously affect their health and productive performance, especially in calves which are more prone to diseases as their immunity level

Table 1. Farmer related variables across the three tehsils of district Lodhran.

Variables	Tehsil Lodhran	Tehsil Kahror Pacca	Tehsil Dunyapur	Overall District Lodhran	P-values
Age of famers (mean, SEM)	39.68±1.867	42.55±2.666	39.75±2.048	40.52±1.246	0.640
Size of household (mean, SEM)	7.92±0.417	7.95±0.741	7.75±0.549	7.88±0.316	0.785
Education level (%)					
No formal education	33.3	40	22.5	32.1	0.407
Elementary	15	27.5	17.5	19.3	
Primary	21.7	10	22.5	18.6	
Secondary	15	15	20	16.4	
Post–secondary	15	7.5	17.5	13.6	
Formal training related to calf rearing (%)	3.3	2.5	2.5	2.9	0.958

Table 2. Housing system of calves.

Variables	Tehsil Lodhran (%)	Tehsil Kahror Pacca (%)	Tehsil Dunyapur (%)	Overall District Lodhran (%)	P-values
Separate calf house					
No	100	100	100	100	N/A
Calves kept in					
Open	0	0	0	0	0.284
Shelter	0	0	2.5	0.7	
Both	100	100	97.5	99.3	
Shelter provided during					
Summers only	0	0	0	0	0.284
Winters only	100	100	97.5	99.3	
Throughout the year	0	0	2.5	0.7	
Shelter provided					
During daytime only	0	0	0	0	0.284
During night only	100	100	97.5	99.3	
All day	0	0	2.5	0.7	
Shelter form					
Permanent pens	58.3	37.5	47.5	49.3	0.120
Semi-permanent stall sheds or pens	41.7	62.5	52.5	50.7	
Shelter floor made up of					
Prepared land	100	100	100	100	N/A
Shelter roof form					
Permanent arrangement	58.3	37.5	45	48.6	0.108
Semi-permanent arrangement	41.7	62.5	55	51.4	
Shelter sidewalls					
Open on all sides	21.7	30	17.5	22.9	0.210
Enclosed on some sides	25	15	37.5	25.7	
Fully enclosed	53.3	55	45	51.4	
Shelter sidewalls made up of					
Nothing (No sidewalls)	21.7	30	17.5	22.9	0.705
Mixture of mud and wheat straw	8.3	12.5	7.5	9.3	
Unbaked bricks	10	12.5	10	10.7	
Baked red bricks	60	45	65	57.1	
Daytime tethering of calves under tree	100	100	97.5	99.3	0.284
Hygiene of calf staying area					
Clean	86.7	87.5	77.5	84.3	0.375
Dirty	13.3	12.5	22.5	15.7	

is low (Tiwari *et al.*, 2007). According to Radostits *et al.* (1994), calf mortality was associated with the type of housing, feeding, management practices, weather conditions, external and internal parasitic infestation and bacterial infection especially those causing septicemia and enteritis. Farmers were cleaning animal housing area at least once a day but without the use of any disinfectant. More than 84% of the farms were found cleaned in our study. Similar cleaning practices were reported by Tiwari *et al.* (2007); Mustafa *et al.* (2010).

Calf feeding management

Land is not available for grazing purpose in district Lodhran because of its scarcity and high cost therefore all farmers practiced stall feeding. Our finding differs from Arif *et al.* (2013) who reported 43.3% farmers grazing their animals in summers and none in winters. 43.6% farmers were offering concentrate feeds to their calves (Table 3). Khan *et al.* (2007) in his study reported that farmers offered concentrate feeds to their calves only after weaning. In our study area, concentrate offering to calves was not dependent on weaning. This is beneficial for calf's health since concentrate feeds are energy rich and good for animal fattening purpose. Mustafa *et al.* (2010) observed that most of the poor farmers were not offering green fodder because of its unavailability and high cost. In contrast, we observed that green fodder was abundantly available at a reasonable cost in district Lodhran except during between seasons time periods and harsh weather conditions. No improvement was seen in milk replacer usage as compared to previous studies (Arif *et al.*, 2013; Saghir *et al.*, 2014). Only 9.3% farmers were using milk replacer in district Lodhran. Some farmers thought that milk replacer will be an extra expenditure ignoring savings in milk. Others, on the basis of previous experience of using milk replacer believe that calves don't like it. Not a single farmer was found using urea treated wheat straw and urea molasses blocks. Most of the farmers were even not aware about these two technologies. Farmers were aware of silage making but did not find any need for it in the presence of wheat straw. Our findings are completely in line with Bilal *et al.* (2008).

Neonatal care

All farmers fed colostrum to their calves. However, timing of colostrum feeding varies. 58.6% farmers fed colostrum to their calves within 2 to 3 h of their birth. 21.4% farmers fed within 4 to 6 h while the rest 20% fed colostrum to their calves only after the release of placenta by the animal (Table 4). It is very important to feed colostrum within 2 to 3 h of birth since it is a well-established fact that delay in feeding of colostrum leads to lowered effectiveness of the colostrum in terms of providing immunity to calves (Sharma and Mishra, 1987). Previous studies have reported farmer's misplaced perceptions about colostrum feeding. These include colostrum feeding immediately after birth is injurious and will cause worm infestation, obstruction of gastro-intestinal tract, diarrhea and will result in animal not releasing placenta (Kumar, 2002; Khan *et al.*, 2007; Bilal *et al.*, 2008; Ahmad *et al.*, 2009). As per our observation, these misperceptions have changed to some extent in our study area, therefore, our findings related to colostrum feeding differ from those reported by Bilal *et al.* (2008); Ahmad *et al.* (2009); Mustafa *et al.* (2010); Saghir *et al.* (2014). Both government and private veterinarians and para-veterinary staff have played their role in this regard. However, more efforts are needed in this regard since there is still a sizable proportion of farmers i.e. 41.4% who are either delaying colostrum feeding or are

Table 3. Variables related to calf feeding.

Variables	Tehsil Lodhran (%)	Tehsil Kahror Pacca (%)	Tehsil Dunyapur (%)	Overall District Lodhran (%)	P-values
Stall feed	100	100	100	100	N/A
Use of milk replacer	10	7.5	10	9.3	0.899
Offer concentrate feeds	53.3	35	37.5	43.6	0.127
Urea treated wheat straw usage	0	0	0	0	N/A
Urea molasses block usage	0	0	0	0	N/A
Silage making and usage	1.7	0	0	0.7	0.511

Table 4. Calf neonatal care practices.

Variables	Tehsil Lodhran (%)	Tehsil Kahror Pacca (%)	Tehsil Dunyapur (%)	Overall District Lodhran (%)	P-values
Colostrum feeding					
Colostrum feeding within 2-3 hours of birth	55	57.5	65	58.6	0.602
Colostrum feeding within 4-6 hours of birth	20	25	20	21.4	0.809
Colostrum feeding after release of placenta	25	17.5	15	20	0.423
Navel cord cutting and disinfection	73.3	75	80	75.7	0.742
Bedding provided	65	52.5	70	62.9	0.243

waiting for animals to release placenta which results in lower immunity levels for their calves.

It is imperative to take proper care of navel cord after the birth of calf since it is a channel through which infectious agents can enter into the blood or underlying tissues which can lead to certain serious diseases in newborn calf (Tiwari *et al.*, 2007). Previous studies have reported abysmal state of affairs regarding navel cord cutting and disinfection (Tiwari *et al.*, 2007; Bilal *et al.*, 2008; Ahmad *et al.*, 2009; Arif *et al.*, 2013; Saghir *et al.*, 2014). Our findings show marked improvement in this regard with more than 75% farmers practicing navel cord cutting and disinfection. However, we noted that farmers were diluting the disinfectant to a great extent. Again, there has been significant improvement in awareness among the farmers about the role of navel cord care in preventing serious diseases in newborn calves. There still is room for improvement in this regard since 25% farmers who are not practicing navel cord care are exposing their newborn calves to diseases. Contrary to Mustafa *et al.* (2010) who reported negligible proportion of farmers providing proper bedding, approximately 63% farmers were providing some form of bedding for the new born calves. The bedding material depended upon whatever was available and affordable at the time of calf birth.

Calf health care practices

All farmers offered treatment to their diseased calves. However, 87.1% farmers reported trying self-medication first since they believe that calling veterinarian every time a calf is sick is completely un-economical (Table 5). This finding is in line with Mustafa *et al.* (2010) who reported that 90% of small farmers were practicing self-medication. Farmers will skip self-medication only if the situation is critical. If self-medication has failed or the situation is critical almost all farmers would call veterinarian who in more than 50% cases would turn out to be a private para-veterinary staff and not a proper veterinarian. This is in sharp contrast to Ahmad *et al.* (2009) who reported that 60.4% farmers never called a veterinarian. There was statistically significant difference at $P<0.05$ in the willingness to eat diseased calf among the three tehsils.

There is heightened awareness among farmers about the importance of vaccination in preventing diseases due to previous losses, awareness generated by veterinary personnel and poor economic condition generally where the loss of even a single calf can put a dent in their economic condition. Due to interplay of these factors, we recorded exceptionally good vaccination rate of 94.3% in comparison to previous studies of Khan *et al.* (2007); Bilal *et al.* (2008); Mustafa *et al.* (2010). Our findings are more in line with Arif *et al.* (2013).

Almost all farmers i.e. 98.6% were performing drenching and dipping on their calves. A major reason of calf mortality is the parasitic load in the calves due to which their health deteriorates and the calf often dies (Sharma and Mishra, 1987). Widespread adoption of drenching and dipping practice in district Lodhran demonstrate farmer's awareness and seriousness about the importance of deworming practices in controlling endo and ecto-parasites to keep their calves healthy. However, more awareness need to be generated about the importance and benefits of scheduled and routine deworming practices since at least half of the farmers were performing deworming only when needed depending upon visual cues. As per our observation, the lesser the number of calves a farmer had, the more serious he was about adopting practices to keep his calves healthy and to avoid

losses due to diseases.

Due to improved adoption rates of practices like vaccination, deworming, colostrum feeding and navel cord care we recorded much lower calf mortality rate (18.78%) than those reported by Tiwari *et al.* (2007); Bilal *et al.* (2008); Ahmad *et al.* (2009). Our finding regarding calf mortality rate is completely in line with Khan *et al.* (2007) who reported calf mortality rate of 17.98%.

Weaning and selling age

In modern dairy farming early weaning of calves is necessary in order to save milk for marketing purpose. However, we noted fairly high weaning age in our study where farmers were not weaning their calves at age less than 6 months. Similar findings have been reported previously (Khan *et al.*, 2007; Arif *et al.*, 2014). This situation is unlikely to change unless farmers are convinced for the use of milk replacer which would enhance farmer's profitability. Mustafa *et al.* (2010) noted that most farmers prefer to sell their calves at very young age. Farmers considered male calves as an economic burden and a loss in milk production. Therefore, 90% farmers in his study sold male calves before the age of 6 months, whereas female

Table 5. Variables related to calf health care.

Variables	Tehsil Lodhran (%)	Tehsil Kahror Pacca (%)	Tehsil Dunyapur (%)	Overall District Lodhran (%)	P-values
Treatment offered	100	100	100	100	N/A
Self-medication first	86.7	90	85	87.1	0.792
Consult veterinarian	100	97.5	100	99.3	0.284
Type/types of medicines offered					
Veterinary medicines	100	97.5	100	99.3	0.284
Traditional, desi or ethno-veterinary medicines	96.7	85	90	91.4	0.116
Human medicines	0	0	0	0	N/A
Eat diseased calf if edible	$93.3^{a,b}$	100^{b}	85^{a}	92.9	0.033
Vaccination	96.7	92.5	92.5	94.3	0.576
Drenching performed					
Routinely	48.3	55	45	49.3	0.738
Only when needed	50	42.5	55	49.3	
Never	1.7	2.5	0	1.4	
Dipping performed					
Routinely	38.3	25	30	32.2	0.558
Only when needed	60	72.5	70	66.4	
Never	1.7	2.5	0	1.4	
Calf mortality rate of last 12 months	20.261	16.494	18.75	18.784	N/A

calves were sold with buffaloes. Contrary to his findings, in our study overwhelming majority of farmers i.e. 90% indicated their willingness to sell calves whether they are male or female between 24 to 36 months of age provided there are no pressing needs. Farmers were not willing to sell calves at a very young age because of unfavorable prices. Secondly, calves aged between 2 to 3 years normally are getting a good price at the occasion of Eid-ul-Adha. Therefore, there is a tendency among farmers to keep calves till the age of 24 months. Due to recent trend of getting good prices at Eid-ul-Adha calf rearing is considered profitable by the farmers and instead of being considered as an economic burden calves have become valuable assets in farmer's eyes.

CONCLUSION

Our findings shows that there have been marked improvements in some parameters like vaccination, colostrum feeding, navel cord care and deworming practices culminating in a lower calf mortality rate. However farmers are still following traditional methods and practices in many aspects of calf rearing especially in feeding, housing and weaning due to severe lack of training. Calf productivity and farmers profitability will not improve without the adoption of modern and scientific methods and practices of calf rearing. Smallholder calf rearing is labor intensive and can provide employment opportunities to the unemployed rural youth. The need of the hour are proper policy interventions and appropriately designed training programs to promote calf rearing on modern and scientific methods keeping in mind the local conditions. This if done properly, can become an engine of growth in rural areas thus providing employment opportunities in rural areas and checking population shift from rural to urban areas. Furthermore, this will help in meeting ever increasing demand of milk and meat products and may also contribute to foreign exchange earnings through exports.

ACKNOWLEDGEMENT

The authors would like to thank all participating calf rearing farmers in district Lodhran for their cooperation, time and hospitality during the study.

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LACTATION CURVES OF MILK, FAT AND PROTEIN IN EGYPTIAN BUFFALO USING TEST-DAY MODEL

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ABSTRACT

Data on 4971 test-day milk yield traits [milk (TDMY), fat (TDFY) and protein (TDPY)] for 691 Egyptian buffalo cows, daughters of 120 sires and 532 dams were used for fitting the lactation curve parameters of milk, fat and protein yields using multiple-trait animal model. The random effects included in the model were direct additive genetic, permanent environment and error, while the fixed effects were herd test-day, year and season of calving and parity as well as days in milk as a covariable.

The means for TDMY, TDFY, TDPY and lactation curve parameters [initial (a), ascending slope (b), descending slope (c), persistency (P), and maximum milk production during lactation (Ymax) and the peak test-day (PY)] were estimated. Heritabilities of TDMY, a, b, c, P, PY and Ymax were 0.22, 0.37, 0.38, 0.39, 0.37, 0.37 and 0.38, respectively. The corresponding heritabilities for TDFY and fat curve parameters were 0.21, 0.41, 0.40, 0.39, 0.38, 0.36 and 0.42, while the estimates for TDPY and protein curve parameters were 0.22, 0.38, 0.40, 0.40, 0.38, 0.40 and 0.43, respectively. Genetic correlations among TDMY and curve parameters of a, b, c, P, PY and Ymax were 0.31, -0.23, -0.34, 0.52, 0.48 and 0.87, respectively. Genetic and phenotypic correlations between milk yield traits were high (mostly of 0.83 and 0.99), like the correlations between curve parameter in milk, fat and protein (from 0.71 to 0.96). In practice, genetic selection for lactation curve parameters (a, P and Ymax) in the Egyptian buffalo would improve total milk yield traits.

Keywords: *Bubalus bubalis*, buffaloed, Egyptian buffalo, lactation curve parameters, genetic parameters

INTRODUCTION

The total number of buffalo in Egypt is estimated to be 3.9 million. It is a very well adapted animal to the small-holder conditions and is raised under the extensive production system. Therefore, it plays as important role in Egyptian agriculture. It is the main dairy animal in Egypt; its contribution to the country's milk production is nearly 45.5% (FAOSTAT, 2013). In general, lactation curves in dairy animals reach the peak yield after calving and then decrease steadily after peak yield to the drying off (Swalve and Guo, 1999). Based on the information obtained from the curve (e.g. days in milk to peak, maximum milk production during lactation and lactation persistency), it can be used as a tool for evaluating and selecting the lactating

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herds (Swalve, 1995). Persistency of lactation has direct economic value as it is the ability of a buffalo cow to continue producing milk at a high level after the peak of her lactation.

Some researchers derived the lactation curve parameters of milk, fat and protein traits. Aziz et al. (2006) tried to fit the linear logarithmic transformed form of the Incomplete Gamma function Wood (1967) to weekly milk yield records to describe the shape of the lactation curve for the first four lactations of the Egyptian buffaloes. The author mentioned that Wood's function seemed to be suitable for Egyptian lactation data and might be used for predicting the whole lactation yield from part lactation data. This result agrees with other work presented by Fooda *et al.* (2010). Abdel-Salam *et al*. (2011) for Egyptian buffalo found that, in comparison between Wood, Wilmink and Guo and Swalve, the goodness-of-fit statistics of the expected curves for daily milk, fat and protein yield, for the best-fit models, appear that Wood model gave the best fit for the studied criteria. The objective of this study was investigating the genetic improvement possibilities for test-day milk yield traits and their lactation curve parameters in the Egyptian buffalo.

MATERIALS AND METHODS

Dataset

A total of 4971 test-day milk, fat and protein yield records was used in this study and they were collected at monthly intervals over the period from 1999 through 2009 from four buffalo experimental herds (El-Nattafe El-Gadid, El-Nattafe El-Kadim, Mahalet Mousa and El-Gemmiza) belonging to the Animal Production Research Institute (APRI), Ministry of Agriculture, Egypt. Test day milk yield (TDMY) records were measured following an alternative am-pm monthly recording scheme. Milking was practiced twice a day at 7 am and 4 pm throughout the lactation period. In general, using TD models could have advantages over a 305-day model (Swalve, 1995). All the known relationships among the individuals were considered in the animal model employed in analysis. The structure of the data analyzed is shown in Table 1.

Measuring the fat and protein percentages in milk

Fat and protein quantities were measured by the automated method of infrared absorption spectrophotometry (Milk-o-Scan; Foss Electric, Hillerød, Denmark) at the Dairy Services Unit,

Table 1. Structure of test day data analyzed for Egyptian buffaloes.

Item	Data
No. of sires	120
No. of dams	532
No. of cows with records	691
No. of base animals	469
No of non-base animals	684
Total number of animals	1153
Total number of lactation records	4971

Animal Production Research Institute, Sakha, Kafr El-Sheikh Governorate. The device needs a set of solutions: The first solution is used to wash the device after the analysis of the samples and before turning it off the name of this solution non foaming stella 0.5% (Foss company electric Denmark). The second solution is used to reset the device which gives the readings 0.000 so it is ready to read the new samples and its name is Triton x-100 and we use only 1 cm/liter of distilled water, and finally we have to give the device the order Prog 2 then Prog 3 then Prog 4, and then the device is programmed to read the buffalo milk samples. After that has been converted the percentages of fat and protein to yields in grams.

Estimating the curve parameters of milk, fat and protein yields

In this work, the shape of the milk, fat and protein curves of Egyptian buffaloes were studied using the gamma type function (Wood, 1967) which was described as sufficiently good for modeling extended lactations (Abdel-Salam *et al*., 2011). The following gamma-type function was used for describing the lactation curve of all parameters:

$$Y_n = an^b e^{-cn}$$

The constants a, b and c were calculated by using a general linear model (GLM) procedure of SAS software (SAS, 2002); where Y_n is the test-day milk (kg), fat (g) and protein yields (g), in the n^{th} month of lactation, a is the initial yield, b describing the rate of production increase up to the peak during the ascending phase, c describes the rate of yield decrease during the descending phase and e is base of natural logarithms. The NLIN procedure of SAS software was used for fitting the gamma type function. After fitting the function, the following components were estimated from the primary components of the equation. Persistency of lactation (P = -(b+1)Ln(c)) was also estimated. Days in milk (DIM) at peak yield (PY) was defined as b/c and the maximum production during lactation (Ymax) was calculated as $a(b/c)^b e^{-b}$ according to Wood (1967).

Genetic analysis for lactation curve parameters

Variance and covariance components (direct additive genetic, permanent environmental, error and phenotypic) and heritabilities were estimated using the following linear multi-trait repeatability animal model of the VCE6 program (Groeneveld *et al*., 2010):

$$Y_{ijklmno} = \mu + A_i + Pe_j + HTD_k + Ye_l + Pa_m + S_n + b(A) + e_{ijklmno}$$

Where: Y_{ijklmn} = The recorded trait of test-day yields (milk, fat and protein), initial yield, ascending phase, descending phase, persistency, DIM at peak yield and maximum production during lactation; μ = The overall mean; A_i = The additive genetic random effect of buffalo, assumed to be NID (0, σ^2_a); P_{ej} = The permanent environmental random effect, assumed to be NID (0, σ^2_{pe}); HTD_k = The fixed effect of the k^{th} herd-test-day (k = 40 levels for all paritie); Ye_l = The fixed effect of l^{th} year of calving (l = 10 levels for all paritie); Pa_m = The fixed effect of m^{th} parity (m = 5 levels for all paritie); S_n = The fixed effect of n^{th} season of calving (n = 2 level); b(A) = The covariable for days in milk; $e_{ijklmno}$ = The random residual term associated with each observation. The previous repeatability animal model could be written in the following matrix structure:

$$y = Xb + Za\ a + Zc\ c + e$$

Where: y = The vector of lactation observations, X = The incidence matrix relating the fixed effects to y, b = The vector of an overall mean and the fixed effects of herd test day, parity, year and season of calving and days in milk (as a covariable), Za = The incidence matrix relating the direct additive genetic effects to y, a = The vector of the random direct additive genetic effect associated with the incidence matrix Za, Zc = The incidence matrix relating the permanent environmental effect, c = The vector of permanent environmental effect associated with the incidence matrix Zc and e = The vector of random residual effects N (0, Is^2e) where I is an identity matrix. The variance-covariance components of the random effects were as follows:

$$Var\begin{pmatrix} a \\ c \\ e \end{pmatrix} = \begin{pmatrix} A\sigma_a^2 & 0 & 0 \\ 0 & I_c\sigma_c^2 & 0 \\ 0 & 0 & I_n\sigma_e^2 \end{pmatrix}$$

Where: a = Numerator relationship matrix, Ic, In = identity matrix with order equal to number of animals and number of records, respectively, σ^2_a, σ^2_c, σ^2_e, and are the variances due to effects of diract additive genetic, permanent environmental and random error, respectively. Occurrence of local maxima was checked by repeatedly restarting the analyses until the log-likelihood did not change beyond the first decimal. The heritability (h^2) was computed as:

$$h^2 = \frac{\sigma^2 g_i}{\sigma^2 g_i + \sigma^2 p_i + \sigma^2 e_i}$$

Where σ^2_{gi} is the additive genetic variance of the i^{th} TD milk traits, σ^2_{pi} is the permanent environmental variance and σ^2_{ei} is the residual variance. Similarly, the genetic correlation coefficients (r_{gij}) between any two TDMY traits were calculated by dividing the additive genetic covariances (σ_{gij}) between any two TD's milk yield traits (i^{th} and j^{th}) by the square root of the product of their additive genetic variances of (σ^2_{gi} and σ^2_{gj}). Then, the genetic correlation between the i^{th} and j^{th} TDMY traits is calculated as:

$$r_{gij} = \frac{\sigma g_{ij}}{\sqrt{(\sigma^2 g_i)}\sqrt{(\sigma^2 g_j)}}$$

The estimates of phenotypic correlation coefficients (r_{pij}) between any two TDMY traits were calculated as:

$$r_{pij} = \frac{\sigma p_{ij}}{\sqrt{(\sigma^2 p_i)}\sqrt{(\sigma^2 p_j)}}$$

The estimates of permanent environmental coefficients (rpe_{ij}) between pairs of TDMY traits were calculated as:

$$r_{pe_{ij}} = \frac{\sigma pe_{ij}}{\sqrt{(\sigma^2 pe_i)}\sqrt{(\sigma^2 pe_j)}}$$

Predicted breeding values

The predicted breeding values (PBVs) were estimated by REML using the computer PEST package (Groeneveld *et al*., 2001) for test-day milk, fat and protein yields according to the repeatability animal model matrix structure. The solutions for the equations of animals were computed from the pedigree file, one animal at a time for animals with records and animals without records (sires and dams). The diagonal element (d_t) and the adjusted right-hand side (y^*_t) were accumulated with each

pedigree file record for the t^{th} animal. For the animals with and without records, the formula used to estimate the PBV was (Kennedy, 1989):

$$PBV = [{}^{y}_{t}/d_{t}]$$

The lactation yield traits curves were measured as the regression of least squares means and breeding values on test day.

Plotting the lactation curve from the phenotypic values and the breeding values for test-day milk, fat and protein yields

The lactation curves from the phenotypic values were measured as the regression of least squares means on test-day. As stated before, the breeding values of the animals with records and without records were estimated using the PEST program (Groeneveld *et al.*, 2002). Accordingly, the breeding values were measured by regressing the breeding values on test-day.

RESULTS AND DISCUSSION

Means and variations of lactation curve parameters

The test-day lactation curve parameters were calculated using the gamma-type function according to Wood (1967) for initial milk yield (a), the rate of yield increase up to peak (ascending phase, b), the rate of yield decrease during the descending phase (c), persistency (P), the days in milk to peak or the time required to attain this peak (PY) and the maximum peak milk yield during lactation (Ymax).

The estimates of means, standard deviations, coefficients of variation (CV), minimum and maximum are shown in Table 2 for TDMY, TDFY and TDPY, a, b, c, P, PY and Ymax for the whole data that were calculated by the logarithmic gamma-type function (Table 2).

The means for TDMY (7.00 kg), showed a lactation curve initializing with 5.59 kg, followed by an increase in milk yield until the peak of the lactation, occurred in the third and fourth test-day (0.99 kg), and a decrease until the end of lactation for 0.19 kg for parameter c. Means of persistency of lactation was also estimated to be 6.09 kg.

The result of parameter a in TDMY was the same for those reported by Kianzad *et al.* (2013); Shokrollahi and Hasanpur (2014), higher than those reported by Atashi *et al.* (2009), but lower than those reported by Bouallegue *et al.* (2013); Sahoo *et al.* (2014); Sahoo *et al.* (2015).

The rate of increase to reach the peak during the ascending phase (b) and the rate of decrease (c) were the same trend by Fooda *et al.* (2010) for the Egyptian buffalo. The ascending phase (b) was faster and bigger as reported by Fooda *et al.* (2010); Bouallegue *et al.* (2013); Kianzad *et al.* (2013); Shokrollahi and Hasanpur (2014), differ those reported by Sahoo *et al.* (2014); Sahoo *et al.* (2015) in buffalo. This trend was attributable to the improvement of nutrition for post-partum, and since 2005, a correct methodology of elimination and replacement activities was performed (Fooda *et al.*, 2010). Therefore, the breeders had a trend to increase the nutrition, vitamins and minerals at the pre, post-partum and the whole lactation period which lead to an increase in the milk yield and the income.

The average persistency in milk yield was 6.09 kg, indicating the deteriorating status of the herd's persistency of the Animal Production Research Institute, which required more work based on a selection index including persistency trait. This estimate of persistency is lower than that

of Fooda *et al*. (2010); Bouallegue *et al*. (2013) on the same population of Egyptian buffalo, lower than reported by Atashi *et al*. (2009); Kianzad *et al*. (2013); Shokrollahi and Hasanpur (2014), but greater than Sahoo *et al*. (2015); Şahin *et al*. (2015) in buffalo.

The peak milk yield averaged 10.77 kg and the peak test day (the time required to attain this peak) was 54.97 days. The peak time estimate is the same as that reported by Atashi *et al*. (2009); Shokrollahi and Hasanpur (2014), but lower than that of Bouallegue *et al*. (2013); Kianzad *et al*. (2013); Şahin *et al*. (2015), and higher than that Aziz *et al*. (2006). The maximum peak milk yield is the same as that of Kianzad *et al*. (2013); Shokrollahi and Hasanpur (2014), increased more as reported by Aziz *et al*. (2006), but decreased as cited by Atashi *et al*. (2009); Bouallegue *et al*. (2013).

The means for fat and protein yields and parameters curves were estimated and illustrated in Table 2. The means for fat and protein yields were 45.59 and 26.87 g, respectively, and these results were the same of those obtained by Silvestre *et al*. (2009), but less than that Bouallegue *et al*. (2013) in cattle. The initialized by estimates were 3.79 and 3.31 g, followed by an increase of 1.06 and 1.01 g in yields until the peak of the lactation occurred in the third and fourth test-day, and decreased for the parameter c again at the end of lactation with a production of 0.33 and 0.32 g, respectively. Means of persistency were also estimated to be 2.38 and 2.33 g for fat and protein, respectively. The peak of fat and protein yield (Ymax) being 4.68 and 3.92 g and the peak test day (PY) was 3.47 and 3.31 days, respectively.

The largest coefficient of variations (CV) among the lactation curve traits were for parameter c and the smallest for parameter a in all traits. These results differ from described by Boujenane and Hilal (2012) that the largest for parameter b but the smallest for parameter P of milk.

The phenotypic and genetic estimates for the lactation curve

For the phenotypic values, it could be observed that the initial yield was 5.59 kg for milk and 32.8 and 20.3 g for fat and protein for the first test day (Figure 1, 2 and 3), then it gradually increased as the lactation period advanced (parameter b) till reached 7.7 kg for the animals in the third and fourth test day in milk and 49.7 and 29.4 g in fat and protein (peak yield or persistency), and reached 4.06 kg (parameter c) in milk and 27.81 and 16.03 g in fat and protein for the animals dried off at the tenth test day. The curve parameters of a and c in milk yield showed lower estimates than that reported by Silvestre *et al*. (2009) and unlike to b parameter, but a, b, c estimates in fat and protein showed higher than Silvestre *et al*. (2009). The Ymax showed a higher estimate than that of Silvestre *et al*. (2009) but unlike PY. However, the curve parameters for fat and protein traits were lower than Bouallegue *et al*. (2013).

For the lactation curve plotted from the breeding values, it could be observed that the initial breeding value was 0.109 kg for milk and 1.125 and 0.280 g for fat and protein for the first test day (Figure 4, 5 and 6), then it gradually increased as the lactation period advanced (parameter b) till reached 5.3 kg for the animals in the third and fourth test day in milk and 3.5 and 0.81 g in fat and protein (peak yield or persistency), and reached 0.026 kg (parameter c) in milk and 0.9 and 0.17 g in fat and protein for the animals dried off at the tenth test day.

Table 2. Means, standard deviations (SD), standard error (SE), coefficients of variation (CV), minimum (min.) and maximum (max.) for test-day milk, fat and protein curve parameters.

Variables	Mean	SD	SE	CV	Min.	Max.
Milk yield						
TDMY, kg	7.00	2.37	0.03	33.91	3.00	13.50
a, kg	5.59	1.60	0.02	28.55	2.50	7.50
b, kg	0.99	0.37	0.005	36.60	0.01	1.50
c, kg	0.19	0.16	0.002	83.09	0.03	0.50
P, kg	6.09	2.64	0.04	43.36	4.30	11.63
PY, day	54.97	20.06	0.28	36.49	45.00	97.00
Ymax, kg	10.77	4.69	0.07	43.53	6.50	17.00
Fat yield						
TDFY, g	45.59	16.66	0.24	36.53	13.86	113.88
a, g	3.79	0.31	0.004	8.15	2.88	5.05
b, g	1.06	0.46	0.01	43.71	0.10	3.26
c, g	0.33	0.18	0.002	53.03	0.10	1.51
P, g	2.38	0.66	0.01	27.84	0.05	5.48
PY, day	3.47	1.33	0.02	38.31	1.07	19.54
Ymax, g	4.68	0.87	0.01	18.64	2.18	8.04
Protein yield						
TDPY, g	26.87	9.16	0.13	34.08	7.89	59.80
a, g	3.31	0.28	0.004	8.41	2.30	4.28
b, g	1.01	0.43	0.01	42.37	0.05	2.81
c, g	0.32	0.17	0.002	50.98	0.01	1.38
P, g	2.33	0.61	0.01	26.16	0.01	5.43
PY, day	3.31	1.02	0.01	30.99	0.92	18.99
Ymax, g	3.92	0.73	0.01	18.62	1.76	7.38

Variables are defined before.

Heritabilities Parameters

The heritability estimates of test-day (TD) milk traits (milk, fat and protein) and the lactation curve parameters are presented in Table 3.

Estimates of heritability (h^2) of the Wood's function for yields milk, fat and protein were 0.22, 0.21 and 0.22, respectively. These results are in the ranges as reported by Flores and van der Werf (2015). But, these estimates were greater than that cited by El-Bramony *et al.* (2010). The h^2 estimates for lactation curve parameters of a, b, c, P, PY and Ymax were 0.37, 0.38, 0.39, 0.37, 0.37 and 0.38, respectively (Table 3). These estimates were greater than those obtained by Gebreyohannes and Koonawootrittriron (2013). The h^2 estimates for fat curve parameters of a, b, c, P, PY and Ymax were 0.41, 0.40, 0.39, 0.38, 0.36 and 0.42, respectively, while the estimates for protein curve parameters were 0.38, 0.40, 0.40, 0.38, 0.40 and 0.43, , respectively. Linde *et al.* (2000) estimated the heritability of milk curve parameter (b) to be 0.13, 0.20, and 0.18, in the first three lactations, respectively. These estimates are much lower than the estimates found here, but are similar in pattern, where the second lactation heritability having the highest magnitude.

Genetic and phenotypic correlations

Genetic correlations in Table 4 for milk yield between initial (a) and increasing phases (b) (-0.8) is comparable with that of Macciotta *et al.* (2005), but differ with Boujenane and Hilal (2012), however, the decreasing phase slope (c = -0.43) estimate with a was similar to those from Tekerli *et al.* (2000); Boujenane and Hilal (2012), but unlike with that of Macciotta *et al.* (2005). The negative genetic correlation between the parameters a and b implies that a higher initial yield is associated with a slower rate of increase until peak yield. Tekerli *et al.* (2000), based on a moderate to large positive correlation estimates of the lactation yield with peak yield and persistency, suggested that one of these traits should be used as a criterion to improve all the three traits. Similarly, considering the large negative correlation among initial yield with increasing and decreasing slopes, Moradi Shahrbabak (2001) recommended the selection based on initial milk yield in order to decrease the increasing slope and the decreasing slope of the lactation curve and to produce steadier lactation and reach peak yield early. Although Ymax had a favorable high genetic correlation with TDMYs (0.87) as Boujenane and Hilal (2012). PY presented a relatively high genetic correlation

Table 3. The estimates of heritability (h^2) and their standard errors (SE) for test-day milk traits and lactation curve parameters.

Trait	h^2±SE	Trait	h^2±SE	Trait	h^2±SE
TDMY	0.22±0.002	TDFY	0.21±0.00011	TDPY	0.22±0.00002
a	0.37±0.006	a	0.41±0.00014	a	0.38±0.00003
b	0.38±0.007	b	0.40±0.00014	b	0.40±0.00003
c	0.39±0.006	c	0.39±0.00014	c	0.40±0.00003
P	0.37±0.006	P	0.38±0.00014	P	0.38±0.00003
PY	0.37±0.006	PY	0.36±0.00014	PY	0.40±0.00003
Ymax	0.38±0.007	Ymax	0.42±0.00015	Ymax	0.43±0.00003

with TDMY (0.48), but Boujenane and Hilal (2012) showed this correlation equal one. TDMY was positively correlated with initial milk yield (parameter a) similar to the results of Boujenane and Hilal (2012). The moderate genetic correlation coefficient between TDMY and initial milk yield was (0.31). This means that initial milk yield seems to be the best predicator of total milk yield (Table 4).

Genetic and phenotypic correlations in Table 4 between b and c curve parameters in the milk yield (0.5 and 0.88, respectively), differ with Boujenane and Hilal (2012); Macciotta *et al.* (2005) indicate that buffalo cows that peaked more rapidly also had a quicker decline after peak. Similar results have been reported by Tekerli *et al.* (2000). The rate of milk yield increase was negatively correlated with persistency of lactation (-0.71), except Boujenane and Hilal (2012), this means that selection for persistency of lactation decreases the rate of increasing milk production till the peak (parameter b).

Genetic correlation in Table 4 between PY and P of milk yield (0.87) is comparable to results obtained by Boujenane and Hilal (2012), suggesting that buffalo cows that reached their peak yield early during their lactation had higher persistency.

Genetic correlations between parameter c with each PY and P of milk yield were -0.48 and -0.26, respectively (Table 4), indicating that selecting for Ymax early in lactation would improve persistency by lowering the rate of decrease after peak yield. Genetic correlation between TDMY and P (0.52) unlike Boujenane and Hilal (2012), suggested that buffalo cows with higher estimated breeding value (EBV) for persistency would be expected to have higher EBV for TDMY. These findings are supported by previous research by Ferris *et al.* (1985).

Phenotypic correlation in Table 4 between initial milk yield (a) with parameters b, c and PY were negative. This result is supported by those of Boujenane and Hilal (2012); Bouallegue *et al.* (2013). On the other hand, positive phenotypic correlations were found between initial milk yield and TDMY, persistency and Ymax as also reported by Mansour *et al.* (1993) on Egyptian buffaloes.

The rate of milk production increase till peak (b) was highly phenotypically correlated (0.88) with the rate of milk yield decrease (c) similar to results of Atashi *et al.* (2009); Boujenane and Hilal (2012). Positive and moderate phenotypic correlation (0.47) was found between b and PY similar to the results of Atashi *et al.* (2009), opposite with Bouallegue *et al.* (2013), however, a low estimate (0.10) was found between b and Ymax unlike Boujenane and Hilal, 2012. The phenotypic correlation between b and persistency was negative (-0.78) similar to those found by Atashi *et al.* (2009); Boujenane and Hilal (2012).

Positive phenotypic correlation in Table 4 was detected between persistency of lactation and TDMY (0.49) as reported by Atashi *et al.* (2009); Bouallegue *et al.* (2013) but differ with Boujenane and Hilal (2012).

The estimates of genetic and phenotypic correlations (Table 4) between all curve parameters in fat and protein were close to a large degree with lactation curve parameters. These results are in line with those reported by Bouallegue *et al.* (2013).

Genetic and phenotypic correlation between milk, fat and protein traits and every curve parameter and its match in each trait

Genetic and phenotypic correlations among test-day lactation traits (milk, fat and protein yields) and curve parameters are presented

Table 4. The estimates of additive genetic correlations and their standard errors (SE) (above the diagonals), and phenotypic correlations (below the diagonals) for yields of milk, fat and protein, and the lactation curve parameters for each trait of milk, fat and protein separately.

Milk yield	**TDMY**	**a**	**b**	**c**	**P**	**PY**	**Ymax**
TDMY	-	0.31±0.002	-0.23±0.009	-0.34±0.009	0.52±0.002	0.48±0.001	0.87±0.009
A	0.50	-	-0.80±0.009	-0.43±0.003	0.49±0.001	-0.30±0.009	0.50±0.001
B	-0.34	-0.75	-	0.50±0.001	-0.71±0.002	0.45±0.002	0.15±0.003
C	-0.38	-0.68	0.88	-	-0.26±0.004	-0.48±0.003	-0.33±0.001
P	0.49	0.54	-0.78	-0.46	-	0.87±0.005	0.35±0.005
PY	0.45	-0.61	0.47	-0.15	0.70	-	0.37±0.009
Ymax	0.83	0.18	0.10	-0.08	0.45	0.42	-
Fat yield	TDFY	a	b	c	P	PY	Ymax
TDFY		0.33±0.0003	-0.28±0.0003	-0.38±0.0002	0.52±0.0002	0.48±0.0003	0.79±0.0003
A	0.55	-	-0.65±0.0003	-0.43±0.0002	0.48±0.0002	-0.30±0.0003	0.46±0.0003
B	-0.38	-0.80	-	0.49±0.0002	-0.63±0.0002	0.45±0.0003	0.16±0.0003
C	-0.41	-0.72	0.89	-	-0.28±0.0002	-0.48±0.0003	-0.38±0.0003
P	0.50	0.49	-0.82	-0.48	-	0.88±0.0003	0.32±0.0003
PY	0.52	-0.58	0.49	-0.18	0.81	-	0.41±0.0003
Ymax	0.93	0.22	0.13	-0.16	0.54	0.46	-
Protein yield	TDPY	a	b	c	P	PY	Ymax
TDPY		0.38±0.00005	-0.28±0.00005	-0.37±0.00005	0.55±0.0005	0.47±0.00005	0.85±0.00005
A	0.52	-	-0.85±0.00005	-0.45±0.00005	0.51±0.00005	-0.34±0.00005	0.41±0.00005
B	-0.40	-0.82	-	0.49±0.00005	-0.60±0.00005	0.39±0.00005	0.17±0.00005
C	-0.32	-0.64	0.79	-	-0.20±0.00005	-0.47±0.00005	-0.41±0.00005
P	0.50	0.60	-0.80	-0.48	-	0.84±0.00005	0.37±0.00005
PY	0.47	-0.59	0.48	-0.18	0.79	-	0.36±0.00005
Ymax	0.84	0.26	0.15	-0.11	0.51	0.47	-

Traits are defined before.

Table 5. Estimates of genetic correlations (r_G±SE), and phenotypic correlations (r_P) among test-day lactation traits (milk, fat and protein yields) and lactation curve parameters.

Traits correlated	r_G±SE	r_P
TDMY and TDFY	0.98±0.02	0.86
TDMY and TDPY	0.99±0.01	0.92
TDFY and TDPY	0.99±0.03	0.83
Lactation curve parameter (a):		
a-milk and a-fat	0.71±0.007	0.86
a-milk and a-protein	0.86±0.007	0.88
a-fat and a-protein	0.85±0.007	0.87
Lactation curve parameter (b):		
b-milk and b-fat	0.82±0.13	0.86
b-milk and b-protein	0.87±0.12	0.92
b-fat and b-protein	0.88±0.15	0.95
Lactation curve parameter (c):		
c-milk and c-fat	0.85±0.005	0.86
c-milk and c-protein	0.82±0.005	0.87
c-fat and c-protein	0.84±0.005	0.87
Lactation curve parameter (P):		
P-milk and P-fat	0.85±0.005	0.90
P-milk and P-protein	0.87±0.005	0.92
P-fat and P-protein	0.86±0.005	0.91
Lactation curve parameter (PY):		
PY-milk and PY-fat	0.74±0.006	0.75
PY-milk and PY-protein	0.73±0.006	0.88
PY-fat and PY-protein	0.72±0.006	0.83
Lactation curve parameter (Ymax):		
Ymax-milk and Ymax-fat	0.94±0.007	0.95
Ymax-milk and Ymax-protein	0.91±0.007	0.96
Ymax-fat and Ymax-protein	0.92±0.007	0.95

in Table 5.

These results showed the high genetic and phenotypic correlation between all traits (milk, fat and protein) as reported by El-Bramony *et al*. (2010) on Egyptian buffalo. The results showed a high genetic and phenotypic correlation between the same curve parameters of milk, fat and protein. The results of lactation curve parameters fully apply to fat and protein curve parameters (Cismaş *et al*., 2012). Genetic and phenotypic correlations of favorite milk, fat and protein curve parameters (a, P and Ymax) are presented in Table 6.

Moderate genetic and phenotypic correlation were found between the favorable curve parameters (a, P and Ymax) with their corresponding traits for all traits. These results may indicate that the use of any of these parameters (a, P, Ymax) can be used to improve the curve of these traits and therefore total yields of these traits.

CONCLUSIONS

Selection for persistency of traits decreases the rate of parameter b (increasing production till peak), the rate of decreasing production, increasing the maximum milk production during lactation and finally total yields. High genetic correlation between TDMY and P suggested that buffalo cows with higher estimating breeding value (EBV) for persistency is expected to have higher EBV for TDMY. High and moderate genetic and phenotypic correlation between all traits (milk, fat and protein yields), between every curve parameter and its match in each trait (e.g. a-milk, a-fat and a-protein, b-milk, b-fat, etc) and the favorable curve parameters (a, P and Ymax) in each trait with the same curves in the other traits, indicate that fat and protein moving in the same direction of genetic parameters of lactation curve parameters. Genetic selection for curve parameters (a, P and Ymax) especially P would improve total milk yield traits.

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Table 6. Estimates of genetic (r_G), and phenotypic correlations (r_P) among favorable lactation curve parameters (a, P and Ymax).

Traits correlated	r_G±SE	r_P
a-milk and P-fat	0.45±0.006	0.56
a-milk and Ymax-fat	0.46±0.007	0.55
a-milk and P-protein	0.44±0.006	0.55
a-milk and Ymax-protein	0.44±0.007	0.54
P-milk and Ymax-fat	0.44±0.007	0.55
P-milk and Ymax-protein	0.42±0.007	0.53
a-fat and P-protein	0.43±0.005	0.49
a-fat and Ymax-protein	0.44±0.007	0.48
P-fat and Ymax-protein	0.39±0.007	0.49

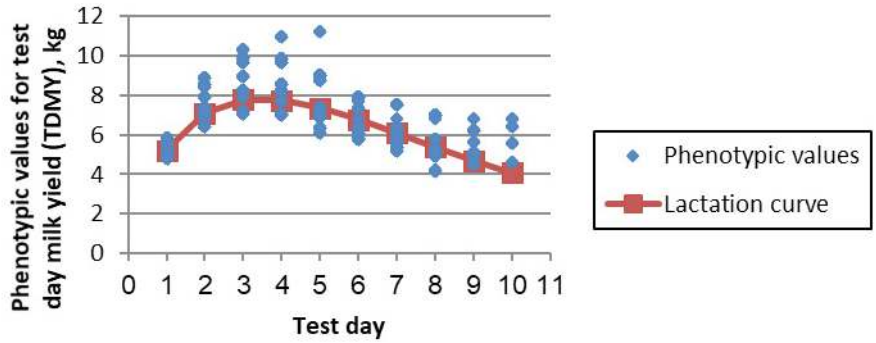

Figure 1. Lactation curve plotted from the phenotypic values for test-day milk yield (TDMY) in the Egyptian buffalo.

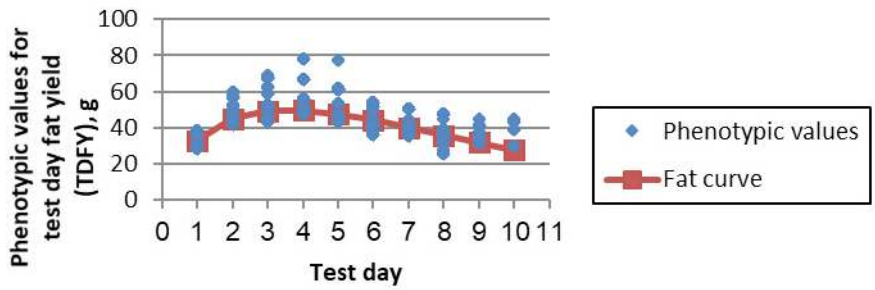

Figure 2. Fat curve plotted from the phenotypic values for test-day fat yield (TDFY) in the Egyptian buffalo.

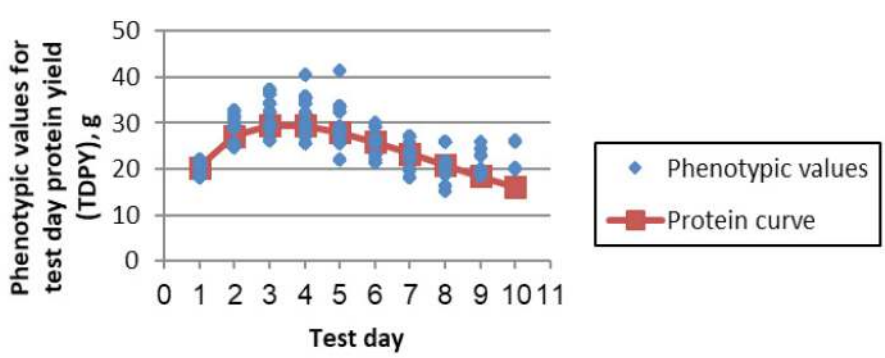

Figure 3. Protein curve plotted from the phenotypic values for test-day protein yield (TDPY) in the Egyptian buffalo.

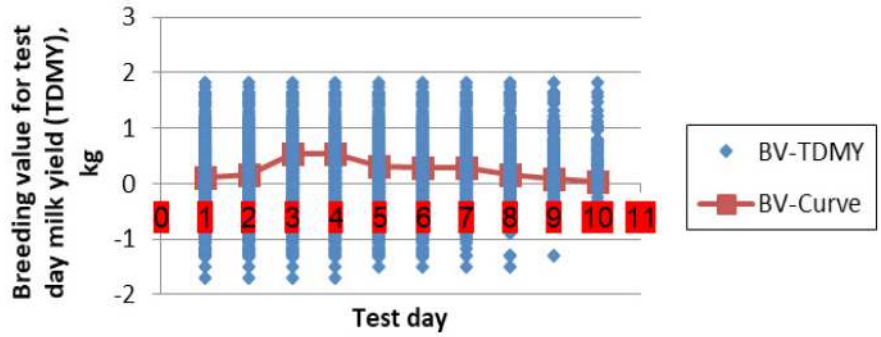

Figure 4. Lactation curve plotted from the breeding values for test day milk yield (TDMY) in the Egyptian buffalo.

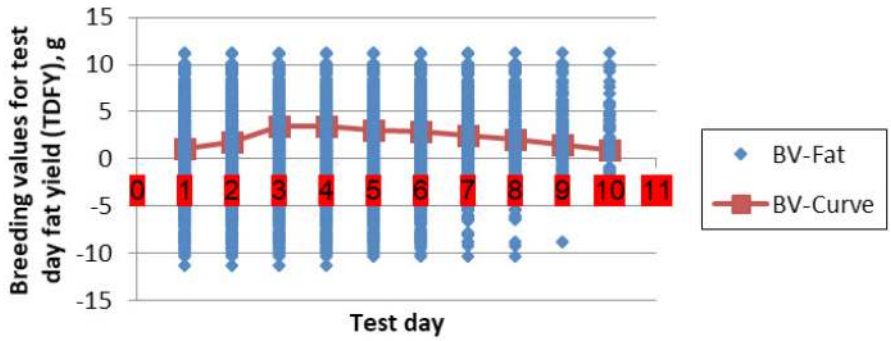

Figure 5. Fat curve plotted from the breeding values for test-day fat yield (TDFY) in the Egyptian buffalo.

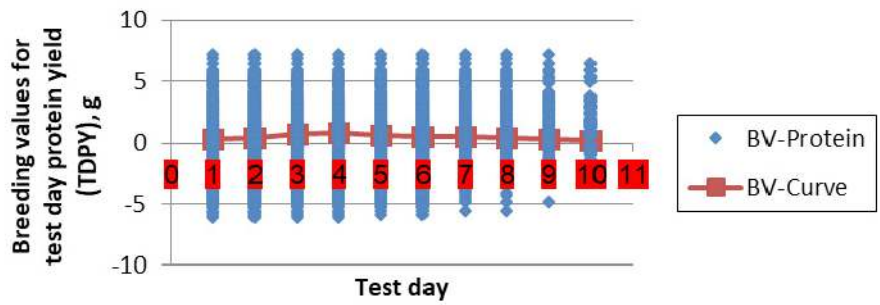

Figure 6. Protein curve plotted from the breeding values for test-day protein yield (TDPY) in the Egyptian buffalo.

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DAIRY BUFFALO PRODUCTION UNDER INTENSIVE SYSTEM IN SEMI ARID AREA OF BANGLADESH

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ABSTRACT

An attempt was made to know the management system of intensive buffalo farming and buffalo derived -problems under semi-arid area of Bangladesh. Direct interview with farmers, farm observation, community discussion and key informant interview (KII) had been followed over six months in Dinajpur district. It was observed that buffaloes were reared under two types of management system namely household yard intensive (HYI) and completely intensive (CI). In HYI system, farmers purchased heifer or pregnant buffalo from local market and sold mother with calf after one year. Buffaloes were kept shed at night and maintained feeding, showering and other activities in the household yard at day. Those were showered by once-two times/week in cool day, but most of them were showered in a day. On the other hand, in CI system, buffaloes were reared inside the shed all time and maintain feeding, showering and other activities in the shed. Buffaloes were showered twice in a day in winter period while three times in summer period. Average milk production for each buffalo was 5 to 8 liter/day. Major productive and reproductive traits e.g. lactation Length (day), average milk yield (L/day), dry period (day), age at first pregnancy (month) and age at first calving (month) were found better in CI system than that of HYI system and those were found highly significant ($P<0.001$). Significant difference were found on total solids, fat and protein content but no significance difference were observed on SNF, lactose and ash of buffalo milk between two farms. Insignificant difference ($P>0.05$) was found for the value of electric conductivity (ECms/cm) that showed both farms produced healthy milk (3.25±0.49 vs 3.09±0.89). Both systems showed as a profitable venture (BCR 1.33 vs 1.25). Problem matrix revealed that the top ranked order of problem were "inadequate knowledge on buffalo rearing", "high price of feed" and "low milk price" mentioned as 1, 2 and 3. Shortage of feeds and fodder, high temperature and lack of cow boy were ranked as 4, 5 and 6. Limited breeder bull, lake of AI workers and inadequate quality semen and credit facilities mentioned as 7 ranked problem. It was concluded that ensuring training and marketing could help to improve intensive system of dairy buffalo that meet up the growing demand of milk in urban people. Simultaneously AI facilities with quality semen, HYV fodder cultivation and credit facilities should be promoted for sustainable dairy buffalo farming.

Keywords: *Bubalus bubalis*, buffalo, buffalo farming, feeding system, milk quality

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INTRODUCTION

The rural farming system of animal agriculture consisting buffaloes (*Bubalus bubalis*) and other livestock resources in Bangladesh. Buffalo is a less prioritized dairy species in Bangladesh though it has potential health beneficial due to quality of milk and meat. The buffalo meat is of good quality as compared to the cattle beef because of the low cholesterol. Buffalo meat contains 40% less cholesterol, 55% less calories, 11% more protein and 10% more mineral in comparison to bovine meat (Nanda and Nakao, 2003). Buffalo milk has 43% less cholesterol than cow's milk on the basis of per gram butterfat and having 58% and 40% more calcium and protein than cow's milk respectively. In addition to the significant cholesterol and calcium benefits of buffalo milk, is also a rich source of iron, phosphorus, vitamin A and of course protein (Bilal *et al*., 2006). Considering its composition, it may be advisable over cattle beef for the cholesterol sanative people (Bilal *et al*., 2006). It has been never addressed in before by policy level in Bangladesh despite their important role in the national economy. The buffaloes are 14.71 million heads (DLS 2016) in Bangladesh. Though the trend of buffalo population (2.20%) in recent year is relatively higher than cattle (0.44%) but buffalo milk production is not contributed significantly to national production in Bangladesh (Islam, 2017). Total milk production of Bangladesh is about 7.27 million metric tons per year (MMT) against the requirement of 14.69 MMT (DLS 2016). To address the gap of demand and availability of milk, another dairy species e.g. buffalo should be prioritised along with cattle.

Several production system based on management practices and feeding system are found in Bangladesh. At the village level production is usually based on a small herd of mixed ages and sexes generally for milk and breeding purposes. In a semi-intensive production system, buffaloes are kept mainly for specific purposes, i.e., either for milk or for meat production. In an extensive production system, a larger herd is kept for both meat and meat production (usually for sale or slaughter). In the village production systems, buffaloes and cattle are kept within the shed at night, and spend the daytime household yards for feeding and resting, together with other animals. Many buffaloes rear in saline coastal region under extensive farming in *Char areas*. In river basin areas, buffaloes are reared under semi-intensive system. In this system, buffaloes are kept within the shed at night and spend daytime for grassing in the *Bathan.* Besides, a few number of intensive buffalo farming also been seen in some institutional herd as well as farmer level as business purpose where buffaloes are kept in shed whole time. Household intensive system are very scattered and mainly seen at Northern part of Bangladesh, e.g. Dinajpur, Rangpur which is reared mainly under the intensive irrigated agriculture system. The national strategies for the irrigated intensive agricultural system in developing countries should focus upon producing less expensive milk from dairy buffaloes that, efficiently, utilize the limited expensive produced feed resources (Soliman, 2007). Milk production has a comparative advantage in agricultural resource use, in terms of net costs per 100 grams animal protein under irrigated intensive agricultural system in developing countries (Soliman, 2007). The total milk yield per lactation and lactation period varied among the systems due to availability of feed resources, management systems and genotypes of buffaloes. The buffalo raised under household / semi-intensive system produced higher milk than those raised under bathan /extensive system which

results household farming is more attractive as it gives milk and meat both. The lactation yield in the household farming/ semi-intensive system and bathan farming /extensive system were 712 to 799 and 435 litres, respectively (Faruque *et al*., 1990; Uddin *et al*., 2016).

There is several research works in Bangladesh which is demonstrated about productive, reproductive and management system of dairy buffalo under extensive and semi- intensive system, but there is very few information about intensive system of buffalo farming. Moreover India, Pakistan, Trinidad, Italy and some other country have been started intensive system buffalo farming to increase the national milk production in the country. Farmers of Bangladesh believe that extensive system is essential for buffalo farming due to its feeding and wallowing behaviours. This study is therefore undertaken to know the different information regarding feeding, breeding, management practice and associated problems under intensive system of dairy buffalo farming; so that policy makers could be given attention for development of milking buffalo farming which would lead to improve milk production in Bangladesh.

MATERIAL AND METHODS

Location of study areas

Dinajpur district was selected for the study as a semi-arid aria. Moreover greater Dinajpur and Rajshahi district are second buffalo concentrate areas holding 5.1 to 10 buffalo for 1000 people (Huque and Khan, 2017). Birganj, Kaharol and Dinajpur sadar upazilla under Dinajpur district were selected for the study.

Climate of Study area

Dinajpur experiences a hot, wet and humid tropical climate. Under the Köppen climate classification, Dinajpur has a tropical wet and dry climate. The district has a distinct monsoonal season, with an annual average temperature of 25°C (77°F) and monthly means varying between 18°C (64°F) in January and 29°C (84°F) in August. Paddy, Maize, wheat and potato are main crops (Wikipedia 2017).

Farmer selection

Based on the information of Upazila Livestock Office of Birganj, Kaharol and Dinajpur Sadar buffalo farmers were considered for the study. Farmers were randomly selected based on management system and on the experience of dairy buffalo farming having at list one milking buffalo and one calf.

Preparation of questioner

Structure questionnaire was developed according to variable of the objectives. Variables like productive, reproductive, feeding and management practice with related problem for buffalo farming were considered. Data was collected using standard procedure. The questionnaire was pre-tested in the selected area. Some addition and subtraction had been made in the preliminary schedule on the basis on experienced gained in pre-tasting and finalized the questionnaire. The schedule contains were both open and closed-ended question.

Data collection

Qualitative and quantitative information were collected from household yard intensive and full intensive farm. Data were collected directly from 30 household yard intensive (HYI) farms and visited by the researcher through face to face

interviews. Moreover more than 60 milking buffalo farmers were discussed for validate the information of household yard intensive system (HYI). Discussion was also conducted with the cowboys (hired labours) who were taken care of buffalo farm to confirm and recheck the information.

Information is collected from two completely intensive (CI) system buffalo farms (who were rearing buffaloes since three decade) over six months period through direct interview with a prescribed format which was kept in record book. Researcher also kept communication with the farm owners by over mobile phone during study period. Based on record book, different information/ variables were collected from individual farm e.g. gestation period, calving interval, lactation period, dry period, milk production, breeding system and profitability. Feeding and management practices were documented based on questioner. Problem associated to buffalo farming were collected and rechecked with cowboys, Veterinary Field Assistant (VFA) and Upazilla Livestock Officer (ULO). The study therefore follows the mixed methodology mode of Greene *et al.* (1989) focusing mainly on triangulation which can investigate the same issue with the same unit of analysis (Mitchell, 1986).

Period of study

Farm visit, farm observation, secondary review, community discussion were held in the period of six moths which was from October 2016 to March 2017.

Milk sample collection and analysis

Milk sample were collected from both type of intensive farm of Dinajpur district. Just after milking milk samples were collected by ice box and frize at minus twenty degree centigrade (-20°C) due to bring the sample to Dairy Science Laboratory, Bangladesh Agricultural University, Mymensingh. Milk samples were analysed after reaching normal temperature. The milk compositions (TS, SNF, Fat, Protein, Lactose, Ash, P^H, Density and conductivity) were analyzed by using Lactoscan milk analyzer (Milktronic Ltd., 600 Stara Zagora, Bulgaria) in the Dairy Science Laboratory, Bangladesh Agricultural University.

Determine of the problem matrix of dairy buffalo farming

In order to understand local perceptions of the problem of dairy buffalo farming, a simple proportional pilling tool was used with two different groups of informant consisting sixteen buffalo farmers. The informants were asked to think about related problem that might have influenced the reduction of dairy buffalo production. Ten factors were identified and then recognized first five factors using 16 small stones.

Data analysis

After collecting data from field, these were edited and coded. The data was then transfer to MS Excel for processing and summarizing. The tabular technique mainly used to analyze the data and derived meaningful finding by using simple statistical measures like mean, percentage and ratio. Statistical analyses were carried out through SPSS version 16 computer package and analysis of variance of treatment (three locations) means was done by ANOVA in case of productive, reproductive and milk quality. Duncan Multiple Range Test (DMRT) was used to the level of significance the treatment means.

RESULT AND DISCUSSION

Dairy buffalo model

Dairy buffalo farm were very scattered in the study areas. Eighty-eight percent (88%) farmers reared dairy buffaloes that were purchased pregnant or heifer buffalo for business purpose. All of them reared buffalo under household yard. After calving, farmers sold buffalo with calf when age of calf became for two-three months. Among them, a total of 12% farmers maintained full cycle; they reared milking buffaloes with calf for selling milk as a means of family income. Buffalo were reared in shed at night and whole day in household yard allocation with feeds. On the contrary, a few number of intensive dairy buffalo herds were shown in study areas that were reared buffalo all time in the shed. They were rearing buffaloes since two-three decades. Herd size was consisting with 6 to 22 buffaloes by maintaining stall feeding with cooling fan and showering, called as completely intensive (CI) system.

Housing system

Most of the house made by muddy, bamboo and straw. At night farmer kept animal in house. During day, they kept animal in household yard (HYI) where animal took rest and fed to straw, grass, water and small amount of concentrate feed under HYI system. But buffaloes were passed day-night in brick and tin made shed under completely intensive system (CI). Uddin *et al.* (2016) observed that dairy buffaloes were kept under semi-intensive system in the wet season and only 15% farmers provided shed having only roof but no concrete floor. Akbar *et al.* (2009) mentioned that most of the dairy buffalo were not housed in extensive system of bathan areas but buffaloes which were in the plain land sometime had an enclosure, only roof made by straw or tin without wall and floor was always muddy. Seventy four percent respondents reported that the average housing facilities were unavailable for buffalo rearing (Siddiki, 2017) which was not agreed to present study as in Dinajpur both type of farm provided houses for buffaloes. According to Siddiki, 2017, 100% of the farmers didn't care about the housing for buffalo rearing in Subornochar upozila, followed by Trishal, Bagha and Lalpur were 80%, 70% and 45%, respectively and differed significantly ($P<0.01$) between the locations.

Feeding system

The main diet for the buffalo was roughage such as natural grass, and paddy straw. The roughage could be fed either fresh as pasture or in a cut-and-carry-system. The roughage was often complemented with grains, concentrate and agro-industrial by-products such as of wheat bran, oil cake and broken rice etc. Concentrate feed, paddy straw, local grass and water were provided in the manger in a household yard in day time under HYI system. But in night only straw and water kept in the shed where passed whole. Forage was found as insufficient during the dry season and abundant during the rainy season.

Different types of feed base identified under intensive household yard (HYI) system. Average rice gruel ready feed, broken maize, maize flower, rice bran, wheat bran , broken rice after cook, mustard oil cack, molasses, paddy straw green grass cultivated/cut and carry and fresh water were 1.71, 0.03, 0.14, 0.16, 0.14, 1.53, 0.86, 0.19, 0.09, 10.69, 6.22 and 25 to 60 kg/day/buffalo provided respectively. All farmers provided paddy straw and half of them were provided green grass. A total of 75% farmers provide wheat bran, 62.5% farmers provide cack, 62.5% farmers provide

broken rice after cooking and 50% farmers provide broken maize to milking buffalo (Table 1). Under completely intensive system (CI), supply of feed resources depend on season and available of feeds and fodders Table 2. Buffaloes were given more grass (10 to 14 kg/day/each) for three-five months (March to July) during flash period and less grass (1 to 6 kg/day/each) for seven months (August to February) during lean period. During that time paddy straws were also given at the rate of 7.5 to 9 and 11 to 14 kg/day respectively depending on the season. Allocation of concentrate feeds were 5 to 7 kg/milking buffalo. Broken maize, wheat bran, broken rice, and oil cake mainly were used for concentrate mixture. Ahmed (2006) showed that common concentrates of wheat bran, rice polish, pea bran, khesary bran, matikalai bran, lentil, sesame oil cake, coconut oil cake and mustard oil cake were available round the year for dairy animal which support our findings. In the rainy and winter season some legumes, green grasses were grown sporadically in some areas of the country and next six months availability of green grass was very limited, during this time rice straw alone contributes 87% of roughage portion of the dairy feed (Tareque, 1991) (Table 3).

The dry matter intake (DMI) (kg/d) of milking buffaloes in different locations was varies from 13 to 15 (kg/d) (Siddiki, 2017) but in our study dry matter supply (DMI) (kg/d) of milking buffaloes in study area was near to similar as dry matter basis (kg/day).

Watering

All farmers (100%) provided fresh drinking water to buffalo in three times a day under HYI and CI system. Average water intake was 26 to 60 litter under HYI. But 45 litters and 60 litters were found in winter and summer period respectively under CI system. Drinking water is the most important water source of buffalo for body functions, e.g. maintaining body temperature, milk production and maintaining blood plasma volume. A restricted water intake leads to a decrease in dry matter intake and thus affects milk production and growth negatively (Buffalopedia, 2018).

Wallowing

Due to keep cool body temperature and control of insect, buffaloes showed wallowing behavior. In case of absence of water or mud hole, the buffaloes behave more likely to cattle. They need shade and shower. Very few numbers of buffaloes were allowed for wallowing at pond or river in summer season under HYI system. Wallowing was done in river/pond for 1 to 2 h during July to October in this system. Buffaloes were showered once/two times during a week in high cool period (December to January) and other time, most of the farmers' showered buffalo at home once time in a day for both HYI and CI system. When buffaloes enter the water, they defecate and/or urinate to maintain temperature (buffalopedia, 2018). According to farmer reaction, without showering, buffalo feed intake was reduced, skin disease, delay heat and early abortion might be occurred.

Breeding

A total of 96.3% buffalo cow received natural breeding under HYI system whereas, AI was covered only 3.7% in semi-arid areas of Bangladesh. Most of the farmers were facing problem to breed their buffaloes during heat. As of notification of farmers buffaloes were missing heat several times due to lack of breeding bull and far distance of bull station from farm. Moreover farmers bring the heated buffalo far way by small van/track which was very costly (BDT 25000 to

Table 1. Location of study areas.

Name of the upazilla	Location in Bangladesh	Human population density	Type of farm
Birganj	26°0′0″N 88°35′0″E	560/km² (1,500/sq mi)	Full Intensive and Intensive household yard
Dinajpur sadar	25°38′N 88°39′E	520/km² (1,300/sq mi)	Full Intensive
Kaharole	25.7917°N 88.6000°E	576/km² (1,490/sq mi)	Intensive household yard

Source: Wikipedia, 2017.

Table 2. Available feed-base and Feed supply (kg/day) for dairy buffalo under HYI system.

Name of feed-base	Amount Kg/day/buffalo	Percent of farmer provided feed items
Rice gruel	1.71	12.5
Ready feed	0.03	25
Broken maize	0.14	50
Maize flower	0.16	25
Rice bran(kg)	0.14	25
Wheat bran (kg)	1.53	75
Broken rice after cook (kg)	0.86	62.5
Mustard oil cake (kg)	0.19	62.5
Molasses	0.09	25
Straw (kg)	10.69	100
Green grass cultivated/cut and carry	6.22	50
Fresh water	25-60	100

30000). On the other hand natural breeding were followed in CI system as breeding bull were kept in this farm (Table 4).

The natural mating system was practiced by the most farmers though few number of breeding bull having in the herd (about 1%) both in household and bathan farming in Bangladesh (Uddin *et al*., 2016) which agreed to our present finding. Sawarkar *et al.* (2001) reported that, most of the farmers preferred natural service with the expectation that it would increase the conception rate. More than half of the farmers used natural service to their buffalo in heat at the right time, while 42% of farmers were unable to inseminate their animals in time due to various reasons (Uddin *et al*., 2016).

Milk production and quality

Average milk production for first, second and third stage were 6.80, 4.30 and 2.00 litter/day/ buffalo respectively under HYI system and it was 11,6.5 and 2.5 litter/day/ buffalo respectively for CI system (Table 5).

Table 6 showed that chemical quality of buffalo milk for household intensive (HYI) and completely intensive (CI) farm in study areas. Significant difference were found on total solids, fat and protein content but no significance difference were observed on SNF, lactose and ash of buffalo milk between two farms. Insignificant difference ($P>0.05$) was found for the value of electric conductivity (ECms/cm) that showed both farms produced healthy milk (3.25±0.49 vs 3.09±0.89). Milk quality of buffalo agreed with the result of Xiao-YanLing *et al*., 2013 who found that the average milk yield per lactation in 305 day, average milk fat, protein, lactose, total solid and non-fat solid of dairy buffalo were 1162 kg ,7.52%, 4.32%, 5.19%, 17.81%, and 10.11%, respectively in Mang city of China.

Milk man was milking from buffalo cow and purchased milk but price was not well (40 to 42 Tk/L) in HYI system. Milk sold to local market and sweetmeat shop. Some time Milk man didn't come to collect milk and farmer became looser. They gave money to farm owner quarterly or monthly basis. Buffalo farmers therefore depend on middle man for pricing of milk. But in completely intensive system, farmer was sold milk directly to sweetmeat shop (50 to 55 Tk/L) and took relatively better price than HYI system.

Productive and reproductive characters

Productive and reproductive characters of dairy buffalo were exposed in Table 7 under household intensive (HYI) and completely intensive (CI) system. Lactation Length (day), average milk yield (L/day), dry period (day), age at first pregnancy (month) and age at first calving (month) were found highly significant ($P<0.001$) between the system. Service per conception (number), service per conception and post Partum heat (month) period of dairy buffalo were found as significant ($P<0.05$) different between household intensive (HYI) and completely intensive (CI) system. But insignificant different were shown in case of weaning age (days) and gestation length between those system (month). The average dry period (day), weaning age (day), service per conception (Number), age at first pregnancy (month), age at first caving (month), calving interval (month), gestation period (month), postpartum heat period (month) were 172.67±22.73 and 97.22±6.18, 199.33±19.28 and 190.56±8.81, 2.00±0.58 and 1.33±0.50, 35.42±3.48 and 29.33±0.86, 45.60±3.60 and 39.33±0.86, 14.00±1.30 and 12.89±1.16, 10.01±0.17 and 10.00±0.00 and 4.23±0.89 and 3.44±0.72 for HYI and CI system, respectively

Table 3. Average feed allocation(kg/day/buffalo) base on fodder availability under CI system.

Month	Rice gruel (kg)	Brokenmaize	wheat bran	Broken rice	Mustard oil Cake	Molsses	Straw (kg)	Green grass from own
March-July (Five months)	6	1.25	2.25	1	0.75	0.3	8.75	13.5
August-February (Seven months)	6	1.25	3	2	0.75	0.3	13.25	5.5

Table 4. Breeding system and time of heat showed by buffalo cows.

Variables	Semi-arid (Dinajpur)
Type of breeding	
Natural breeding (% of buffalo)	96.3
Artificial insemination (AI) (% of buffalo)	3.7
Time of signing heat	
Early morning (% of buffalo)	11
Noon (% of buffalo)	3.2
Afternoon (% of buffalo)	5.7
Evening (% of buffalo)	12.4
Early night (% of buffalo)	47
Late night (% of buffalo)	20.7

Table 5. Average milk production (L/day).

Management system	First stage (L/day)	Second stage (L/day)	Third stage (L/day)
HYI	6.80	4.30	2.00
CI	11	6.5	2.5

Table 6. Chemical quality of buffalo milk under Household Intensive (HYI) and Completely Intensive (CI) system.

Parameter (%)	Household intensive	Completely intensive	Level of significance
Total solid	16.43±1.03	18.20±0.98	**
Fat	6.86±0.65	8.62±0.97	***
SNF	9.42±0.70	9.49±0.29	NS
Lactose	4.54±0.18	4.66±0.14	NS
Ash	0.67±0.05	0.70±0.03	NS
Protein	4.32±0.35	3.86±0.22	**
pH	6.20±0.42	6.09±.30	NS
Conductivity	3.25±0.49	3.09±0.89	NS

(Table 7). Similar findings were also reported by other authors (Karim *et al*., 2013; Faruque *et al*., 1990; Shabede *et al*., 2003) which were more or less similar to our findings.

The lactation yield in the household farming/ semi-intensive system and bathan farming /extensive system were 712 to 799 and 435 litres, respectively (Faruque *et al*., 1990; Uddin *et al*., 2016) which are much lower than the Nili-Ravi buffaloes reported by Mudgal (1989); Khan (1995); ICAR (2000) and also lower than present finding. Lactation yield of our finding also more than the finding of Hussen (1990); Faruque and Amin (1995). Lactation yield of indigenous buffaloes were low in Khulna region (280 litres, Faruque and Amin, 1995) and Tangail district (830 litres, Hussen, 1990). Cross breed buffaloes were reared in HYI and CI system in Dinajpur district which were migrated from India.

Calf management

Most of the farmers fed colostrums and milk to new born buffalo calf. After one month letter, wheat bran, cake and newly grown soft grass were fed to calf (50 to 60 gm/day). Calves were kept in separate dry and clean place in same room of mother at night and kept at day time in raise pit under tree shed. Timely feeding of colostrums to calves is essential and it should be fed within 2 to 3 h after birth and delay in feeding of colostrums lead to lowered effectiveness of the colostrums in terms of providing immunity to calves (Sharma and Mishra, 1987). Tiwari *et al*. (2007) found that the disease incidence in India Diarrhoea, pneumonia, parasitic infestation, dysentery was found as common diseases in study areas. Tiwari *et al*. (2007) also found those type of disease incidence in India in buffalo calves which reveals that the most common and frequent occurring disease in calves

Table 7. Productive and reproductive traits of dairy buffalo under Household Intensive (HYI) and Completely Intensive (CI) system.

Parameters	Household Intensive (HYI) system	Completely Intensive(CI) system	Level of significant
Lactation Length (day)	188.67±13.06	217.22±6.18	***
Lactation yield (L)	1028±256.68	2001±332.97	***
Average milk yield (L/day)	5.46±1.33	9.22±1.56	***
Dry period (day)	172.67±22.73	97.22±6.18	***
Weaning age (days)	199.33±19.28	190.56±8.81	NS
Service per conception (number)	2.00±0.58	1.33±0.50	*
Age at first pregnancy (month)	35.42±3.48	29.33±0.86	***
Age at first calving (month)	45.60±3.60	39.33±0.86	***
Calving interval (month)	14.00±1.30	12.89±1.16	*
Gestation length (month)	10.01±0.17	10.00±0.00	NS
Postpartum heat (month)	4.23±0.89	3.44±0.72	*

*** = Significance at 0.1% level of probability; * = Significance at 5% level of probability;
NS = Non significant; ± = Standard deviation

was diarrhoea which was reported by 82.2% of the dairy farms followed by endoparasite infestation (80%), ectoparasite infestation (78.9%), naval ill (66.7%) and pneumonia in calves (26.7%). Calf mortality was found as 8% and 7% respectively for HYI and CI system.

Primary health care

A total of 31% farmers used anthelmintics drug for internal parasite for buffalo but 23% farmers followed vaccination practice under HYI system. In CI system farmers were used for controlling both external and internal parasite. Vermic injection which introduced two times in a year (1 ml/40 kg body weight). Deworming drug was used for all type of internal parasite. Vaccine was used for HS and FMD under CI system

Economics of buffalo farming

Yearly expenditure, gross income and net income were BDT 140000, BDT 200000 and BDT 60000 respectively and yearly Benefit Cost Ratio (BCR) was found as 1.33 for in HYI system. On the contrary, yearly expenditure, gross income and net income were BDT 968810, BDT 1220525 and BDT 251715 respectively in CI system farm. Yearly Benefit Cost Ratio (BCR) was found as 1.25. Moreover total remaining assets were BDT 1060000 which represents of present value of buffalo herd of CI system (Table 8). Hasan *et al*., 2016 found that buffalo rearing in the coastal areas of Bangladesh was highly profitable that was crucial pathway for poverty alleviation. Rahman *et al*., 2008 stated that the benefit cost ratio was 1.31, indicating that buffalo rearing was profitable in Bangladesh which is agreed with the findings of Islam *et al*., 2017; Siddique *et al*., 2017 and also support to present study.

Problems associated with dairy buffalo production

According to farmer reaction ten bellow problems had been identified for milking buffalo production system resulted reason for decreasing buffalo population.

Shortage of feeds and fodder

Lack of feed and fodders at January to February as during this time there was no fellow land for grassing. The situation of pasture land was aggravated day by day because of crops were occupied most of the land in those areas. Maize was cultivated most of the land for grain production but they could not fed maize leaf to their animal due to lack of awareness.

High price of feed

High cost of concentrate feed of animal. Farmer could not provide balance feed due to high price of feeds.

Low milk price

Most of the farmers did not get good price of milk (30 to 40BDT). In some cases a few number of farmer get good price (BDT 50 to 52) where milkman collects milk from several farmers and sold to district level sweat meat shop.

High temperature

High temperature was a hindrance for buffalo rearing. Temperature was gradually increased and reducing water source. During high temperature; buffalo could not to take feed as required and abortion might be happen if not showering properly done during hot summer season.

Table 8. Cost -benefit of intensive buffalo farming (1USD = BDT83).

Herd composition and items		**Unit price**	**Yearly total cost**	**Yearly Gross income**	**Yearly net income**	**Net income Monthly**	**Remaining asset (Unit Price)**	**Total Remaining asset**
Milking buffaloes	8	Total milk production 45 L/ day, BDT 53/L	-	870525	-	80000	640000	-
Dry buffaloes	4	-	-	-		70000	280000	-
Heifer	1	-	-	-	-	65000	65000	-
Bull	1	-	-	-	-	75000	75000	-
calf	7	50000	-	350000	-	-	-	-
Concentrate feed	-	18	643860	-	-	-	-	-
Straw	-	3	229950	-	-	-	-	-
Labour	1	7500	90000	-	-	-	-	-
Other	-	-	5000	-	-	-	-	-
Total	-	-	968810	1220525	251715	20976	-	1060000
BCR	1.25							

Decrease of buffalo ploughing

One decade ago, land was cultivated by buffalo ploughing but now day it is done by power tiller.

Limited breeder bull

Few number of breeder bull was main hindrance to bred the buffalo. Farmer could not reach to bull station to breed the buffalo cow in time.

Lake of AI workers and inadequate quality semen

Artificial Insemination (AI) facilities were not available, even yet not popularized because of insufficient AI worker as well as unavailable of buffalo semen and low conception rate.

Lack of financial institutes

There was NGO office but did not provision to disburse credit to purchase buffalo.

Lack of cow boy

Now a day cow boy was not found as before. Labor did not wanted to work in buffalo farm as a cow boy due to opportunities of several jobs.

Inadequate knowledge for buffalo rearing

Government/ NGOs/ private sector did not provide still training or awareness program on Buffalo rearing.Problem matrix represented total score and percentage of score to assess dairy buffalo-derived problems illustrated in Table 9. The top three problems were found "inadequate knowledge for buffalo rearing/no training facilities on buffalo farming", "high price of feed" and "low milk price" which were ranked as 1, 2 and 3. Shortage of feeds and fodder, high temperature and lack of cow boy were ranked as 4, 5 and 6. Limited breeder bull, lake of AI workers and inadequate quality semen and lack of financial facilities as credit were ranked for 7. Decrease of buffalo ploughing shown as 8 (Table 9).

Table 9. Problem matrix for assessing dairy buffalo-derived problems.

Name of the problem	% of the score	Ranked order
Inadequate knowledge for buffalo rearing/no training facilities on buffalo farming	18	1
High price of feed	15	2
Low milk price	14	3
Shortage of feeds and fodder	11	4
High temperature	10	5
Lack of cow boy	9	6
Limited breeder bull	7	7
Lack of AI workers and inadequate quality semen	7	7
Lack of financial facilities as credit	7	7
Decrease of buffalo ploughing	4	8
-	100	-

CONCLUSION

Social and technological transformation can boost up the milk production from this species. Development of management practise along genetic improvement and policy intervention could be enhanced the productivity of buffalo and it would be contribute significantly in national economy.

ACKNOWLEDGEMENT

The authors are highly encouraged due to financial support from BAS/USDA. The authors also grateful to Government Livestock Office for supporting in the field.

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FACTORS AFFECTING SWAMP BUFFALO PRODUCTION IN SONGKHRAM WET LAND, NAKHON PHANOM PROVINCE, THAILAND

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ABSTRACT

The current study was conducted to find out the factors affecting to swamp buffalo production in Songkhram wet land area (consisting of 3 districts: Si Songkhram, Tha Uthen, and Na Wa Districts), Nakhon Phanom province, Thailand. The data were collected from 370 farmers and analyzed by using Chi-square to test association of the studied factors between farm groups (Group 1: farmers who produced the swamp for fancy buffaloes and showing, and Group 2: farmers who produced the buffaloes for power work and meat production) in the study population. The results showed that many factors that affected to buffalo production, such as source of knowledge used for buffalo production, patterns of buffalo production, kinds of land, objectives for buffalo production, record keeping, types of roughage, person who suggest to select buffalo, mating systems, changing sire, information for considering for replacement dams selection, age at first mating, and problem and obstacle related to the achievement of swamp buffalo production ($P<0.01$) excepted for gender of farmers, educational level and type of labor for buffalo production ($P>0.05$). This information implied the need of increasing efficiency of buffalo production, and also improvement of knowledge, understanding and chance in sire selection of the farmers.

Keywords: *Bubalus bubalis*, buffalo, buffalo production, Songkhram wet land, Nakhon Phanom, Thailand

INTRODUCTION

Nakhon Phanom province was ranked as the sixth biggest province in Thailand for buffalo production (accounted for 5.09% of the whole country, 6.97% of north eastern Thailand and 18.32% in term of land area (Department of Livestock Development, 2017). In another report by the Department of Livestock Development (2016), Nakorn Phanom owned proximately 62,800 buffaloes and most of the buffalo farms located along the Songkram river, of which Si Songkhram district was leader with the largest number of buffaloes with 11,857 heads (7.0 buffaloes per family averagely), followed by Na Wa and Muang districts with 7,800 and 3,700 buffalo respectively. Having abundance of water with a large area of wet land was an advantage for the buffalo production

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in the region. The original source of water is from the Poopan Mountain in Nonghan district which is located in Udon Thani povince. The river flows through Sakon Nakorn and Nong Khai and then the river traces its way back to the east and flows into Nakhon Phanom Province in the areas stated above and becomes the Songkram river at Na Wa and Songkram districts before it goes down to the Mekong river at Chai Buri sub-district, Tha Uthen district (Nakhon Phanom Province office, 2016).

According to Sarakul *et al.* (2016), who carried out a study in the same area and reported that nearly 80.00% of farmers raised buffalo as source of power for work. However, income from buffalo production was ranked as the second important source for 67.30% of farmers in the region, after rice cultivation. Even though buffalo production was one of main activities for farmers in the region, it was still facing many difficulties which can influence to number of buffalos, revenue and income of farmers such as knowledge, attitude of farmers and local veterinary services. This study was conducted in order to explore and classify the factors that affecting buffalo swamp production in Songkhram wet land, Nakhon Phanom province, Thailand, supporting to build up a guideline for maintenance and development of buffalo production in the region.

MATERIALS AND METHODS

Population, sampling and study area of dataset

In current study, data was collected by using the survey research method. The tools in gathering the data were the questionnaires and interviews from November 2014 to July 2015. In-depth study was applied along with field studies. The areas focused for the information were 3 districts located around the Songkram river. Each district was subdivided into three sub-districts (9 sub-districts in total). In Si Songkram district the areas focused were Ban Uang, Nadua, and Sampong sub-districts. For Tha Uthen district there were Na Wa, Row Pattana and Ta Rue sub-districts. In Na Wa districts, the areas covered Ta Jampa, Panom and Ramrat sub-districts at Nakhon Phanom province, Thailand. Three hundred and seventy farmers were obtained by the purposive sampling. The farmer samples were categorized into two groups by the purposes of the buffalo production: Group 1 was the farmers focused in fancy buffalo and shows whereas people in Group 2 aimed to gain the draught buffalo and meat production.

Statistical analysis

Data in the study was classified to 4 catalogues. 1) Farmer's personal information consisted of gender of farmer, educational level (no education, primary school, high school, and bachelor degree), type of labor (family and hired people), and source of knowledge and information used for buffalo production (book and magazine, seminar and training, from other farmers and governmental officers). 2) factor of buffalo production and management was defined as patterns of buffalo production (rounded up the herd, tied up with housing, raised in wall round stall, and cut and carries fresh grasses or rice straw to their housing), kinds of land (land belongs to themselves, land from rent and land from public), objectives for buffalo production (produce calves for selling, for fancy and value added, for fertilizer and for as the heritage), record keeping (no kept record, sometimes kept record, and kept record), and types of roughage (fresh grasses and rice straw). 3) factor of decision making on selection was classified as person who suggest to select buffalo

(farmers themselves, lectures, farm personnel, government officials, and head of group), mating systems (natural mating, artificial insemination, and both natural mating and artificial insemination), changing sire (no changing sire and changing sire), information for considering replacement dams selection (no selection, phenotype characteristics, pedigree of sire and dam, and fertility of buffalo), and age at first mating (less than 22 months, 22 to 24 months, 25 to 27 months, and more than 27 months). 4) factor that related with the problem and obstacle of buffalo production (lack of area for buffalo production, lack of fresh grasses and rice straw, lack of labor for buffalo production, lack of knowledge to produce buffalo production, lack of knowledge to select sire and dam, and reproductive and disease problem). All this information was analyzed to evaluate the effects on buffalo production between two groups of farmers (Group 1 who raised buffalo as fancy buffalo and shows, and Group 2 who raised buffalo for draught and meat production). Least square means of the studied traits were estimated by the considering factors, and then were compared using a chi-square test, at an $\alpha = 0.05$.

RESULTS AND DISCUSSION

Farmer's personal information

Factors of farmers' characteristics were gender, education level, type of labor and source of knowledge used for buffalo production. It could be classified the purposes of raising buffalo into two different groups: Group 1, the group of farmers who produced the swamp for fancy buffaloes and showing (accounted for 20.27%); and Group 2, who produced the buffaloes for power work and meat production (79.73%). It was interesting to find that sources of knowledge were significantly important ($P<0.01$), but gender, education level and type of labor did not strongly relate to achievements of buffalo production ($P>0.05$). The results showed that swamp buffalo production depended on sources of knowledge applied to buffalo production. In the Group 1, half of the farmers obtained knowledge from seminars and training (50.00%), followed by learning from other farmers (48.00%). There were only 2.00% of farmers in this group who received knowledge from governmental officers, and book and magazine. On the other hands, most farmers who raised their buffaloes as draught buffalo and meat production obtained knowledge from other farmers (64.00%), followed by from governmental officers (32.00%), book and magazine (2.00%), and seminar/training of the buffaloes (2.00%).

This indicated that the farmers in Group 1 could actively receive knowledge and practice as being leant from seminar and training. This was different from farmers in Group 2, who received the secondary information from other farmers mostly and was not able to earn the knowledge personally. This may lead to the inability to apply the knowledge in practice directly. For farmers in the Group 1, to become a fancy buffalo for shows, the animal needs to meet many special requirements on phenotype as well as behavior and genetic characteristics. Thus, the buffalo was not only high valuable, but also was their hobby and concern. This can explain why farmers in this group paid more attention to their products, motivated in accumulation of new knowledge and willing to spend more for any innovation (Suhachavalit *et al*., 2013; Yaemkong *et al*., 2017). However, in the past, all farmers raised buffalo either as a source of power work for agricultural activities or source of meat for human. Since mechanism growing and increasing demand of human for cultural and

social entertainment, they depend less on animal power and lead to the increasing proportion of buffalo for shows. The differences of productive purpose resulted in differences of farmer's attitude, interests, and behavior in buffalo production (Chantalakhana, 1991 and 1994; Bunyavejchewin, 1995).

Buffalo farming production and management

Among all the factors, patterns of buffalo production, kinds of land, the objectives of buffalo production, record keeping and types of roughage that were related significantly to the achievement of buffalo production in the region for both groups ($P<0.01$). The study also found the differences of patterns used for buffalo production, kinds of land, objectives for raising buffalo, record keeping, and types of roughage between two groups.

Most of farmers in the Group 1 (51.00%) cut fresh grasses or rice straw and carries them to feed their buffaloes which were kept in cage. Proximately (27.00%) of them raised in wall round whereas the others stall buffalo rounded up the herd (16.00%) and tied up with housing (6.00%). These farmers mainly used their own land for buffalo raising (57.00%), while 40.00% of farmers used public land for raising buffalo and the others (3.00%) had to rent land to raise their buffaloes. In opposite, only 4.00% of farmers in the Group 2 cut and carries fresh grasses or rice straw for their buffaloes. Most of them (55.00%) kept their animals rounded up the herd, followed by tied up with housing (28.00%), and the others raised buffalo in wall round stall (13.00%). This group of farmers also relied more on public land with 53.00% of surveyed farmers often release the animals to public land. Only 32.00% of them used their own land for buffalo and the remain (15.00%) needed to rent land for their animals.

To maintain the farm, most of farmers in the Group 1 (57.00%) stated that they could produce calves for selling and got added value from fancy buffalo to generate income. Besides, they could get benefit from this activity by using manure as fertilizer for plantation (25.00%) and the other raising buffalo as the way of cultural conservation and heritage for their descendant (18.00%). In the contrast, only 29.00% of farmers in the Group 2 could earn money from selling calves. Besides of using buffalo as working power, most of them considered raising buffalo for collecting fertilizer (55.00%) whereas the others considered this activity concerning to cultural conservation and heritage (16.00%). The result from Group 2 agreed with those from Sapanan *et al*. (2013) who reported that the main objectives of raising buffalo were utilizing of buffalo's manure (38.00%), selling their animals (35.20%) or maintaining the activity from their parent (26.80%).

The number of farmers keeping farm record was low in Group 1. the results showed that 60.00% of farmers did not keep the records, 25.00% of them sometimes keep it and only 15.00% of them always did. In the contrast, most of farmers in the Group 2 (92.00%) always kept the farm record, 7.00% kept it sometimes and only 1.00% never did it. Although the value of buffalo in the Group 2 was lower than that of Group 1, farmers in Group 2 paid more attention for keeping record. Buffalo seemed to be more important for this group of farmers when more people realized the necessary of this habit. Probably, it was not only because of they were poorer, they were also a tool for them to maintaining the agricultural farm (rice cultivation and power for transportation on the field).

The results of the study also found that farmers in the Group 1 used mostly roughage as fresh grass (accounted for 81.00%) and remain

used rice straw (19.00%). As consequent, farmers spent more time to either grow or collect grass for their buffalo in order to serve them better food. In contrast for farmers in Group 2, the number of farmers used fresh grass and rice straw seemed to be equal (48.00 and 52.00% respectively for fresh grasses and rice straw). It indicated that farmers in Group 2 did not care much for the type of roughage used for their buffalo.

Decision making in breeding selection

Factors of decision making on selection were determined by person who decided or strongly influenced to the decision to select buffalo, mating systems, changing sire, information for considering replacement dams selection, and first mating age. All of these factors were related significantly to the achievement in buffalo production of farmers ($P<0.01$). In the Group 1, almost half of the farmers (48.00%) need help from governmental officers to select sire, 41.00% of them consulted from neighbor farmers, the others looked for help from lecturers (8.00%), and materials the head of Group (3.00%). In contrast, most farmers in the Group 2, 9.00% of the farmers could give decision by themselves in selection of buffalo. Most of them get consult from other farmers (56.00%), from governmental officers (25.00%) and from head of group (10.00%). Farmers of both groups primarily used natural mating (64.00 and 87.00%), followed by both natural mating and artificial insemination (20.00 and 8.00%), and artificial insemination within their farms (16.00 and 5.00%, respectively for Group 1 and 2). All of farmers in the Group 1 frequently changed sire to mate their dam whereas 35.00% of farmers in the Group 2 changed sire but within their farms, the remain (65.00%) have never changed sire at all.

In the factor information for consideration of dam replacement, 60.00% of farmers in both groups considered phenotype characteristics as a parameter to decide replacement of dams. However, 32.00% of farmers in Group 1 and 16.00% farmers in Group 2 considered fertility of buffalo as indicator to replace dams.

The majority of farmers in the Group 1 (54.00%) stated that their buffalo got the first mating at the age of 25 to 27 months whereas 41.00% reported that the first mating of their buffalo was more than 27 months and the other recorded for 22 to 24 months (5.00%). While farmers in the Group 2 reported that their buffaloes got longer time for this parameter. Most of farmers (82.00%) reported that their buffaloes needed 27 months to get first maturity, followed by 25 to 27 months (9.00%), less than 22 months (8.00%) and 22-24 months (1.00%), respectively.

It was noticed that this group used male buffaloes to control the buffalo herd in their farms but sires have never changed. This might cause the reduction of body size in the next generation. All though farmers of both groups substituted female buffaloes by considering their phenotype, the heredity of buffalo herd also depended on genealogy of animals with many genetic parameters needed to be considered. Furthermore, expertise and knowledge with accurate manipulation could influence also to the successes. Nevertheless, 21.00% of farmers never substituted female buffaloes in the study was still high.

In another study, Punsawat *et al.* (2007) who studied the buffalo rearing in Nakhon Si Thammarat found that a large number of the farmers reared the buffaloes in order to continue habits or traditional activities from their predecessors. In that system, natural method of breeding without selection of heredity was mainly adopted. This technique could affect to the size of buffaloes, resulted in smaller

size and inability to off-springs (Satchaphun *et al.*, 2005). The results in current study agreed with another one carried out by Sarakul (2010) who reported that the level of education, experiences, labor, source of knowledge, pattern of rearing, feed and the experts who advised for the decision making could affect to the quality of breeding selection.

Factor that related with the problem and obstacle of buffalo production

The problem and obstacle of buffalo production was the factor that inhibit the achievement in buffalo production of farmers ($P<0.01$). The results revealed that most important problems and obstacles for farmers of both groups were lack of area for buffalo production (56.00% and 36.00% respectively for Group 1 and 2), followed by lack of labor for buffalo production (19.00 and 22.00%). Lack of knowledge in buffalo production seemed to be a problematic for farmers in the region also when 12.00% and 11.00% of farmers in the Group 1 and 2 stated it. Some farmers considered lack of knowledge to select sire and dam (7.00 and 12.00%), lack of fresh grasses and rice straw (3.00 and 10.00%), and reproductive and disease problem (3.00 vs 9.00%), respectively.

This result was similar to those reported in many previous literatures (Pookduang and Pinyotepprathan, 2008; Phothong *et al.*, 2013; Sapanan *et al.*, 2013; Jumrasboonhirun and Akkrajun, 2015; Phasuk and Ruangchoengchum, 2016). These studies pointed out that the biggest problem and obstacle of buffalo farmers was the lack of pasture, forage and knowledge. This could be occurred because the land in this area was mainly used for cultivation of rice, sugar cane and cassava or rubber tree (Sarakul *et al.*, 2016). In the study area, farmers used the natural grass growing around the field and rice straw - a byproduct from rice production as feed for buffalo. Some farmers could use a part of their land for growing grass but it was not enough (Phasuk and Ruangchoengchum, 2016). However, since buffalo production was not the major activity, they could not get priority for land use that led to lacking of pasture, space and resulted in the lack of feed and inadequate of roughages. Facing to this limitation, farmers should know how to collect, process and store roughages for their buffaloes in dry season. This point agreed with other studies by Pookduang and Pinyotepprathan (2008); Yaemkong *et al.* (2017); Yaemkong *et al.* (2018a); Yaemkong *et al.* (2018b) who reported that lack and inadequate of roughages were the problematic for raising buffalo, beef and dairy cattle. All of these problems and obstacles should be suggested to governmental authorities, policy makers who could support and promote the development of buffalo production in the region.

This study also illustrated that there were many factors could affect to the buffalo production in the region, in which the main factor affected to farm management relating to the knowledge of farmers was the capacity in updating new innovation and sources of information on buffalo production. The other factors such as rearing methods, space and feed as well as making decision for breeding selection also influenced to buffalo production in the region. This parameter involved to the persons (guru's or expert's) who advised farmers technique in breeding selection, breeding methods, sire selection and when farmer should change sires. The farmers in the study area also suggested that the Department of Livestock Development should provide financial support in terms of training and promoting sire selection; improving public land for raising buffaloes; forage production; using animal for agriculture; and using buffalo's manure

efficiently.

CONCLUSION

The conclusion for this research was confirmed that source of information used for buffalo production, patterns of buffalo production, kinds of land, the objectives for buffalo production, record keeping, types of roughage, person who suggest to select buffalo, mating systems, sire selection, information for considering replacement dams selection, age at first mating, and the problem and obstacle of buffalo production were related to the achievement in swamp buffalo production of farmers.

ACKNOWLEDGMENTS

The authors would like to thank the National Research Council of Thailand for funding this study. The research would not be completed without providing data from the Department of Livestock Development as well as participating of the buffalo farmers in Nakhon Phanom province. Thus, authors would like to give special thanks for their cooperation. Finally, authors also would like to thank Pibulsongkram Rajabhat University for other supports.

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MILK AND FATTY ACID COMPOSITION OF ANATOLIAN WATER BUFFALO (*BUBALUS BUBALIS*) FROM DIFFERENT PROVINCES

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ABSTRACT

The present study was undertaken (1) to characterize the fatty acid (FA) composition particularly on the concentration of conjugated linoleic acid (2) to investigate of physicochemical properties of Anatolian water buffalo milk, and from six different provinces in Turkey. The fat amount in water buffalo milk samples were in the range of 5.97±0.30% to 9.19±0.57% and the mean fat was 6.96±0.25%. The main individual FA in water buffalo milk were in the order 16:0, 18:1 *cis*-9, 14:0, and 18:0. The linoleic acid (CLA), bovinic acid (*cis*-9, *trans*-11), represented 1.09±0.06 in water buffalo milk. Saturated fatty acids (SFA) were the potent fraction in water buffalo milk fat (70.63±0.7); MUFA and PUFA were 29.37±0.7 and 0.2±0.03, respectively. The data exhibit statistically differences ($P \leq 0.05$) in the proportions of individual FA were detected among different provinces in Turkey. Anatolian water buffalos were poor in terms polyunsaturated FA compared to other water buffalos from different countries. Therefore, these results may provide useful information about the nutrient composition of buffalo milk and further studies are warranted to improve the technological and nutritional characteristics of Anatolian buffalo milk.

Keywords: buffalo milk, fatty acids, physicochemical properties, linoleic acid

INTRODUCTION

The principal sources of fat in the human diet are visible fats and oils, red meats, poultry, fish and dairy products. Fats forms a large group of nutrients, making a main component of dairy products, which provide vital components for the human diet. The effects of dietary fats generally reflect the cumulative effects of multiple fatty acids in the diet (Chandrasekharan, 1999). Milk fat contains ~400 different fatty acids (FA), which make it the most complex among natural fats. The milk fatty acids (FA) are produced almost equally from two sources, first in the rumen as a feed and the microbial activity and secondly in the mammary gland as *de novo* synthesis in animals. For FA in milk, short chain fatty acids (SCFA) (4 to 8 carbons) and medium chain fatty acids (MCFA) (10 to 14 carbons) derived almost exclusively from *de novo* synthesis, and long chain fatty acids (LCFA) (>16 carbons) are produced from the uptake of circulating lipids, while FA with 16 carbons originate from both sources (Liu *et al.*, 2016). Associations between consumption of fats and the incidence of some chronic diseases such as coronary artery disease, diabetes, cancer and obesity has been shown (Lawrence, 2013).

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An estimated 17.5 million died of cardiovascular disease in 2012 accounting for 46% of all noncommunicable disease deaths (Mendis, 2017). However, other studies prove that consumption of milk may favourably modify the body composition and cardiometabolic risk factors due to milk's conjugated linoleic acid (CLA) content (Lehnen *et al.*, 2015). Therefore, the production of dairy products from water buffalo milk with enriched mono- and polyunsaturated FA content, especially CLA and omega-3, benefits for high nutritional properties of produced food.

In the last three decades, milk production in the world has incremented by more than 50%, from 500 million tons in 1983 to 811 million tons in 2017 (FAO, 2018). Globally, 16.9% of milk consumed by humans comes from livestock species other than cattle (Faye and Konuspayeva, 2012) and water buffalos (*Bubalus bubalis*) are the world's second massive milk producer with 14% behind the dairy cow. There are two main groups of water buffalos: river buffalo and swamp buffalo in the world (Kumar *et al.*, 2007). In Turkey, only one breed, the Anatolian water buffalo is present, and it is classified as Mediterranean river type (Soysal *et al.*, 2010) which represents the best characteristics for milk production. Water buffalo milk products (WBMP), such as mozzarella cheese, curd, yogurt, cream and ice cream, are getting more and more popular due to WBMP's higher concentrations of protein, fat, lactose, minerals and vitamins in buffalo milk (Han *et al.*, 2012). Water buffalo milk and its derived products could be a good source of CLA for humans, like other food products from ruminants because bacteria present in the rumen may biohydrogenate dietary PUFA to form CLA. Despite its nutritional value, currently, information on chemical composition including CLA content in water buffalo milk is very limited in the world and kind of vague in Turkey. Therefore, the objective of this study was to investigate physicochemical composition and FA content of Anatolian water buffalo milk from six different provinces in Turkey.

MATERIALS AND METHODS

Animals and milk sampling

Water buffalo milks corresponded to individual milks taken from 57 buffalos of Mediterranean breed of *Bubalus bubalis* from six different provinces where most intensive water buffalo breeding present in Turkey (Table 1). Provinces were selected according to water buffalo population. All milk samples were collected in same season (April and May, 2018). All animals were grazed in the pasture and swamp area during this period. In Turkey, the distribution of plants in pastures; 15% leguminous, 10% grasses and 75% other plants (belonging to other families) have been reported (Avağ, 2018). In addition, all animals were allowed to consume at least 60% of the roughage during the sampling. Drinking water was always available in pasture. All the water buffalos were milked once a day during the milking period and milk samples were taken from this milking time. The farms were located in Afyonkarahisar (38° 45' 24.787" N 30° 32' 19.334" E), Balıkesir (39° 39' 11.873" N 27° 53' 25.231" E), Diyarbakır (37° 55' 29.903" N 40° 12' 39.539" E), İstanbul (41° 10' 48.155" N 28° 44' 17.426" E), Kayseri (38° 40' 29.572" N 35° 18' 28.177" E) and Samsun (41° 16' 46.931" N 36° 20' 9.841" E) provinces. Forty mL of fresh milk was then drawn and released into 50 mL falcon tube where they were preserved with antibiotic tablet (Bronolab Broad Spectrum Microtabs, 18 mg tablet: comprises 8 mg Bronopol and 0.30 mg Natamycin) and transferred via

insulated foam containers with cooling cassettes. Milk samples were stored 4°C until portions were analyzed the following day. Prior to analysis of FA content, water buffalo milk samples were stored at -20°C.

Determination of physicochemical and fatty acid composition in the water buffalo milk

All milk samples were analysed for, fat, protein, lactose and non-fat dry matter by Milkana Multi - Test (Mayasan Gıda, İstanbul, Turkey). Milk fat extraction was prepared according to Gerber method (James, 1995). One-step extraction-transesterification process (Sukhija and Palmquist, 1988) was used to prepare fatty acid methyl esters (FAME). The FAME profile for a 0.6-µl sample at a split ratio of 1:50 was generated using a Schimadzu, GC 2010 plus gas chromatography equipped with a flame ionization detector (Schimadzu, Kyoto, Japan), a capillary column (60 m × 0.25 mm ID × 0.250 µm (cat. # 13199)) and H_2 as the carrier gas. The FAMEs were separated using a temperature gradient program (Injection: 2.0 µL split (split ratio 200: 1)), 4 mm inlet liners (cat # 20814), injection temperature: 225°C, carrier gas: hydrogen, flow rate: 1.2 ml / min, oven temperature: 100°C (4 minutes) to 240°C (10 minutes) 3°C / minute) and the peaks were identified based on comparison of retention times with authentic standard (Supelco #37, Supelco Inc., Bellefonte, PA, USA; L8404 and O5632, Sigma Aldrich, St. Louis, MO, USA). Milk fat CLA level was identified based on comparison of retention times with CLA standard (cat # 16413 Sigma-Aldrich, St. Louis, MO, USA).

Statistical analyses and calculations

One-way ANOVA was done for assessment of the differences in the FA composition among provinces. The means were determined by using Duncan's multiple range tests. The results of the statistical analysis were presented as mean values and standard error of the means (SEM). Level of significance was considered as $P<0.05$. ANOVA was performed in IBM SPSS Statistics for Windows, version 17 (IBM Corp., Armonk, N.Y., USA). In order to analyze the influence of the whole composition of the measured FAMEs on provinces a principal component analysis (PCA) was used. PCA is a multivariate statistical method that are widely used in situations when the effect of many possibly correlated predictor variables into a set of values of linearly uncorrelated variables. PCA analysis was performed in paleontological statistics software package (PAST) version 3.20 (Hammer, Harper and Ryan, 2001).

Atherogenicity indices were calculated as the content ratio of SFA / unsaturated FA using the following formula proposed by (Ulbricht and Southgate, 1991):

$$\text{Atherogenecity index } (AI) = [C12{:}0 + 4(C14{:}0) + C16{:}0)] / \Sigma (MUFA + PUFA)$$

The Δ^9 desaturase ratio were used as an indicator of the Δ^9 desaturase activity using FA that are substrates and products for Δ^9 desaturase and calculated using the following model proposed by (Lock and Garnsworthy, 2003):

$$\text{Desaturase index } (DI) = C14{:}1 / C14{:}0$$

RESULTS

The physical characteristics such as fat, non-fat dry matter, lactose, protein and pH, are crucial parameters for the physicochemical properties and nutritional attitude of milk.

Different physical properties of the different milk samples from 6 provinces in Turkey belongs Anatolian water buffalo were shown in Table 1. The fat amount in water buffalo milk samples were in the range of 5.97±0.30% to 9.19±0.57% and the mean fat was 6.96±0.25%. In the present experiment, the lowest fat content was identified for the P5 province water buffalo milk while for P2 province showed the highest value (Table 1). Among the water buffalo milk samples, the lowest value was identified for non-fat dry matter in P2 province, followed by P5 province milk and the highest was demonstrated in P1 province milk samples (Table 1). For the protein, the lowest value was recorded for P2 province 3.12±0.06% and the highest was for the P3 province milk samples 3.77±0.07%. In general pH is the measure of sample acidity and alkalinity and small differences were detected for the pH measurement in all the milk samples. The pH values for the milk samples were in between 5.20 and 5.26. P3 milk samples showed the highest pH among all investigated milk samples (Table 1). Lactose which is known as milk sugar and is consisted of galactose and glucose. In all investigated milk samples, P3 province water buffalo milk contained the highest and P2 province milk samples lowest amount of lactose (Table 1).

Milk fat concentration, mean concentration of some individual FA and the sum of FA in different provinces are summarized in Table 2. Milk FA was quantified by gas chromatography fitted with a flame-ionization detector and a FA standard mix (contain 37 FA). Nineteen FA could not be detected with enough accuracy thus they were excluded in the statistical analysis. Those FA have been indicated as unknown followed by their retention time (in minutes) in the chromatographic run. In present study, the major FA (expressed as mean ± SEM in g / 100 g fat) were C16:0 (palmitic acid, 34.90±0.5), *cis*-9 C18:1 (oleic acid, 25.97±0.74), C18:0 (stearic acid, 14.33±0.48), and C14:0 (myristic acid, 11.13±0.27) accounting for approximately 86% of the total milk fat. C4:0 (butyric acid, 2.66±0.08), C12:0 (lauric acid, 2.09±0.07), C15:0 (pentadecanoic acid, 1.54±0.13) and C10:0 (capric acid, 1.52±0.07) were also relatively abundant in water buffalo milk samples which was approximately 7.5% of the total milk FA (Table 2). The main CLA, bovinic acid (*cis*-9, *trans*-11), represented 1.09±0.06 in water buffalo milk (Table 2).

Saturated fatty acids (SFA) were the potent fraction in water buffalo milk fat (70.63±0.7); MUFA and PUFA were 29.37±0.7 and 0.2±0.03, respectively. Considering the classification based of the carbon chain length: medium-chain fatty acids (MCFA) exhibited the greater part (51.77%) of the water buffalo milk fatty acid fraction, whereas long chain fatty acids (LCFA) and short chain fatty acids (SCFA) represented 41.81% and 6.21%, respectively. Among the unsaturation index and ratios, the atherogenicity, had the highest mean percentage 2.07±0.07. In the study, three ratios (DR14, DR16 and DR18) were calculated to estimate FA acid desaturase activity and get an indication of the syntheses of unsaturated FAs. Ratios given in the present study represent the product / substrate relationship for desaturase. Highest desaturase, desaturase ratio was found for DI14, DI16 and DI18; 0.08±0.00, 0.02±0.00 and 0.64±0.01 respectively.

The results are shown in Table 2 by applying one-way ANOVA which FA differences according to the provinces. However, potential coherences of different FAs are not considered in this analysis. Therefore, the principal component analysis (PCA) was used in order to determine principal components (PC) based on the whole FA

composition. The data consisted of 57 rows (milk samples) and 18 FA measurement including fat and protein. Loadings and PCA scores plot of the first and the third PC of the auto-scaled data showed in Figure 1. The data matrix explained approximately 91.3% of the total variance. The difference between the samples from six provinces is clear. P5 province lay mainly in the NE quadrant with positive scores on the first PC and positive scores on the third PC, whereas P3 occupied the SE quadrant with negative scores on the first PC and positive scores on the third PC. West Anatolian samples lay mainly in the NW quadrant with positive scores on the first PC and negative scores on the third PC (Figure 1).

DISCUSSION

The milk quality is greatly determined by its chemical composition such as protein, lactose and fat and FA contents due to its direct relation to human health, and the organoleptic characteristic. The level of physicochemical compositions in the measured milk samples were also compared with the previously reported literature from Anatolian water buffalo (Ermetin, 2017) and as well as from different countries (Khan, Islam and Siddiki, 2007; Imran *et al.*, 2008; Kashwa, 2016). Similar milk fat percentages were described for Anatolian water buffalos (Şekerden, 1999), although sometimes lower values were reported (Tonhati *et al.,* 2011), likely reflecting the effect of different management, feeding, and environmental conditions. The results show that values for the fat in all the milk samples are in good agreement with the reported literature. Şekerden (1999) reported Anatolian water buffalo fat content as 7.1% which is lower than western Anatolia samples but higher than central and east Anatolia water buffalo milk samples. In the current study, fat content was found to be much higher in P2 and P1 samples and slightly higher in P4 samples compared to the Mediterranean water buffalos which are reared in Sweden (Kashwa, 2016). Khan *et al.* (2007); Imran *et al.* (2008) and reported that the fat in the milk was 7.3% and 7.6% in Bangladesh and Pakistan water buffalo milk samples, respectively which are less than what we found in the present study (Table 1). Fat content of water buffalo milk samples were also found similar in Western Anatolia samples but lower in central and eastern Anatolia samples compared to Italian and Bulgarian water buffalo samples (Mihaylova and Peeva, 2007; Tufarelli, Dario and Laudadio, 2008). The concentration range of total solids (non-fat dry matter) was from 8.12±0.41% to 9.96±0.53% as given in Table 1. The amount of non-fat material is 9.6±0.8% in Anatolian water buffalo (Şekerden, 1999) and 9.8±0.1% (Imran *et al.*, 2008) in Pakistan buffalo samples. The results show that values for the total solids in all the milk samples are in good agreement with the reported literature. In the current milk samples, lactose was in the range of 4.38±0.27% to 5.44±0.59% (Table 1). Tufarelli, Dario and Laudadio (2008); Imran *et al.* (2008); Mihaylova and Peeva (2007) reported that the lactose in the milk was from 4.6% to 4.85% which is slightly lower than found in the current study. Not relevant differences seemed to be detected in total SFA and UFA.

This is the first comprehensive data on the FA content of Anatolian water buffalos. Although data about physical properties were reviewed in literature no data was available for the FA content of Anatolian water buffalo (Abd El-Salam and El-Shibiny, 2011). Main FA profile in the present study coincided with that reported for Mediterranean buffalos in (Pegolo *et al.*, 2017) in which the major individual FA in water

buffalo milk were 16:0, 18:1 *cis*-9, 18:0, and 14:0. Similarly, SFA were the predominant fraction in Mediterranean water buffalo milk fat (70.49%); and monounsaturated, PUFA were at 25.95 and 3.54%, respectively (Varricchio *et al.*, 2010; Pegolo *et al.*, 2017). However, we identified lower mean PUFA for Anatolian water buffalos (0.20, Table 2) compared to Mediterranean buffalos, reared in Italy (Varricchio *et al.*, 2010; Pegolo *et al.*, 2017). The same FA pattern was also observed in Nili-Ravi buffalos in Pakistan, but they also identified PUFA as 4.91 (Qureshi *et al.*, 2010) which is higher than that of which was identified in the current study. PUFAs play a crucial role in cells' physiological and biochemical processes and decreasing the risk of many diseases via resolving their inherent inflammation condition (Zárate *et al.*, 2017). Various studies showed that the FA profile of milk and its dairy products can be changed due to feeding system and stage of lactation in sheep (Cividini *et al.*, 2018), dairy cattle (Odongo *et al.*, 2007) as well as in Mediterranean water buffalo (Pegolo *et al.*, 2017). Chilliard *et al.* (2000; 2001) showed that feeding animals with different types of forages and animal fat or marine oils had potential effects on polyunsaturated FA (Chilliard *et al.*, 2000).

Palmitic acid (PA) (C16:0) was found as the major SFA in the current study (Table 2) and almost same mean values were detected in Mediterranean water buffalos (Bergamo *et al.*, 2003; Ménard *et al.*, 2010; Varricchio *et al.*, 2010; Pegolo *et al.*, 2017) and silage fed water buffalos in Bulgaria (Penchev *et al.*, 2016) by various researchers. However, compared to Anatolian water buffalos in the present study, PA acid content in Murrah buffalos found lower (Fernandes *et al.*, 2007; Shelke and Thakur, 2011). Although PA is known for putative detrimental health effects, it has multiple crucial physiological activities in order to maintain membrane phospholipids balance (Carta *et al.*, 2017). However, more than palmitic acid' uptake, balance of dietary PA / PUFA ratio is crucial to avoid unwanted physio-pathological conditions (Carta *et al.*, 2017). The relative high content of lauric acid (C12:0) among detected MCFA in the present study which may display antibacterial activity against various Gram-positive strains (Batovska *et al.*, 2009).

Concerning the unsaturated fatty acids (UFAs), substantial proportions of oleic acid (OA) (C18:1ω-9) and conjugated linoleic acid (CLA) isomer, C18:2 *cis*-9 *trans*-11 (rumenic acid) acids were identified in raw milk lipids. Evidences in the last years have showed the effects of OA in human health and disease in which OA may improve the immune response associated with elimination of pathogens such as bacteria and fungi (Sales-Campos *et al.*, 2013). CLA is attracting interest because of its expressed effects on body composition specifically a reduction in body fat mass together with anticarcinogenic, antiatherogenic, antidiabetogenic, and immune modulating properties (Rainer and Heiss, 2004). The mean CLA in Anatolian water buffalos (Table 2) were higher compared water buffalos reared in Italy. Pegolo *et al.* (2017) found CLA *cis*-9, *trans*-11 isomer as 0.45 and Menard *et al.* (2010) found the same isomer as 0.90 more similar to findings of the current study. Murrah buffalos in Brazil showed higher CLA contents (1.242) as compared to the results obtained in the present study. The concentration of CLA in milk fat can be enhanced by changes in the livestock nutrition via ruminal biohydrogenation (Chilliard, Ferlay and Doreau, 2001; Daley *et al.*, 2010). However, endogenously production of CLA from adipose tissue and the mammary gland in lactating dairy

Table 1. Least squares means and standard errors for the physicochemical parameters in water buffalo milk from different provinces.

Traits	Provinces								
	P1	P2	P3	P4	P5	P6	Mean	SEM	P-value
n	7	11	10	11	8	10	57		
Fat, %	8.14^{ab}	9.19^{a}	6.81^{bc}	7.66^{abc}	5.97^{c}	6.83^{bc}	6.96	0.256	**
Non-fat dry matter, %	9.58^{ab}	8.12^{d}	9.96^{a}	9.32^{abc}	8.87^{c}	9.04^{bc}	9.14	0.118	***
Lactose, %	5.20^{ab}	4.38^{c}	5.44^{a}	5.06^{ab}	4.83^{b}	4.92^{b}	4.97	0.066	***
Protein, %	3.65^{ab}	3.12^{d}	3.77^{a}	3.55^{abc}	3.37^{cd}	3.44^{bc}	3.48	0.043	***
pH	5.21^{bc}	5.21^{b}	5.26^{a}	5.20^{c}	5.21^{bc}	5.21^{b}	5.21	0.003	***

[abcd] Means in the same raw for each type of capsule without common letter differ significantly (P≤0.05); n = number of samples. SEM: standard error of means; NS P>0.05; *P≤0.05; **P≤0.01; *** P≤0.001.
P1: Afyonkarahisar; P2: Balıkesir; P3: Diyarbakır; P4: İstanbul; P5: Kayseri; P6: Samsun.

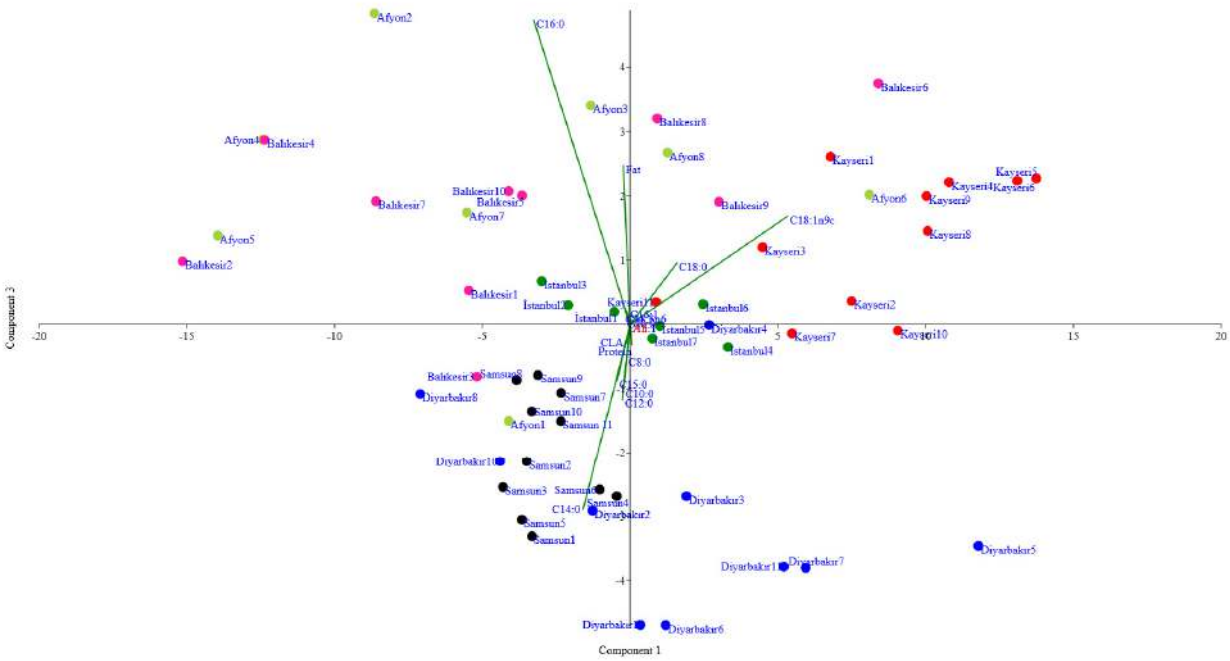

Figure 1. Principal component analysis (scores and loading plot) of Anatolian water buffalo milk FA profile. Loading plot describing the relationship among milk FA derived from a principal component analysis based on proportions (% of total FA) in milk from six provinces (n = 57). Three clusters of FA were distinguished. Conjugated linoleic acid (CLA) and milk protein percentage were loaded opposite (negatively related) to milk fat percentage whereas saturated palmitic and unsaturated oleic acid were loaded with milk fat percentage.

Table 2. Least squares means and standard errors for FA content (g/100 g fat) in water buffalo milk from different provinces.

Fatty acids	Provinces								
	P1	P2	P3	P4	P5	P6	Mean	SEM	P-value
C4:0	2.67^{ab}	2.71^{ab}	2.20^{b}	2.88^{a}	3.03^{a}	2.54^{ab}	2.66	0.08	*
C6:0	0.59^{b}	0.63^{b}	1.95^{a}	1.67^{a}	1.80^{a}	1.29^{ab}	1.25	0.14	*
C8:0	0.84^{b}	0.51^{c}	0.88^{b}	1.06^{a}	0.64^{c}	0.67^{c}	0.77	0.03	***
C10:0	1.70^{b}	1.00^{c}	1.79^{b}	2.13^{a}	1.23^{c}	1.29^{c}	1.52	0.07	***
C12:0	2.27^{b}	1.54^{c}	2.39^{b}	2.75^{a}	1.76^{c}	1.84^{c}	2.09	0.07	***
C14:0	11.97^{ab}	8.02^{c}	12.02^{ab}	12.73^{a}	10.98^{b}	11.46^{ab}	11.13	0.27	***
C14:1	0.88^{bc}	0.58^{d}	1.06^{b}	0.64^{cd}	1.16^{ab}	1.43^{a}	0.95	0.06	***
C15:0	0.99^{cd}	0.73^{d}	1.47^{bcd}	2.56^{a}	1.84^{b}	1.53^{bc}	1.54	0.13	***
C15:1	0.53^{b}	0.35^{c}	0.55^{b}	0.49^{bc}	0.47^{bc}	0.74^{a}	0.52	0.03	***
C16:0	35.07^{b}	31.89^{c}	32.05^{c}	34.91^{b}	38.53^{a}	38.06^{a}	34.90	0.50	***
C16:1	0.65^{b}	0.44^{c}	0.65^{b}	0.50^{c}	0.78^{a}	0.86^{a}	0.63	0.02	***
C18:0	13.76^{b}	18.48^{a}	14.37^{b}	12.67^{b}	12.90^{b}	13.10^{b}	14.33	0.48	***
C18:1n9*c*	26.82^{b}	32.23^{a}	26.48^{b}	23.51^{b}	22.90^{b}	23.14^{b}	25.97	0.74	***
CLA (*c*-9*t*-11)	0.87^{bc}	0.65^{c}	1.49^{a}	1.10^{ab}	1.26^{ab}	1.18^{ab}	1.09	0.06	***
C20:0	0.25^{c}	0.16^{c}	0.51^{b}	0.31^{c}	0.74^{a}	0.59^{ab}	0.42	0.04	***
C18:3n6	0.14^{bc}	0.07^{c}	0.14^{bc}	0.09^{c}	0.61^{a}	0.31^{a}	0.21	0.04	***
∑SFA	70.11^{ab}	65.67^{b}	69.63^{ab}	73.66^{a}	72.82^{a}	72.34^{a}	70.63	0.70	**
∑MUFA	29.89^{ab}	34.33^{a}	30.37^{ab}	26.34^{b}	27.19^{b}	27.66^{b}	29.37	0.70	**
∑PUFA	0.14^{b}	0.07^{b}	0.14^{b}	0.09^{b}	0.61^{a}	0.23^{b}	0.20	0.03	***
SCFA	5.80^{bc}	4.86^{c}	6.81^{ab}	7.75^{a}	6.07^{bc}	5.78^{bc}	6.21	0.21	***
MCFA	52.36^{ab}	43.56^{c}	50.18^{b}	54.58^{a}	55.52^{a}	55.90^{a}	51.77	0.80	***
LCFA	41.71^{bc}	51.51^{a}	42.86^{b}	37.59^{c}	37.80^{c}	38.00^{c}	41.81	0.92	***
AI	1.84^{a}	1.92^{a}	1.72^{a}	1.76^{a}	1.01^{b}	2.07^{a}	3.05	0.13	***
DI C14	0.10^{ab}	0.11^{a}	0.07^{cd}	0.08^{bc}	0.07^{cd}	0.05^{d}	0.08	0.00	***
DI C16	0.02^{b}	0.02^{a}	0.02^{ab}	0.02^{b}	0.01^{c}	0.01^{c}	0.02	0.00	***
DI C18	0.63	0.63	0.66	0.65	0.64	0.65	0.64	0.01	NS

SCFA: short-chain fatty acids; MCFA: medium-chain fatty acids; LCFA: long-chain fatty acids; CLA: conjugated linoleic acid; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids; ^{abcd} Means in the same raw for each type of capsule without common letter differ significantly (P≤0.05). SEM: standard error of means; AI: atherogenicity index; DI: desaturase index; NS P>0.05; *P≤0.05; **P≤0.01; *** P≤0.001. P1: Afyonkarahisar; P2: Balıkesir; P3: Diyarbakır; P4: İstanbul; P5: Kayseri; P6: Samsun.

cows by Δ^9-desaturase has been also demonstrated (Griinari *et al.*, 2000). Therefore, to assess the nutritional quality of lipids desaturase and atherogenicity indexes (AI) are calculated. The AI showing the inhibition of the aggregation of plaque and diminishing the levels of esterified FA, cholesterol, and phospholipids, thereby preventing the appearance of micro- and macro-coronary diseases (Ulbricht and Southgate, 1991). Among unsaturation indexes, Varricchio *et al.* (2010) stated that Mediterranean buffalos reared in Italy exhibits a range between 2.15 and 2.61% which is lower than the samples measured in the current experiment. Higher AI value implies a lower PUFA / SFA ratio and this is in accordance with the present findings that Anatolian water buffalos showed lower PUFA compared to Mediterranean buffalos (Varricchio *et al.*, 2010; Pegolo *et al.*, 2017).

CONCLUSIONS

Taken together this study presents a detailed analysis of FA profile of Anatolian buffalo milk, including FA present in small concentrations those may have a positive effect on human health. We confirmed Anatolian water buffalo milk samples showed variation in the FA profile due to the specific FA origin and metabolic pathway most probably related with their environmental conditions. Interestingly, East Anatolian water buffalos exhibit higher CLA content compared to West Anatolian ones and milk fat vice versa. Generally, Anatolian water buffalos were poor in terms polyunsaturated FA compared to other water buffalos from different countries. Therefore, these results may provide useful information about the nutrient composition of buffalo milk and further studies are warranted to improve the technological and nutritional characteristics of Anatolian buffalo milk.

ACKNOWLEDGEMENT

This work was supported by Research Fund of the Erciyes University, Kayseri, Turkiye. Project Number: FHD-2018-7909. We also appreciate Balıkesir, Diyarbakır and Kayseri branches of the Buffalo Breeders' Association of Turkey for their helps during sampling.

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PREDICTORS OF SUCCESS OR FAILURE IN ARTIFICIALLY INSEMINATED BUFFALO COWS IN BAYBAY CITY, LEYTE, PHILIPPINES: AN UNMATCHED CASE-CONTROL STUDY

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ABSTRACT

A case-control study was conducted to identify the factors associated with the success or failure of artificial insemination (AI) of buffaloes in Baybay City, Leyte, Philippines. The cow-calf pair was used as the unit of interest in this study regardless of breed and number of buffalo cows the farmers own. Of the 24 selected barangays, an equal number of cases (38 failed AI) and control (38 successful AI) were selected from 78 farmer-cooperators of the Philippine Carabao Center (PCC) AI program and data were collected using a questionnaire. On the one hand, our study found that every year increment beyond the age at first breeding of the cow could predispose the animals to fail by as much as 2.5 times when compared to younger cows. On the other hand, a monthly increment in the calf weaning age may increase the likelihood of AI success by as much as 50%. These results imply that the age at first breeding must be conscientiously considered to allow optimal sexual and physical maturity of the breeding cows while ensuring that first breeding does not occur with too much delay. Moreover, while more mature calves have higher chances of success at weaning, provision of wallow during pregnancy also appears to support AI success as an effective method of cooling.

Keywords: *Bubalus bubalis*, buffalo, artificial insemination, PCC, Philippines

INTRODUCTION

Large ruminants like cattle and buffaloes are an integral part of Asian agriculture. Millions of farmers particularly in Asia rely on cattle and buffaloes as source of meat, milk and draft power in agricultural operations (Nanda and Nakao, 2003). In East and Southeast Asia, it is not uncommon to find about 1 to 5 swamp type buffaloes raised per household as source of draft power and meat; or milk from riverine-type buffaloes in South and Southwest Asia (Cruz, 2007). It is therefore necessary that these animals receive considerable attention including optimal management, good nutrition and improved breeding practices to enhance their production potential and uplift the livelihood of smallholder farmers.

Buffaloes (*Bubalus bubalis*), considered as the farmer's docile friend in agriculture and a walking fertilizer factory is inseparable among traditional farming systems in Asia (National Research Council 1981; Roxas-Lim 1998; Murtaza *et al*., 2017). In the Philippines, a working buffalo works for about 84 to 98 days per year particularly in rice-based farming system (Alviar, 1987). Crossbred water buffaloes also provide

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considerably more edible and non-edible by-products after slaughter with several characteristics of buffalo meat including crude protein, ash, fat, cholesterol comparable to the more preferred beef (Lapitan *et al*., 2007).

The vast majority of buffaloes worldwide can be found in Asia representing nearly 2 million in 2004 (Cruz, 2007). Unfortunately, a decline in carabao inventory requires considerable attention brought about by low productivity, poor nutrition and indiscriminate slaughter among others. Unlike cattle, buffaloes only received minimal attention from the government and researchers in the past possibly due to inherent merits of this species, along with the massive introduction of farm mechanization (Nanda and Nakao, 2003).

In Pakistan, buffaloes appear to be affected more often by repeat breeding syndrome than in cattle (Khan *et al*., 2016) causing about 3 to 6% of the herd being culled annually (Bartlett *et al*., 1986; Yusuf *et al*., 2010). Potential reasons for this are due to higher occurrence of silent estrus in buffalo cows as well as their docile nature which can be aggravated by several factors including breed, and agro-climatic and management conditions (Khan *et al*., 2016). Ovulatory disturbances and reproductive tract infection (Kutty and Ramachandran, 2003) as well as hormonal aberrations alongside a combination with other factors were also identified to influence repeat breeding syndrome (Singh *et al*., 2008). In Egypt, typical repeat breeders accounted for 7.25% of total reproductive disorders in buffalo cows (Ahmed *et al*., 2010) and an incidence rate of 8.82% was reported in India (Kumar *et al*., 2011).

The Philippine government through R.A. 7307 better known as the Philippine Carabao Act of 1992 established the Philippine Carabao Center (PCC) with satellite stations throughout the country. It was mandated to develop and promote the buffalo industry though modern breeding techniques (i.e., artificial insemination or AI) and optimum management practices. The PCC dispersed Bulgarian-Murrah buffaloes to farmers including their native local stocks are direct recipients of PCC AI service. As reproductive efficiency in water buffaloes is notably lower than in cattle (*Bos taurus* and *Bos indicus*; Drost 2007), this study was aimed to identify putative factors associated with the success or failure of artificial insemination of buffalo cows in Baybay City, Leyte, Philippines.

Methodology

Survey design

An unmatched case-control study was conducted and administered using a written questionnaire on farmer-cooperators who are recipients of the PCC at VSU AI service as respondents. Of the 76 farmers from 24 representative barangays, 54 cow-calf pairs were qualified for the study: 27 cases and 27 controls. These respondents have had their buffalo cows bred using AI as method of breeding during the most recent breeding cycle as identified by the PCC technician.

The cow-calf pair was the unit of interest in this study regardless of what breed and number of buffalo cows the farmers owned. From the given population, cases (failed AI) and control (successful AI) were drawn as many as possible. Cases were identified as those cow-calf pairs that have any or all of the following: 1) the buffalo cow failed to get pregnant at first AI attempt within that specific breeding cycle; 2) either or both the dam and the calf died within 3 days of calving; and 3) occurrence of parturition disorders such as but not limited to fetal abnormalities, embryonic mortality, mummification, abortion, fetal death

and/or dystocia. Control cow-calf pairs included those other than the above.

Construction and administration of the questionnaire

A questionnaire was formulated following extensive and thorough review of the literature and upon close coordination with assigned experts concerning the buffaloes' reproductive efficiency. The questionnaire was comprised of three main parts. The first includes the demographic background of the farmer directly in-charge of the rearing and management of the animal. This also includes the production systems employed during breeding/insemination, during pregnancy and during parturition and the reproductive management that were also employed specific to that pregnancy or AI cycle. Secondly, the insemination technique was recorded based on the interview with the PCC technician. This comprised the detailed procedure and materials used during AI including timing, number of inseminations, semen used, thawing procedure, and drugs/hormones given. Finally, the general cow-calf information was recorded which include the assessment of the cows' reproductive performance, breed, age at breeding, and whether problems were encountered during parturition. The questionnaire was written in English but translated in the local dialect during the conduct of the interview.

Data management and analysis

Data from the questionnaire were consolidated using Microsoft Excel and transferred to a series of computer data files. Data entry errors were identified and outlying values were compared against the original data collection sheets.

Statistical analyses were conducted using Epi Info™ version 7.2.2.2 (Centers for Disease Control and Prevention, U.S. DHHS). Unconditional association between the failure in AI (dependent variable) and the plausibly related independent variables were assessed for crude association using the Chi-square test for categorical independent variables and Kruskal-Wallis test for non-normally distributed independent variables ($P \leq 0.25$). Variables found to have significant crude associations were examined further using the backward stepping logistic regression. All independent variables found to have unconditional association with the dependent variable were initially included in the model and the over-all *p* value evaluated. Thereafter, the least significant variables were identified and removed from the model one after the other and the model was run all over again. This procedure was repeated until all the remaining variables in the model and the over-all regression was significant ($P \leq 0.05$). In addition, standard descriptive statistics were conducted for the famers' demographic background, production systems used, reproductive management and AI procedure.

RESULTS

Both the age of calves at weaning and the provision of cow wallow during pregnancy were found to support AI success. A monthly increment in the calf weaning age may increase the likelihood of AI success by as much as 50%. Meanwhile, every year increment beyond the age at first breeding of the cow could predispose the animals to fail in AI by as much as 2.5 times when compared to younger cows (Table 1; $P \leq 0.05$).

Of the 76 farmer-respondents, 93.4% were males and 6.6% were females. Most the farmers did not complete high school (90.8%) and only 9.2%

reached college. Majority of the farmers (86.8%) were engaged in farming while the others (13.2%) were into fishing. More than half (57.9%) of the farmers spent about 2 to 3 h per day looking after their cows and 42.1% spent more than 3 h. Majority of the farmers (92.1%) had more than one-year experience in handling their cows. Interestingly, only 27.6% of the farmers had attended seminars regarding AI. Most of the farmers (89.5%) felt confident of the AI system (>60% of the rating scale).

As to the feeding management, most of the farmers practiced the tethered feeding system (89.5%) as compared to cut and carry (30.3%). One-hundred percent of the cows were fed with common grasses, half of them were given untreated straws and legumes in only 14.5%. Common grasses found in the study areas included Carabao grass, Paragrass and Napier grass. Notably, the majority of the cows were housed under trees and only about 2.6% were kept in the shed. All the animals have access to a wallow (rivers or man-made).

Most of the cows used in this study were native (81.6%) and about 18.4% were crosses. Inseminations were conducted mostly during natural onset of heat (81.6%) while 18.4% were induced. Nearly 95% of the cows were inseminated twice, 5.26% thrice and 1.32% once, conducted at early estrus (76.3%). Cows were first bred between 2 to 9 years old with a mean of 3.77 years for control and 4.52 years for cases. Cows' age during the AI cycle when the study was conducted ranges from 2 to 15 years with a mean of 7.98 and 6.10 years for control for cases, respectively. Mostly (84.56%), farmers did not give any assistance to the cow during calving.

Semen used for insemination mainly came from frozen-thawed semen from Bulgarian Murrah buffalo (BMB) bulls. Temperature and thawing time were either at 38ºC for 15 seconds or at 70ºC for 4.5 seconds, respectively. Most of the PCC technicians deposited the semen at the late cervix (80.3%) and took about 1 to 3 seconds (81.6%) to completely release the full semen from the AI instrument.

DISCUSSION

Poor breeding efficiency is often cited a major obstacle that leads to a lower production potential in water buffaloes (*Bubalus bubalis*) than in cattle (*Bos taurus* and *Bos indicus*). Nevertheless, despite many factors that hamper reproductive success in water buffaloes including seasonality, poor expression of estrus and long calving interval, its reproductive potential can be significantly

Table 1. Logistic regression showing predictor variables unconditionally associated with failure in artificial insemination of buffalo cows.

Predictor variables	Odds ratio	95% C.I.		Coefficient	S.E.	P-value
		minimum	maximum			
Cow age at first breeding	2.5384	1.1493	5.6062	0.9315	0.4043	0.0212
Calf age at weaning	0.4749	0.3219	0.7006	-0.7446	0.1984	0.0002
Cow wallow provision during pregnancy	0.0059	0.0002	0.1945	-5.1244	1.7791	0.0040

improved (Gwazdauskas *et al*., 1981; Drost 2007). This can be accomplished following established reproductive management techniques used in cattle given the vast similarities in the anatomy and physiology of the reproductive systems between the two species (Drost, 2007). Our study demonstrated that the age of buffalo cows at first breeding must be conscientiously considered to allow optimal sexual and physical maturity of the breeding cows. Moreover, considering the feeding practiced in smallholder cow-cafl operations, calves should be given sufficient time to grow and mature before weaning is initiated. The provision of cow wallow during pregnancy may also provide beneficial effects to mitigate potential impacts of heat stress on the reproductive health of buffalo cows.

The significant impact of the age of buffalo cows at first breeding puts a greater emphasis on the appropriate sexual and physical maturity of animals needed to successfully carry out pregnancy considering that buffaloes have later onset of puberty than cattle (Drost, 2007). While first calving in buffaloes is possible as early as 39 months (Tonhati *et al*., 2000) with an average of 53.88±0.48 months in Murrah and 51.51±1.18 months in Surti buffaloes (Gogoi *et al*., 2002), delaying first pregnancy until the animal is mature enough and have attained an optimal breeding weight would ultimately favour conception rates and thus AI success (Coleman *et al*., 1985). Our results suggest that the older the buffalo cows, the higher is the likelihood to fail in AI by as much as 2.5 in every year increment. This amount of time at first breeding as seen in our results appear to support the report of Nanda *et al*. (2003) saying that a large proportion of buffalo heifers reach puberty at 3 to 5 years of age (Nanda and Nakao, 2003). Since age at first calving trait has a low estimate of heritability (Seno *et al*., 2010), our results implies that management strategies including judicious provision of balanced nutrition and the use of performance modifiers might be helpful in promoting maturity of buffalo cows as previously described both in buffaloes and Sahiwal cows (Bhatti *et al*., 2007).

It appears that buffaloes are especially attracted to water and have been observed to wallow between 10 am and 3 pm where water is available, although buffaloes may wallow anytime in the presence of severe insect attack (Tulloch and Litchfield, 1981). It is not easily understood how the provision of wallow during pregnancy is associated with the success of artificial insemination as observed in our study but peculiar features of buffaloes such as dark skin, spare hair coat and poor sweating ability make these animals particularly susceptible to the effects of direct solar radiation (Ahmad and Tariq, 2010; Marai and Haeeb, 2010). Such conditions may predispose the buffaloes to hormonal imbalances, reduced feed efficiency and utilization and issues associated with reproductive performance (Ahmad and Tariq, 2010). It has been reported that during hot season, buffaloes may suffer from disruption in ovarian cyclicity leading 20 to 80% anoestrus (Nanda *et al*., 2003). However, buffaloes having access to a wallow have been demonstrated to have increased feed intake and milk yield (Aggarwal and Singh, 2010). These beneficial effects may be associated with an increase in the average plasma T4 and insulin levels in buffaloes wallowing in a water pond than those provided with water showers only during hot-dry season (THI of 83.6) as well as T3 levels during the hot-humid seasons (Aggarwal and Singh, 2010). Moreover, wallowing was demonstrated to be an effective method of cooling by cutaneous evaporation (Khongdee *et al*., 2011). It was shown that skin and rectal temperatures

were lower in the wallow group compared to those with water showers (Aggarwal and Singh, 2008) although rectal temperature didn't differ between buffaloes in wallow than those in shade (Tulloch and Litchfield, 1981).

Pre-weaning mortality in calves is directly correlated with poor nutrition in the breeding females (McDermott *et al*., 2010). As farmer co-operators may have varying degree of management, supervision of labor and available feed supply, our results suggest that buffalo farmers need to provide enough time for calves to build sufficient body reserves before weaning since every year increment in the weaning age may reduce the likelihood of AI failure by as much as 50%. While the average weaning weight in Nili-Ravi buffaloes have been reported to be 66.12±9.16 kg at 90 days (Akhtar *et al*., 2012), there appears to have several factors that can affect calves in attaining optimal weaning weight. These include year of birth in Swamp buffalo (Thevamanoharan *et al*., 2001), year and season of birth in ranged Mashona cattle (Tawonezvi, 1989) and the age of dam (Akhtar *et al*., 2012).

In conclusion, our study found that the age at first breeding must be conscientiously considered to allow optimal sexual and physical maturity of the breeding cows while ensuring that first breeding does not occur with too much delay. Calves also should be allowed enough time to grow and mature before weaning is initiated while provision of wallow during pregnancy may also provide beneficial effects on the breeding cows that would support AI success.

ACKNOWLEDGMENT

The authors would like to acknowledge cooperation of the PCC at VSU and the farmer cooperators of the PCC AI service.

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COMPARISON OF THE EFFECTS OF KISSPEPTIN-10 OR GNRH ON LUTEINIZING HORMONE SECRETION DURING THE LUTEAL PHASE OF THE OESTROUS CYCLE IN SWAMP BUFFALO COWS

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ABSTRACT

This study compared the effect of kisspeptin-10 or GnRH administration on LH release in swamp buffalo during the luteal phase of the estrous cycle. Six animals were treated with a single intravenous injection of 1,000 pmol/kg b.w. of Kisspeptin-10 or a single intramuscular injection of 10 µg/cow of GnRH agonist buserelin. For plasma LH analysis blood samples were collected every 15 minutes, 1 h before and 6 hour after kisspeptin-10 and GnRH administration. An increase in LH plasma concentrations was observed after GnRH administration but not after kisspeptin-10 administration. The results of this study indicate that during the luteal phase of the estrous cycle, administration of GnRH, but not Kisspeptin-10, stimulate LH secretion.

Keywords: *Bubalus bubalis*, buffalo, Kisspetin-10, GnRH, LH, progesterone, swamp buffalo

INTRODUCTION

In water buffalo cows, as in other domestic animals, attempts to control follicular development during the estrous cycle have been the subject of several studies. However, in buffalo, the neuroendocrine mechanisms that control the reproductive axis is not yet well known (El-Wishy, 2007; Qureshi and Ahmad, 2008). Better understanding of the mechanisms regulating the activity of the hypothalamic-pituitary-ovarian (HPO) axis would contribute to the improvement of fertility or to the development of novel agents that would control reproductive activity.

In buffalo, gonadotropin-releasing hormone (GnRH) plays a pivotal role in controlling reproductive functions and GnRH administration induces LH secretion with a stimulating effect on ovarian activity (Chaikhun *et al*., 2010; Nasir *et al*., 1986; Singh *et al*., 1984). Recently, it has been observed that Kisspeptin-10 produced by the hypothalamic neurons and Kisspetin-10 receptors are highly expressed in GnRH neurons (Irwig *et al*., 2004; Smith *et al*., 2011). Therefore, these neurons are considered to be a master regulator of reproduction in many mammalian species (Oakley *et al*., 2009; Okamura *et al*., 2013; Messanger *et al*., 2005; Hashizume *et al*., 2010; Tanaka *et al*., 2012). Indeed, it has been shown that Kisspeptin-10 administration can stimulate GnRH and LH secretion in rats (Irwig *et al*., 2004), mice (Gottsch *et al*., 2004; Messanger *et al*., 2005), humans (Dhillo *et al*., 2007; George *et al*., 2012; Jayasena *et al*., 2015), sheep (Caraty *et al*., 2007), goats (Hashizume *et al*., 2010; Matsui *et al*., 2004),

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cattle (Kadokawa *et al*., 2008; Whitlock *et al*., 2008; Ezzat *et al*., 2009, 2010), river-type buffalo (Macedo *et al*., 2014) and is able to induce ovarian activity and ovulation (Caraty *et al*., 2007, Matsui *et al.,* 2004; Sebert *et al*., 2010).

Recent data *in vitro* data show in buffalo the presence of kisspeptin-10 receptor expression in GnRH neurons (Chaikhun *et al*., 2016; Chaikhun-Marcou *et al*., 2016; Chaikhun-Marcou *et al*., 2018) and therefore has been suggested that exogenous administration of Kisspeptin-10 could induce LH release and can stimulate ovarian activity.

The aim of this study has been to investigate if kisspetin-10 administration can induce LH release during the luteal phase of oestrous cycle, when the plasma progesterone concentrations are high. and investigate if this effect is different with GnRH administration. If this hypothesis is correct, then the use of kisspetin-10 can be considered for controlling the estrous cycle in swamp buffalo.

MATERIAL AND METHODS

Ethical approval detail

This project has been reviewed and approved by the Certification of Institutional Animal Care and Use Committee (IACUC) in accordance with Chulalongkorn University Animal Care and Use Committee regulations and policies governing the care and use of laboratory animals. The Animal use protocol and approval number is 13310007. The review followed the guidelines documented in Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes, edited by the National Research Council of Thailand.

Animals and treatment

Six healthy swamp buffalo cows (*Bubalus bubalis*) between the ages of 4 and 6 years (determined by using the dental age estimation technique; Moran, 1992) with a mean body weight 360 kg were selected for this study. The experiment was done between December 2016 and February 2017.

During the experimental period the animals were housed in individual pens and fed fresh grass and hay and allowed to graze freely in the fields and bath in the area's natural pounds.

To allow frequent blood sampling for LH determination, all cows were fitted with an indwelling jugular vein catheter 4 to 6 h before the start of blood sampling.

The buffalo were treated during the midluteal phase of the estrous cycle (i.e. day 10 to 11 from estrus). Evaluation of the luteal phase of the estrous cycle was determined by detection of estrus. Estrus was detected by visual observation twice a day. The luteal phase was also confirmed by ultrasound evaluation of the corpus luteum morphology and confirmed, retrospectively, by the determination of progesterone plasma concentrations.

During the luteal phase of oestrous cycle, each animal received a single intravenous administration in the jugular vein of kisspeptin-10 (human metastin 45 to 54 (YNWNSFGLRF-NH2), 4389-V2, Peptide Institute Inc., Osaka, Japan) consisting of 1,000 pmol/kg b.w. or 1.3 µg/kg b.w. per dose dissolved in 2 ml distilled water (Chaikhun-Marcou *et al*., 2014). This method of administration was chosen based on suggestion that peripheral administration of kisspeptin-10 would not allow all the kisspeptin-10 to pass the blood-brain barrier (d'Anglemont de Tassigny *et al*., 2010; Ezzat *et al*., 2010; Suzuki *et al*., 2008) and was calculated on the basis of previous studies in ovariectomized cows (Whitlock *et al*.,

2008). To test the effect of GnRH administration, to each animal was given a single intramuscular injection of 10 µg GnRH (Buserelin, Receptal, Intervet, Netherlands) during the luteal phase ofthe following estruos cycle in which was administered kisspeptin.

Blood sample collection

Blood was collected at 15 minutes intervals 1 h before and for 6 h after Kispeptin-10 or GnRH administration. After centrifugation at 3000 x g for 5 minutes, plasma was harvested and stored at -20°C until analysis for plasma LH and progesterone concentrations were effectuated.

LH and progesterone determination

Plasma samples were measured for LH concentrations by a commercial enzymatic immunoassay test kit (EIA) (LH DETECT for Buffalo, Repropharm, France), following the kit's instruction. The LH intra- and inter-assay coefficients of variation were 32.16% and 39.53%, respectively and the sensitivity was 0.25 ng/ml.

Plasma progesterone concentration was analyzed by radioimmunoassay (RIA). The progesterone intra- and inter-assay coefficients of variation were 7.7 and 13.9, respectively and the sensitivity was 0.01 ng/ml.

Statistical analysis

The effect of treatment on LH plasma concentrations were tested for period (pre- or post-treatment), and treatment by period interaction using ANOVA procedures for repeated measures. The LH data in each treatment group were presented as mean±SEM. Level of significant was $P<0.05$.

RESULTS

The LH plasma profile for each cow after Kisspetin-10 or GnRH analogue administration is represented in Figure 1 and Figure 2, respectively. After Kisspetin-10 administration, there was no increase in LH mean concentrations during the whole sampling time whereas after GnRH administration mean plasma LH concentrations significantly increased ($P<0.05$) beginning about 30 minutes after treatment and lasting for about 130 minutes (Figure 2). The mean plasma LH concentrations were greater ($P<0.05$) for the first 3 h after GnRH compared to Kisspeptin-10 administration (Figure 3).

DISCUSSION

Kisspeptin-10 administration stimulates LH secretion in humans (Jayasena *et al*., 2015), female rats (Adachi *et al*., 2007), prepubertal heifers (Kadokawa *et al*., 2008), ovariectomized ewes (Caraty *et al*., 2007), ovariectomized cows (Whitlock *et al*., 2008) and ovariectomized river buffalo (Macedo *et al*., 2014). Secretion of LH after Kisspetin-10 administration is reduced when compared to GnRH administration. However, the interaction between progesterone and kisspetpin-10 effect on GnRH and LH secretion has been not clarified. In one study in goats (Hashizume *et al*., 2010) during the luteal phase of estrous cycle, there was an increase in LH secretion after Kisspeptin-10 administration, but lower compared to GnRH. Our study is the first to examine the effect of Kisspeptin-10 on LH secretion in the presence of elevated progesterone palsma concentrations, as it occurs during the luteal phase of the estrous cycle. The results show that kisspetin-10 does not induce

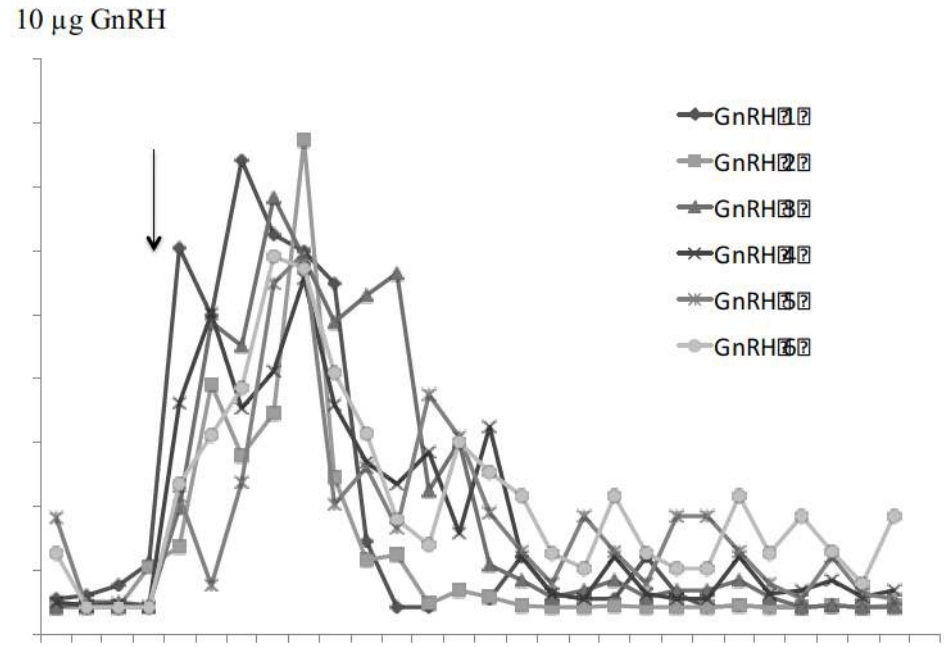

Figure 1. Response of circulating LH in swamp buffalo during the luteal phase of oestrus cycle after administration (arrow) of GnRH analogue (10 µg of Buserelin, Receptal). Time 0 = administration of GnRH.

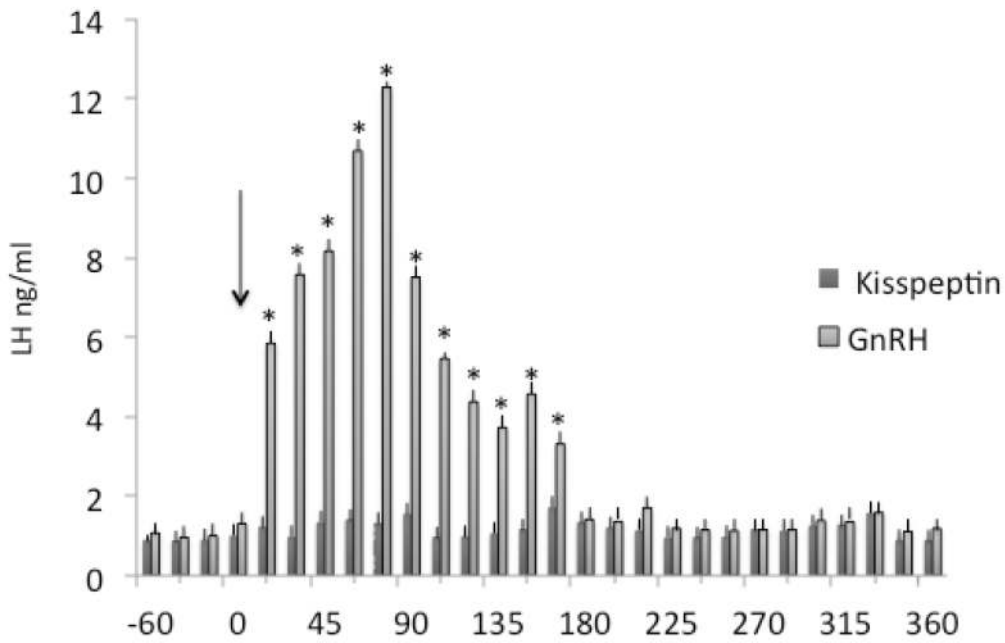

Figure 2. Response of circulating LH in swamp buffalo during the luteal phase of the oestrous cycle after administration (arrow) of Kisspeptin (1.3 µg/kg b.w). Time 0 = administration of kisspeptin.

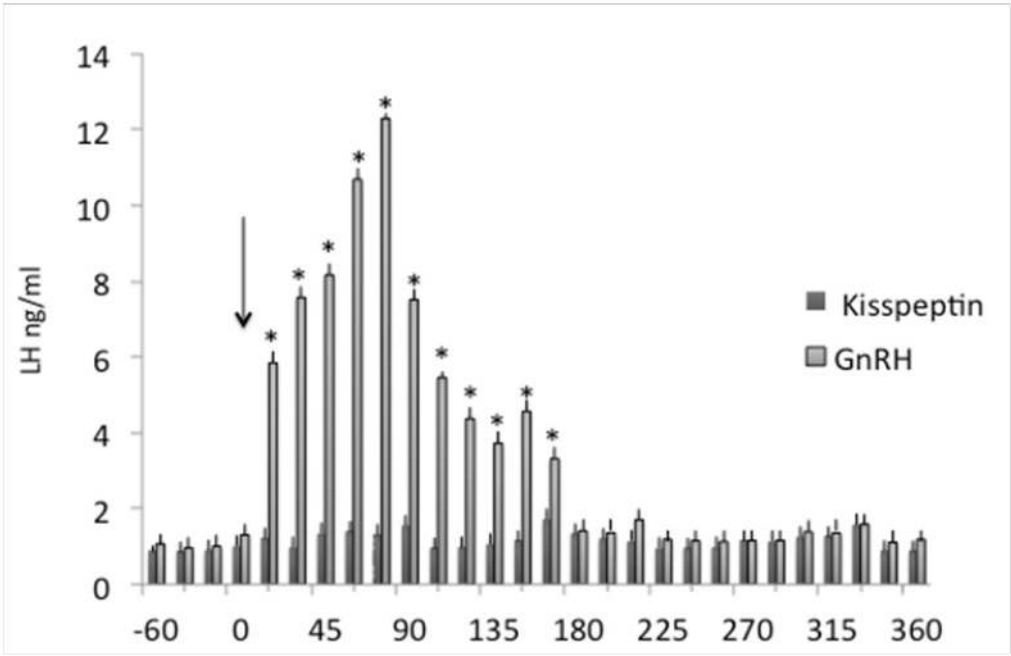

Figure 3. Response of circulating LH (mean ± SEM) in swamp buffalo during the luteal phase of the oestrous cycle after administration of Kisspeptin or GnRH. *indicate differences ($P<0.05$) in mean LH between treatments.

any significant LH release in presence of elevated plasma concentrations of progesterone. The difference between studies is difficult to explained but could be related to the species utilized (swamp buffalo *vs* goats) and the dose utilized because we injected 1.3 µg/kg b.w compare 5,0 µg/kg b.w. dose of Kisspeptin in Hashizume *et al.* (2010) study.

The fact that in overiectomized cows the maximum LH-releasing effect to the i.v. injection of Kp10 was observed at 0.13 µg /kg b.w. (Whitlock *et al.*, 2008). However, Whitlock *et al.* (2008) study were utilized ovariectomized cows while in our study we utilized cows during the luteal phase of estrous cycle.

In conclusion, our study indicate that GnRH, but not Kisspeptin-10 administration during the luteal phase of the estrous cycle can stimulate LH secretion.

ACKNOWLEDGEMENTS

For statistical analysis, we thank Dr. Jamlong Mitchaothai. For English correction and editing, thank you Mr. Philippe Marcou, USA. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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WATER BUFFALO (*BUBALUS BUBALIS*) SUSCEPTIBILITY TO BOVINE TUBERCULOSIS IS INFLUENCED BY G.4002C>T POLYMORPHISM IN INTERLEUKIN-10 GENE

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ABSTRACT

Outcome of bovine tuberculosis (bTB), an infectious disease caused by *Mycobacterium bovis*, is influenced by host genetic background. For this reason, polymorphism discovery association studies are a powerful tool for selective breeding helping disease control. Interleukin-10 (IL-10) is a regulatory cytokine produced by different cells and fine-tune immune response to bTB. Thus, in this research, we evaluated the role of the single nucleotide polymorphism g.4002C>T in IL-10 gene for susceptibility to bTB in Mediterranean water buffalo. We characterized 184 animals grouped in cases and controls and demonstrated that homozygous subjects TT are about 3 times more susceptible to bTB compared to CC homozygous. Indeed this polymorphism is responsible of amino acid substitution p. (Thr 175 Met) in the primary protein sequence which affects protein secondary structure. This polymorphism might represent a valid tool for marker assisted selection against bovine tuberculosis in water buffalo.

Keywords: *Bubalus bubalis*, buffalo, gene polymorphisms, interleukin-10, bovine tuberculosis, Italy

INTRODUCTION

Interleukin-10 (IL-10) is a regulatory cytokine produced by different innate and adaptive immune cells during bovine tuberculosis (bTB) (Dorhoi and Kaufmann, 2016) an infectious disease caused by *Mycobaterium bovis*. *M. bovis* is the causative agent of bovine tuberculosis (bTB), an infectious disease endemic in many countries (Marassi *et al*., 2009; Medeiros *et al*., 2010; Laisse *et al*., 2011) where is responsible of economic losses and i still considered a risk factor for humans (Humblet *et al*., 2009; Laisse *et al*., 2011). The interaction of *M. bovis* with its hosts is long-dated (Alvarez *et al*., 2009) and is therefore plausible assuming that their co-evolution (reciprocal adaptation) influenced the genomes of the pathogen as well as that of its hosts. The genetic makeup of the host may therefore reasonably plays a crucial role in the resistance to the pathogen. Selective breeding for disease-resistant genotypes represents therefore an approach supporting disease control (Bishop and MacKenzie, 2003; Persson and Vance, 2007). For example, SNPs located within the LRR domain of Toll-like receptors 2 (TLR2), Toll-like receptors 4 (TRL4) and Tool-like receptors 9 (TRL9) confer resistance to *M. bovis* (Alfano *et al*., 2014).

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Moreover, the same study identified three genetic polymorphisms - located in the solute carrier family 7 member 13 (SLC7A13); the Interleukin-1 alpha (IL1a); and the Deleted Malignant Brain Tumours 1 (DMBT1) genes - associated with *M. bovis* infection in the African buffalo (Roex *et al*., 2013). Furthermore, also variability in non translated regions have been associated with susceptibility to bTB. Indeed we recently have reported that an intronic polymorphism in Tumor Necrosis Factor-a (TNFa) gene belonging to a potential regulatory sequence, confers susceptibility to bTB (Papaianni *et al*., 2017) indicating that polymorphism in cytokine may have an important role as resistance/ susceptible gene in bTB. A further SNPs in the 3'UTR region of Interferon gamma (IFNg) which is a part of the target sequence recognized by microRNA 125b also increases susceptibility to bTB (Iannaccone *et al*., 2018a) showing that microRNAs have a double role beside the ones as biomarker (Iannaccone *et al*., 2018b) Thus, because polymorphisms in cytokines may have an important role in bTB, we decided to investigate the genetic variability in IL-10 of water buffalo related to resistance/susceptibility to bTB.

MATERIAL AND METHODS

Samples collection

Speciment samples were collected from 184 animals reared in different herds located in Campania region (south Italy) and grouped in cases (positive to hypersensitivity and microbiological test, 59 samples) and controls (negative to multiple hypersensitivity test, 125 samples) according to previous work (Alfano *et al*., 2014). DNA was extracted using QIAamp DNA mini kit according to manufacturer's procedure and purity was analyzed using Nanodro specfotometer. Only samples with ratio A260/280 higher than 1.8 were used for further analysis.

IL-10 amplification and polymorphism discovery

Using primers designed (forward: 5'-TTCATCTCCCAATGCAAGCAAGCTA-3'; reverse:5'-ATCGGATTTCAGAGGTCTTCC GTTTAT-3') on water buffalo genome sequence (GeneBank NW_005783511, 356114. 360857), we amplified and sequenced a genomic DNA region of 300 nucleotides spanning the coding region of the exon 5 in 10 cases and 10 controls. Amplification thermal conditions were the following: 5 minutes at 95ºC and then 30 seconds at 95ºC, 30 seconds at 55ºC, and 40 seconds at 72°C (40 cycles), with a final extension for 5 minutes at 72°C and sequencing was performed by external company (Microgem, S.r.l., Italy) Genotyping for the g.4002C>T polymorphism was carried out using the following primer: C-allele primer: 5'-TACATAGAAACCTACGTGACAAC-3'; T-allele primer: 5'-TACATAGAAACCTACGTGA CAAT-3'; common reverse: 5'-ATCGGATTT CAGAGGTCTTCCGTTTAT-3'.

Bioinformatic and statistical analysis.

Sequence alignments was performed using and results were aligned using by use of Chromas software (Technelysium, Queensland, Australia). Secondary protein structure analysis was carried out using https://zhanglab.ccmb.med. umich.edu/I-TASSER/ (Roy *et al*., 2010). ORs and 95% confidence intervals were calculated by Fisher's exact test using the statistical package GraphPad Prism version 5 (GraphPad, La Jolla, CA, USA). Hardy–Weinberg equilibrium by the Hardy–Weinberg calculator (http://www.oege.org/

software/hwe-mr-calc.shtml).

RESULTS AND DISCUSSION

A genomic DNA sequence spanning the exon 5 of the IL-10 gene in water buffalo was amplified and sequence alignments show a point transversion g.4002C>T. which is responsible of an amino acid substitution p.(Thr175Met) in the primary protein sequence. Using a free web tool for protein secondary structure prediction (https:// zhanglab.ccmb.med.umich.edu/I-TASSER/), we found that the substitution p.(Thr175Met) lengthens the a-helix compared to the wild type sequence (Figure 1).

Thus, to test the possibility that g.4002C>T polymorphism is a bTB, we genotyped using an allele-specific PCR all remaining samples and results are summarized in Table 1.

Homozygous subjects TT are more represented in the cases group (22 out of 59; frequency: 0.37) compared to controls groups (24 out of 125; frequency: 0.19); conversely, the genotype CC is more common in the controls group (52 out of 125; frequency: 0.42) compared to the cases group (14 out of 59; frequency: 0.27). The comparison of the CC *vs* TT ratio between case and control subjects by Fisher's exact test, showed an odds ratio (OR) of 2.97; with the 95% confidence interval (CI) ranging between 1.231 and 6.667; P-value (two sided) = 0.0088. This result strongly suggests a predisposition of TT subjects to bTB because the OR is in the range between 2.1 and 5.7 (Ioannidis et al, 2001). Moreover, as expected in case of association (Anderson *et al*., 2005), genotypic frequencies at the *IL-10* locus were in equilibrium among controls and in disequilibrium among cases (Table 2).

Figure 1. Predictive comparative analysis of IL-10 proteins translated from C-allele at the site g.4002. Sequences are compared based on secondary structure generate by I-TASSER software (http:// zhanglab.ccmb.med.umich.edu/L-TASSER). C: coil H: helix.

Table 1. Association between susceptibility to *M. bovis* infection and the g.4002C>T polymorphism in water buffalo.

Genotypes	Controls	Cases	OR (95% CI)	P-value[aa]
CC	52	14	-	-
CT	49	23	2.14 (0.988 to 4.630)	0.0764
TT	24	22	2.97 (1.231 to 6.667)	0.0088

[aa]Fisher's exact test.

Table 2. Hardy-Weinberg equilibrium based on g.4002G>A in water buffalo infected and not infected with *M. bovis*.

Status	GG	AG	AA	χ^2
Cases	14	23	22	4.65
Controls	52	49	24	3.81

CONCLUSION

In conclusion, our data demonstrated that susceptibility to bovine tuberculosis in water buffalo is influenced by the polymorphism g.4002C>T in *IL-10* gene probably consequent to a change in the protein secondary structure. This polymorphism might represent a valid tool for marker assisted selection against bovine tuberculosis in water buffalo.

ACKNOWLEDGEMENTS

Research was supporting by Italian Ministry of Research (Project number: PON01_00486_ GENOBU) and by the Department of Agriculture, University of Naples Federico II (Progetto Giovani Ricercatori).

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MOLECULAR DETECTION, HAEMATOLOGICAL AND THERAPEUTIC STUDIES ON THEILERIOSIS IN BUFFALOES

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ABSTRACT

Tropical theileriosis poses major threat for buffaloes causing significant economical loss to livestock farmers. Early detection and prompt treatment helps to minimise mortality and economical loss. This study was conducted on 79 female buffaloes presented with the signs suggestive of theileriosis. Evaluation of PCR for detection and buparvaquone for efficacy was undertaken. Overall prevalence of *T. annulata* infection recorded was 22.78 % by PCR. Adult buffaloes showed higher prevalence (13.92 %) compared to young buffaloes (8.86 %). Blood smear examination revealed 38.89 % sensitivity in detection of *Theileria* piroplasms. Haematological observations showed significant decreased values of Hb, TEC, PCV and TLC. Neutropenia, monocytopenia, eosinopenia and lymphocytosis were recorded. Buparvaquone was 100 % effective in complete elimination of *T. annulata* in infected buffaloes.

Keywords: buffaloes, *Theileria annulata*, PCR, Buparvaquone

INTRODUCTION

Tropical theileriosis caused by *Theileria annulata* poses major threat for buffaloes causing significant economic losses to the livestock farmers. Early diagnosis and prompt treatment is essential to prevent the production loss. Diagnosis of *T. annulata* infection is usually based on microscopic examination of blood smears stained with Giemsa. However apart from difficulty in species differentiation blood smear technique is not suitable for detection of infection where parasitaemia is low (Nayel et al. 2012). The methods of detection of Theileria species involving serological tests are not sensitive due to cross-reactions and disappearance of antibodies in long term carriers (Passos et al. 1998). Therefore a sensitive and highly specific method for the diagnosis of piroplasms is required. Recently species specific polymerase chain reaction (PCR) methods have been developed for the detection of *Theileria* species (D' Oliveira et al. 1995; Roy et al. 2000; Ica et al. 2007; Altay et al. 2008; Ali and Radwan 2011; Kundave et al. 2015; and Ganguly et al. 2015). Hence the present study was conducted to know the haematological alterations, use of PCR for detection and suitable treatment for *Theileria annulata* infection in buffaloes.

MATERIALS AND METHODS

The study was conducted on 79 female buffaloes (26 young and 53 adult) presented to Veterinary College Hospital, Bidar, Karnataka, India with the signs of pyrexia, lymphadenopathy,

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pale mucus membrane, anorexia and tick infestation.

Haematological examination

Thin blood smear were prepared from ear vein and stained using Giemsa stain. The parasites were identified according to the characters described by Soulsby (1982). The blood samples were collected from jugular vein in EDTA coated vacutainers for PCR analysis. The blood samples found positive for *T. annulata* on blood smear examination were subjected to haematological examination as described by Schalm *et al*., (1975). Similarly blood samples collected from apparently healthy buffaloes were subjected to haematological examination and served as control.

Isolation of DNA from blood samples

Genomic DNA was extracted from 200 µl of the whole blood using DNA extraction kit (Bio basic) according to the manufacturer's instructions. The isolated DNA was quantified spectrophotometrically and run on 0.8 % agarose gel. Aliquots of extracted DNA were stored at -20°C for PCR analysis.

Polymerase chain reaction

The primer was designed on the coding sequence of the 30 kDa major merozoite surface antigen (Tams 1 gene) of *Theileria annulata*. The forward primer sequence is 5'-CCA GTA ACC TTT AAA AAC GT – 3' and reverse primer sequence is 5'- GTT ACG AAC ATG GGT TT -3'. The PCR reaction on total volume of 15 µl containing 30 ng of template DNA, 7.5 µl of 2x master mix (Fermentus), 0.5 µl of each forward and reverse primer (10 pmol/µl), 5.5 µl of nuclease free water. Reaction condition were initial denaturation at 98°C for 5 minutes, followed by 37 cycles of 95°C for 30 seconds, 45°C for 30 seconds, 72°C for 30 seconds, with a final extension step of 72°C for 1 minute and holds at 4°C in a thermal cycler (Eppendorf). A negative control (Sterile water), a positive control DNA from *Theileria annulata* were included in each amplification run. PCR products were analysed by electrophoresis on 1.5 % agarose gel and documented with documentation system (Gel Doc, Syngene).

Treatment

Buffaloes infected with *T. annulata* confirmed by blood smear examination were treated with dose of Buparvaquone 2.5 mg/kg body weight. Therapeutic efficacy was evaluated based on changes in clinical parameters and parasitological status on 7th day post treatment.

Statistical analysis

The difference of means of haematological values between *T. annulata* infected and healthy control group were compared using student t-test (Snedecor and Cochran, 1994)

RESULTS AND DISCUSSION

Out of 79 blood samples 18 (11 adult and 7 young) were positive for *T. annulata* by PCR indicated overall prevalence of 22.78 %. Higher prevalence (13.92 %) of *T. annulata* was recorded in buffaloes more than 2 year age group when compared to less than two year age group (8.86 %) Similarly higher prevalence of *T. annulata* in adult bovines was recorded by Anand et al (2009) and Kundave *et al*. (2015). Higher rate of disease occurrence in adult buffaloes could be attributed to stress of pregnancy and lactation (Durrani 2003). The age-related resistance in young cattle to most

tick-borne protozoan diseases has been reported by Dumanli *et al*. (2005).

Out of 79 blood samples, Giemsa stained blood smear examination revealed presence of *Theileria piroplasms* in 7 samples (Figure 1) which indicated 38.89% sensitivity compared to PCR as base reference. A desired product size of 721 bp was obtained in *T. annulata* positive samples (Figure 2). Blood smear examination in the present study had shown false negative under light microscope, which shows low sensitivity of the test. It could be attributed to low parasitaemia, destruction of piroplsms in red blood cells due to haemolysis and unsuitable blood smear staining (Hoghooghi *et al*., 2011). Moreover the microscopic detection of piroplasms in blood samples that were negative by PCR was not possible. The samples positive by stained blood smears were found to be positive with PCR assay. This fact confirms the superiority of PCR over blood smear examination. The results of the present study are in agreement with earlier workers (Sanchez *et al*., 1999; Roy *et al*., 2000; Azizi *et al*., 2008; Mohammod *et al*., 2011; Ganguly *et al*., 2015 and Kundave *et al*., 2015)

The haematological values of *T. annulata* infected and healthy control groups have been presented in Table 1. The infected group showed significant decreased ($p \leq 0.01$) values of total erythrocyte count, haemoglobin, packed cell volume than healthy group, indicating normocytic normochromic anaemia. Significant decrease ($p \leq 0.01$) in total leukocyte count and neutrophil count and non-significant decrease in monocyte and eosinophil count was observed in infected group. Similar haematological observations in *Theileria annulata* infected buffaloes were made by Sharma *et al*. (1985), Osman and Al-Gaabary (2007) El-Deeb and Younis (2009). Decreased total erythrocyte count, haemoglobin and PCV in *Theileria annulata* infected buffaloes could be attributed to erythrophagocytosis (Yagi *et al*., 2002). Relative increase in lymphocyte count reflects compensatory mechanism in target cells in response to their invasion with *T. annulata* (Lamia, 1997).

Seven buffaloes infected with *T.annulata*

Table 1. Hemogram in Healthy and *Theileria* infected buffaloes (mean ± S.E).

Variable	Healthy buffaloes (*N*=07)	Infected buffaloes (*N*=07)
TEC (10^6/µl)	5.78 ± 0.13	3.70 ±0.18
Hb (g/dl)	10.11 ± 0.22	7.60 ± 0.19
PCV (%)	32.71 ± 0.83	22.99 ± 0.86
MCV (fl)	56.5 ± 0.50	60.41 ± 1.57
MCH (pg)	17.44 ± 0.21	20.81 ± 0.61
MCHC (g/dl)	30.9 ± 0.50	34.50 ± 0.85
TLC ($10^3 \mu l^{-1}$)	6.45 ± 0.19	5.60 ± 0.28
Neutrophils ($10^3 \mu l^{-1}$)	33.85 ± 0.67	39.86 ± 0.55
Lymphocytes ($10^3 \mu l^{-1}$)	61.42 ± 1.08	56.00 ±0.61
Monocytes ($10^3 \mu l^{-1}$)	2.85 ± 0.50	2.71 ± 0.42
Eosinophils ($10^3 \mu l^{-1}$)	1.85 ± 0.34	1.43 ± 0.20

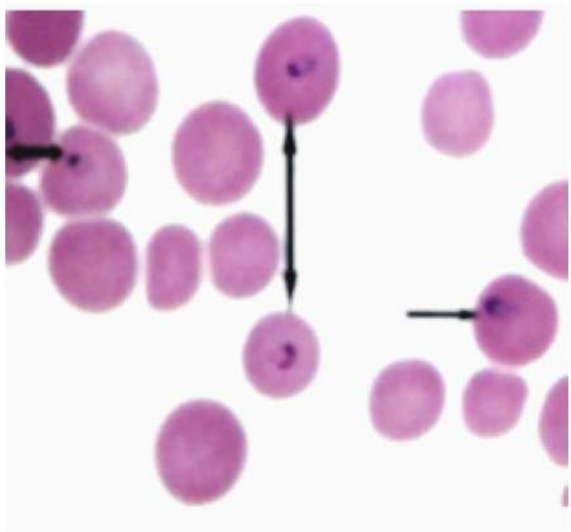

Figure 1. Piroplasmic forms of *T. annulata* by Giemsa staining method.

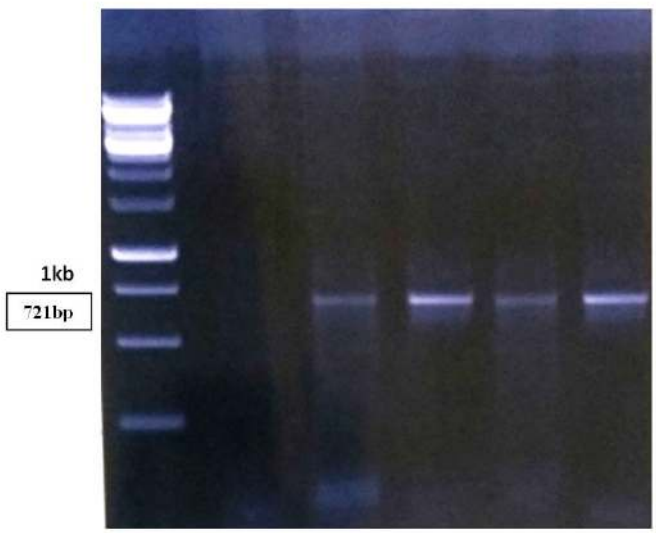

Figure 2. Detection of *T. annulata* in blood by PCR.

confirmed with blood smear examination were treated with single dose of Buparvaquone at the rate of 2.5 mg/kg body weight intramuscularly. All seven buffaloes revealed clinical improvement and absence of intracellular piroplasms by seventh day with the efficacy rate of 100 percent. This is an agreement with the report of Osman and Al-Gaabary (2007).

From the findings of the present study it can be concluded that prevalence of *Theileria annulata* infection was high in adult buffaloes and haematological changes observed are useful in understanding disease pathogenesis and corrective measures for supportive therapy. PCR can reliably be used for accurate detection of *T. annulata*. Buparvaquone treatment is indicated in the treatment of tropical theileriosis in buffaloes.

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POLICY IMPLICATION OF BUFFALO MEAT IMPORTATION TO BEEF MARKETING: CASE OF BOGOR, INDONESIA

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ABSTRACT

Meat is the main source of animal protein. One type of meat that is most consumed by people is beef. The increasing price of beef due to the scarcity of beef in Indonesia, encourages the government to issue a policy of buffalo meat importation, in order to cope with the void of Indonesian meat stock. The problem is that this policy has many pros and cons from various parties. There are parties who support and there are also those who strongly oppose. Those who oppose this policy are governments in several regions and organizations that feel disadvantaged by the buffalo meat importation policy. Based on the phenomenon that occurs with the import of buffalo, in this study the authors are interested in analyzing Policy Implication of Buffalo Meat Importation to Beef Marketing: Case of Bogor, Indonesia. The method used is a Structural Equation Models (SEM) with 150 respondents. The result of this research is respondents were willing to buy and consume buffalo meat as long as the quality, health and halal were guaranteed.

Keywords: *Bubalus bubalis*, buffalo, importation, policy, structural equation model (SEM)

INTRODUCTION

Meat is the main source of animal protein. One of the most widely consumed types of meat is beef, which has an important role in meeting the nutrition of the Indonesian people. Demand for products related to nutrition fulfillment such as beef products is increasing along with population growth and improving living standards of Indonesian people.

Based on data from the Ministry of Agriculture, in general the development of beef production in Indonesia during the 2013 to 2017 grew by 2.56%. The number of beef production that cannot fulfill the needs is one of the causes of a high increase in beef prices. Such higher costs, in comparison to the production of conventional products, usually require comparatively higher prices and thus a higher consumer willingness to pay (Olbrich, Hundt and Grewe, 2014).

The following are data from the Ministry of Trade regarding the growth of beef prices in Indonesia for the period March 2013 to 2017 (Figure 1).

The solution taken by the government to fix the beef deficit is to open slot for buffalo meat importation that are much cheaper than beef. The key suppliers of frozen meat in 2015 were Brazil,

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India, Sudan, United States and Australia. Brazil is the dominant supplier of the frozen beef while India is the sole supplier of frozen buffalo meat (Hassan *et al.*, 2018). With the buffalo meat importation from India, people are expected to be able to make substitution of beef, so that it can reduce the price of beef that is too high. The problem is that this policy has many pros and cons from various parties. There are parties who supports, there are also those who strongly oppose. The opposing parties in this regard are governments in several regions and organizations that feel disadvantaged by the Indian buffalo meat import policy.

There are various areas that are targeted for buffalo meat importation, and one of them is Bogor City. In addition, Bogor is one of the regions that has a high amount of meat consumption. Based on the phenomenon that occurs with the import of buffalo, in this study the authors are interested in analyzing the implications of buffalo meat import policy on the marketing of beef in Indonesia, a case study in the city of Bogor (Figure 2).

MATERIALS AND METHODS

This research was conducted in the area of Bogor City in March to December 2017. The selection of this location was motivated because Bogor City was one of the first cities to get buffalo meat quota from India. This research approach uses descriptive research with survey methods. This method was conducted by distributing questionnaires to selected respondents with certain criteria, such as: respondents ever bought or consumed beef (Non Vegetarian) with minimum 17 years old or over, domiciled in Bogor City. The number of samples to be used in this study is limited according to the rules of Structural Equation Model which is 5 times to 10 times the estimated variable parameters (Hair *et al.*, 2006). This research has 25 variables. So the range of the number of samples used is 125 to 250 respondents, and 150 respondents will be taken. The sampling method has been done with multistage non probability sampling technique with stages (Table 1 and Figure 3):

1. The city of Bogor is divided into 6 sub-districts, namely North Bogor, South Bogor, East Bogor, Central Bogor, West Bogor and Tanah Sareal.

2. In each sub-district, 2 housing will be chosen using purposive sampling technique.

3. The number of respondents in each housing will be divided proportionally by using a sample fraction in accordance with the population in each sub-district.

4. The selection of respondents was done by convenience sampling.

The data obtained were analyzed using descriptive analysis and Structural Equation Modeling (SEM). Structural equation modeling (SEM) is a versatile multivariate statistical technique and application of this method have been increasing since its introduction in the 1980s (Xiong *et al.*, 2015).

RESULTS AND DISCUSSION

General description of the respondents

The majority of respondents in this study belong to the age group of 22 to 26 years (44%). The majority of respondents were dominated by male (55%). The education level of respondents was dominated by undergraduate level (59%).The majority of respondents occupations were private employees (45%) and college students (25%) with total household expenditure per month dominated

Rp 1.000.000 to Rp 2.000.000 (27%).

Characteristics of respondents' buying behavior in this study showed that meat consumption is not necessarily local or imported meat (71%). This means that meat consumers in the city of Bogor are not too picky in relation to the area of origin of the meat, both imported and local. The amount of money spent by respondents to purchase meat> Rp. 100,000.00 (16%) per month. With a price range of meat per kg starting from Rp. 80,000.00 to Rp. 130,000.00 consumers purchase meat as much as ± 0.8 to 1.3 kg per month. The results showed that the respondent frequency of purchasing meat per month was 1 to 2 times (34%). That is, meat consumers must pay a fee of ± Rp 80,000.00 to Rp 260,000.00 to purchase meat. Meat mass for each purchase is the amount of meat in kg per purchase. The results showed that the meat mass for each purchase ranged from 0.5 kg to 1 kg (45%). That is, the respondents in this study paid a fee of ± Rp 50,000 to Rp 200,000.00 to purchase meat. The place of purchase is the location of buying meat (traditional markets, modern markets, slaughterhouses, traveling vegetable makers, others). Basically the place to purchase products depends on the preferences of each consumer. The results of this study indicate that most respondents purchase meat in traditional markets (41%).

Good information will give consumers more knowledge of a product. The results of the study showed that 46% of respondents occasionally find out information on the origin of meat at each purchase.

Measurement model

Reliability testing is the process of measuring the accuracy (consistent) of an instrument. This test is intended to ensure that the instrument used is an instrument that is reliable, consistent, stable and dependable, so that when used repeatedly can produce the same data. To test the reliability of the data, this research uses indicators based on variance extracted (VE) and construt reliability (CR) formulas (Figure 4).

The Variance Extracted (VE) value was used to measure the number of variants that can be captured by the construct compared to the variance caused by measurement error. While the value of composite reliability (CR) showed the consistency of each indicator in measuring the construct. The higher the value of composite reliability (CR) the more consistent the indicator in measuring the construct. Hair *et al*. (2010) stated that the construct reliability that can be received is a coefficient that is worth more than 0.70 while for calculating the variance extracted the recommended number is more than 0.5 (Table 2).

The results of processing in Table 2 show that all values of construct reliability (CR) and variance extracted (VE) in this study are above 0.70 and 0.50, so it can be concluded that the models in this study are reliable for use (Table 3).

The relationship between 'Quality' with the indicators. Quality assurance schemes had been seen as relatively important in the purchase of mince beef and such schemes were valued by consumers (Northen, 2000).Variable indicators that have the highest contribution to form the 'quality' latent variable are Q1 (fresh imported buffalo meat). This indicated by the Q1 load factor value of 0.93. That is, prospective consumers of buffalo meat assess the quality of buffalo meat based on its freshness.

The relationship between 'Safety and Health' variable with the indicators. Food safety is an important issue facing current consumers, the food industry and the government. Variable indicators that have the highest contribution to

form the latent variables of safety and health are SH4 (the cleanliness of buffalo meat imported from India is guaranteed). That is, prespective consumers of buffalo meat consumers assess the safety and health of buffalo meat based on the cleanliness of the meat. Today's consumers are better educated and hence, more updated about issues regarding food safety and compared to those in the past (Liana *et al.*, 2010).

The relationship between 'Price' variable with the indicators. The price has a very important role in influencing consumer decisions to buy products, so as to determine the success of marketing a product or the amount of value in exchange from consumers on the benefits for owning or using a product or service (Aspan *et al*, 2017). Variable indicators that have the highest contribution to form the latent variable price is P3 (cheap production costs cause the price of imported Indian beef is cheaper than other meat).

The relationship between 'Halal' variable with the indicators. The acceptance of the halal products and services among Muslims and non-Muslims is caused by the perception that halal symbolizes healthier lifestyle and clean preparation (Aziz and Chok, 2016). Variable indicators that have the highest contribution to form the latent variable of halal are H2 (the halal status of buffalo meat imported by India is clear even though it is supplied by a non-Muslim majority state). That is, prospective consumers of buffalo meat consider the clarity of the halal status of imported Indian buffalo meat before making a purchase.

The relationship between 'Distribution' variable with the indicators. Variable indicators that have the highest contribution to form the latent variable of distribution are D2 (Indian imported buffalo meat is easy to obtain). That is, prospective consumers of buffalo meat consider that a good distribution is seen from the ease of obtaining buffalo meat.

The relationship between 'Information' variable with the indicators. Variable indicators that have the highest contribution to form the latent variable of information are I3 (I know the quality of meat imported by Indian buffalo).

The relationship between 'Willingness to Pay' variable with the indicators. The variable indicator that has the highest contribution to form the latent variable of willingness to pay is WP2 (I will only buy Indian imported buffalo meat when the price of beef rises. That is, prospective consumers of buffalo meat will only make purchases of buffalo meat if the price of beef goes up.

Respondents perception

This research produced several findings that would be compiled into managerial implications that are expected to be beneficial for the government as the maker of buffalo import policy. Based on the results of the study, respondents' perceptions of the quality of imported buffalo meat are still within the normal limits. In that sense, imported buffalo meat can be received in the market as a substitute for beef. On average the respondents considered that the freshness of the meat sold on the market showed the quality of the meat. Although the perception of buffalo meat as food is still unfamiliar, the average respondent is willing to buy buffalo meat as a substitute for beef as long as the imported buffalo meat is still fresh.

Based on the results of the study, respondents' perceptions of the safety and health of imported buffalo meat showed that on average the respondents were hesitant but believed that the government could not allow imported buffalo meat to circulate in the market if its safety and health were not guaranteed. The average respondent

considers that the cleanliness of imported buffalo meat circulating in the market is very important. The average respondent is willing to buy imported buffalo meat as long as the meat is clean.

Based on the results of the study, respondents' perceptions of the price of buffalo meat showed that the price of buffalo meat circulating in the market was affordable. It's just that what makes the respondents hesitant in assessing the price of buffalo meat is that the respondents cannot distinguish between imported and local buffalo meat because they do not include the origin of the buffalo meat.

Based on the results of the study, respondents' perceptions of halal buffalo meat, the status of halal buffalo meat circulating was clear even though supplied in non-Muslim countries. Although there are still respondents who doubt this, the respondents still believe that imported buffalo meat entering Indonesia has been proven halal. The fact that Indonesia is an Islamic State can be one reason respondents believe in the halal status of imported buffalo meat circulating in the market, because every product sold must pass halal selection first, if there are products that are not halal then the seller must clearly label the product is not halal.

Based on the results of the study, respondents' perceptions of the distribution of imported buffalo meat showed that the average respondent easily found imported buffalo meat in the city of Bogor. Based on the results of the study, respondents' perceptions of information related to imported buffalo meat showed that the government had provided sufficient information regarding imported buffalo meat. Based on the results of the study, the average respondent is willing to buy imported buffalo meat only if the price of beef is too high.

Structural model

The structural model describes the relationship between latent variables in the model. The relationship between quality, Safety and health, price, halal, distribution and information to willingness to pay (Table 4).

The results showed that quality, Safety and health, price, halal and information had a significant and positive influence on willingness to pay, this was indicated by the value of the loading factor and t-test on the five relationships. The values of loading factors for quality, safety and health, price, halal and information are positive with values of 0.20, 0.41, 0.52, 0.54 and 0.67 respectively. Whereas the t-test value produced on the relationship of quality, safety and health, price, halal and information with willingness to pay is 2.73, 1.98, 2.39, 2.93 and 3.96, which means that quality, Safety and health, price, halal and information have a significant effect on willingness to pay.

Distribution has a positive relationship with willingness to pay, so hypothesis 5 can be accepted. However, in contrast to quality, Safety and health, price, halal and information, the relationship of distribution to willingness to pay is not significant. The value of the loading factor of the distribution relationship with willingness to pay is indeed positive (0.01), but the t-test value of the distribution is 0.19, which is smaller than 1.96, which means that the distribution does not affect the willingness to pay significantly. So if the government improves the distribution of imported Indian buffalo meat in the market, it will only slightly affect the willingness to pay consumers.

CONCLUSION

This research showed that health, safety

Figure 1. Beef Prices in Indonesia for the 2013 to 2017.

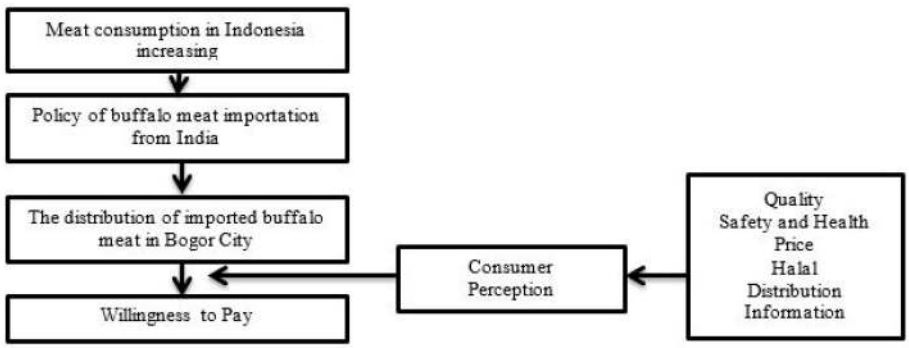

Figure 2. Framework.

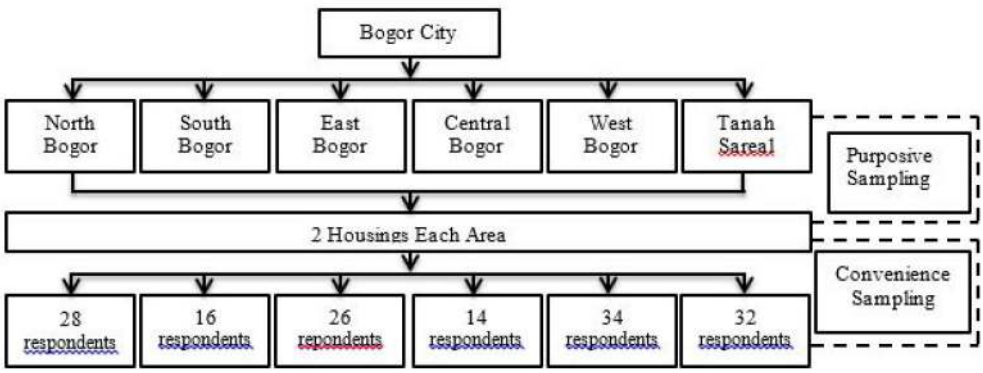

Figure 3. Respondents selection process.

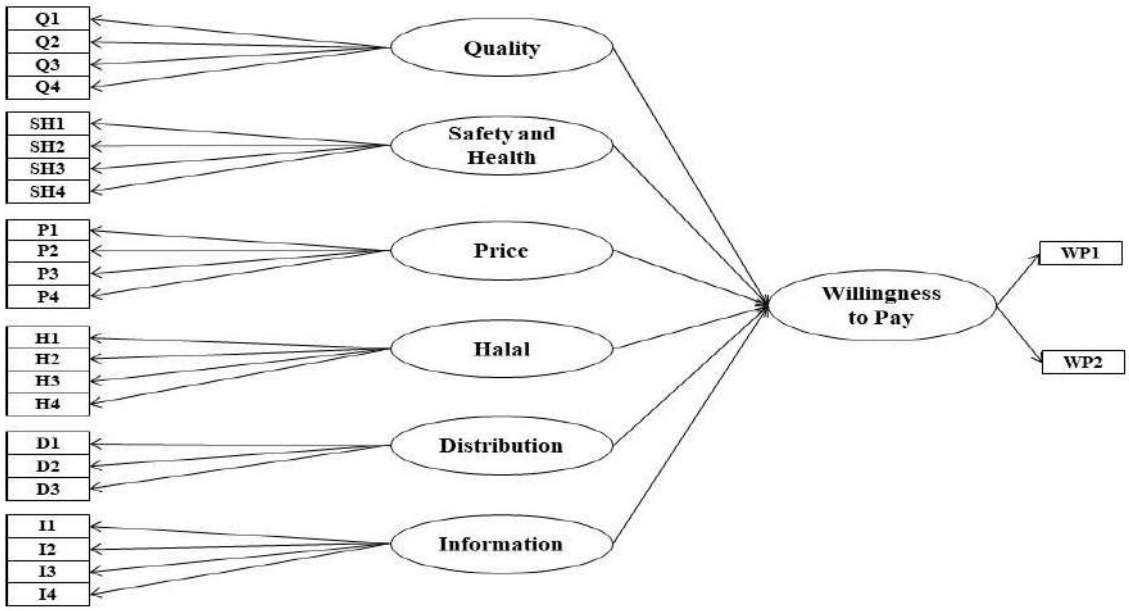

Figure 4. Structural equation model.

Table 1. Bogor city population.

Area	Total population	Sampel fraction	Total respondents
North Bogor	199248	0.19	28
South Bogor	104737	0.10	16
East Bogor	192812	0.18	26
Central Bogor	104682	0.10	14
West Bogor	236302	0.22	34
Tanah Sareal	226906	0.21	32
Total	1064687	1	150

Source: BPS (2017).

Table 2. Reliability test.

Latent variable	CR > 0.70	VE > 0.50	Status
Quality	0,82	0,53	Reliable
Safety and health	0,94	0,79	Reliable
Price	0,81	0,51	Reliable
Halal	0,87	0,63	Reliable
Distribution	0,89	0,72	Reliable
Information	0,81	0,51	Reliable
Willingness to Pay	0,80	0,69	Reliable

Table 3. Operational measures and scale reliability values.

Variables	Code	Indicator	Loading factor	t-table
Quality	Q1	Fresh imported buffalo meat	0.93	17.21
	Q2	The taste of buffalo meat is better than beef	0.64	12.96
	Q3	Soft buffalo meat texture	0.64	12.53
	Q4	The smell of buffalo meat does not smell	0.67	15.85
Safety and health	SH1	Indian's Buffalo meat are safe for health	0.86	22.72
	SH2	Indian's buffalo meat is not guaranteed safe, because it often appears in a illegally imported meat cases	0.88	23.06
	SH3	Government oversight of Indian imported buffalo meat is good	0.87	22.83
	SH4	The cleanliness of buffalo meat Indian imports is guaranteed	0.93	23.96
Price	P1	The price of Indian imported buffalo meat is more affordable than the price of local buffalo meat	0.66	16.06
	P2	The price of imported Indian buffalo meat is in accordance with the promised quality	0.72	14.95
	P3	The price of local buffalo meat is affordable	0.77	15.77
	P4	The price of local buffalo meat is in accordance with the promised quality	0.63	15.64

Table 3. Operational measures and scale reliability values (Continue).

Variables	Code	Indicator	Loading factor	t-table
Halal	H1	Indian imported buffalo meat products sold in the market have halal labels	0.67	17.77
	H2	The halal status of imported buffalo meat is clear even though it is supplied by a majority of non-Muslim countries	0.88	21.11
	H3	The process of halal labeling on imported meat is in accordance with Islamic law	0.80	20.16
	H4	Institutions that issue halal labels can be trusted even though they are in non-Muslim countries	0.80	19.93
Distribution	D1	Many stores sell imported Indian buffalo meat	0.82	16.14
	D2	Indian imported buffalo meat is easy to obtain	0.91	17.03
	D3	Imported Indian buffalo meat is always available in stores	0.82	16.13
Information	I1	Imported meat vendors always tell the origin of the meat they sell	0.63	14.9
	I2	Imported meat vendors always include information labels	0.63	14.61
	I3	I know the quality of Indian beef imported meat	0.79	18.47
	I4	The government has provided sufficient information about meat import policy and all aspects of its health and safety	0.73	17.66
Willingness to pay	WP1	I will still buy imported Indian buffalo meat even though the price is expensive	0.61	8.26
	WP2	I will only buy Indian imported buffalo meat when the price of cattle rises	1.00	4.81

Table 4. Hypothesis.

Hypothesis		Loading factor	t-value >1.96	Conclusion
H1	Quality	0.20	2.73	Supported
H2	Safety and health	0.41	1.98	Supported
H3	Price	0.52	2.39	Supported
H4	Halal	0.54	2.93	Supported
H5	Distribution	0.01	0.19	Not supported
H6	Information	0.67	3.96	Supported

and health, price, halal and information are significantly impacted consumer willingness to pay of buffalo meat in Bogor City. This research also showed a positive effect between distribution and willingness to pay, but unlike other variables, the relationship towards willingness to pay is not significant. That concludes distribution does not affect willingness to pay significantly. So when the Government improve the distribution of imported buffalo meat it will only slightly affect consumer's willingness to pay. This research also found that the lack of information regarding the quality of meat, the origin of meat, Safety and negligence can affect the willingness to pay consumers. Consumers generally want to consume clear information and benefits. Based on the results of this study, it is important for the government to continue to make an introduction to imported buffalo meat. Especially on the quality of buffalo meat which is no less good for health, ensuring that the buffalo meat is safe for consumption, ensuring its reliability and ensuring easy access to import buffalo meat at the nearest market / store, so that people can find this imported buffalo meat in the modern market that is easily accessible by the community.

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ATYPICAL CYCLOPIA ASSOCIATED WITH ARHINIA IN BUFFALO CALF AND ITS MANAGEMENT THROUGH FETOTOMY

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ABSTRACT

A case of dystocia associated with malpresentation of atypical cyclopic monster with arhinic condition was delivered per-vaginally through fetotomy in a buffalo.

Keywords: *Bubalus bubalis*, buffalo, cylopia, arhinia, fetotomy, monster

INTRODUCTION

Cyclopia or cebocephalus is a rare congenital disorder characterized by a single orbit in which global tissue is absent or rudimentary or in which the eyeballs vary from a single apparently normal eye through all degrees of doubling to one consisting of two complete but small adjacent globes (Robert, 1971). Cyclopia most commonly seen in pig and sheep (Roberts, 1971) but also reported in goat (Kanthraj, 2010; Sivasudharsan *et al*., 2010) and cows (Gupta and Anand, 2002; Ozcan *et al*., 2006). However, this anomaly has been rarely reported in buffaloes (Thippeswamy *et al*., 1996; Singh *et al*., 2013). The present paper documents a rare case of atypical cyclopia with arhinia in buffalo calf.

CASE HISTORY AND OBSERVATION

An eight years old, apparently healthy multiparus non-descript she-buffalo at full term presented with a history of straining for the last seven hours and ruptured water bags, was presented to Teaching Veterinary Clinical Complex, Veterinary University, Mathura (Reg. No. 941/25.10.12). Clinical examination revealed an increase in respiration and pulse rate with normal rectal temperature. Obstetrical examination revealed dead fetus in anterior longitudinal presentation and dorso-sacral position with both the forelimb extended in birth canal, and palpation of base of the neck at the level of pelvic inlet suggestive of extreme deviation of neck (right side), found to be major cause of the dystocia.

TREATMENT AND DISCUSSION

The animal was restrained in lateral

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recumbency, effort were made to correct the deviated neck through obstetrical manoeuvres but unsuccessful, then it was decided to amputate the neck at its base. In this course, the deviated neck of fetus was amputated at its base (Figure 1, b) after creating some space by amputing right fore-limb of fetus at scapular level (Figure 1, a) with double barrel Thygeson's fetotome. For this, fetotome was partially threaded on one side, and a sand snare introducer was applied at end of the wire. The wire was then carried into the birth canal with loose loops to pass over the deviated neck and retrieved from other side; the fetotome was then completely threaded outside, and the loop was positioned near the base of the neck. Now, the head of fetotome was carried into the birth canal and positioned at the base of the neck ventrally. Finally, sawing was done, initially with short strokes followed by a continuous full hand strokes till the neck was amputated. The amputated neck along with head was extracted by applying Krey-Schottler double jointed eye hook. After thorough lubrication of the birth canal, forced traction was applied on extended left forelimb & rib cage, remaining dead male fetus was extracted out. Thereafter, buffalo was administered with Inj. Dextrose normal saline 5% (2 Litre, I/V), Inj. Calcium borogluconate (450 ml, I/V), Inj. Intamox (4 gm, I/M), Inj. Meloxicam (100 mg, I/M) and Inj. Dexamethasone (30 mg, I/M) and placenta was expelled out at same night without any assistance.The morphological examination fetal head revealed that there was absence of typical upper and lower jaw leading to flat face, bearing single orbit with fused two eye balls and two separate corneas at fore head made the face of calf just like monkey face (Figure 2, a, b and c). Both upper and lower eyelids rudimentary with scanty eyelashes and absence of eye bro. Muzzle, nostrils and typical nose (arhinia). There was two tubular appendages were placed above the orbit and below the rudimentary oral opening (Figure 3, a and b). In true cyclopia orbital region is grossly deformed, resulting in the formation of a central cavity 'pseudo-orbit', bearing single eye with absence of nasal cavity and presence of a rudimentary proboscis above the pseudo-orbit (Garzozy and Barkay, 1985). If two globes are found in a different degree of fusion in the pseudo-orbit, the condition is called synophthalmos (Garzozy and Barkay, 1985). The present monster bearing fused eye ball (Synophthalamos) with proboscis which was modification of the nose and lower tubular appendages probably modification of chin as hair pattern was similar to chin.

Both ears were normal and hairs were present all over the body. There was rudimentary visible fused eyes which was also reported in non-descript cow (Gupta and Anand, 2002). Ozcan *et al*. (2006) reported an atypical cyclopia in a Brown Swiss cross calf with most significant malformation such as the presence of a median orbita-like opening that did not contain an eyeball and other defects included prosencephalic aplasia, brachygnathia superior and arhinia. Arhinia and cyclopia in a German Fleckvieh calf was also reported by (Schulze and Distl, 2006). Khasatiya (2010) also reported a cebocephalus (cyclopia) monster in a cross-bred cow and noted that epitheliogenesis imperfecta over the forehead and body, having rudimentary and separate eye balls and atypical deformed ears and jaw.

Thus the present report describes relieving dystocia due to severe deviation of neck and head associated with atypical cyclopia along with arhinia in a buffalo calf. Though it is proved that congenital abnormality generally manifestation of genetic abnormalities or extreme environmental adverse effect during the organogenesis of the fetus. These

Figure 1. Extracted dead fetus after amputation of right fore-limb (a) and neck (b).

Figure 2. Single eye orbit (a) with fused two eye balls (b) with two separate corneas (c).

Figure 3. Two appendages placed above and below (a) and centrally located eye (b).

factors were ionising radiation, some of drugs like contraceptives, viraemia plus corticosteroids and salicylates, rubella vaccine, antibiotics, and amidopyrine (aminopyrine) as reported in human (Benawra *et al*., 1980; Mollica *et al*., 1981). In one study (Binns *et al*., 1963) found that cyclopic malformation in newborn lambs in a flock of sheep and stated that this anomaly arises due to ingestion of *Veratrum californicum* in pregnant ewes. In the present case, identification of specific cause will aid in ensuring the preventive measures. This could be achieved by genetic analysis of the dam and sire of a defective fetus and detail account of antenatal exposure of dam to various drugs or other teratological agents to which dam might have been exposed during the early gestation leading to the cyclopic condition in the calf.

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DELIVERY OF A *SCHISTOSOMUS REFLEXUS* MONSTER THROUGH CAESAREAN SECTION IN A MURRAH BUFFALO

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ABSTRACT

A case of dystocia in a Murrah buffalo due to *Schistosomus reflexus* monster is reported and discussed here.

Keywords: *Bubalus bubalis*, buffalo, *Schistosomus reflexus*, Murrah buffalo

INTRODUCTION

Schistosomus reflexus is a rare and fatal congenital disorder. Primarily observed in ruminants, its defining features include spinal inversion, exposure of the abdominal viscera because of a fissure of the ventral abdominal wall, limb ankylosis, positioning of the limbs adjacent to the skull and, lung and diaphragm hypoplasia (Laughton *et al*., 2005). The condition belongs to the family of defects involving incomplete closure of the ventral body wall. The 'schistosomus' aspect of the syndrome i.e. the presence of a congenital schistocoelia is manifested in many species (Bishnoi *et al*., 1987; Pivnick *et al*., 1998). Conversely, the 'reflexus' component of the disorder is limited to only a few species. In fact, it has been suggested by Bezek and Frazer (1994) that this anomaly is restricted to ruminants. The 'reflexus' component alone has been described using such varying terminology as dorsiflexion, dorsal flexion, retroflexion and inversion (Laughton *et al*., 2005). The prevalence of *Schistosomus reflexus* is believed to occur in cattle from as low as 0.01% (Sloss and Johnston, 1967) to a high of 1.3 (Knight, 1996). Such occurrences are costly to the cattle and buffalo owners because of the reduction in number of viable offspring's, loss of milk production and cost of fetal extraction or caesarean section. This monstrosity has been reported in cattle (Jana and Ghosh, 2001) and water buffalo (Murthy *et al*., 1991; Singla and Sharma, 1992). The present case reports a rare occurrence of *Schistosomus reflexus* in a Murrah buffalo.

CASE HISTORY AND OBSERVATIONS

A case of dystocia (Registration No. E-71160 dated 29.07.2016) in a pleuriparous Murrah buffalo was presented to Teaching Veterinary Clinical Complex, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana) with the history of straining and hanging of fetal stomach filled with mucoid fluid and loops of intestines through the dam's vulva (Figure 1) and no progression in parturition for last 9 h. The owner of the animal was cross checked for any mishandling by the veterinary practitioners at

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Fiure 1. Hanging of fetal stomach filled with mucoid fluid and loop of intestines of a *Schistosomus reflexus* monster.

Figure 2. *Schistosomus reflexus* monster delivered through caesarean section.

door step and it revealed that paraveterinary staff referred the animal as a case of rupture of maternal uterus leading to prolapse of intestines. Following epidural anesthesia and sufficient lubrication, animal was examined per vaginally. Per-vaginal examination revealed that the birth passage was completely relaxed, fetal reflexes were absent and the fetus was confirmed as dead. Fetal thoracic and abdominal contents were present in the birth canal. No remnants of fetal ventral body wall were apparent. The case was diagnosed as dystocia due to *Schistosomus reflexus*.

TREATMENT AND DISCUSSION

Epidural anaesthesia with 2% lignocaine was given to the dam, followed by evisceration of protruding fetal contents and forced traction was applied. But could not succeed due to deformed fetal pelvis. The owner was advised for caesarean section. The caesarean section was performed as per routine surgical method (paramedian, lateral to milk vein) and a *Schistosomus reflexus* monster was delivered (Figure 2). On the basis of external genitalia, sex of monster was female. The dam was administered systemic broad spectrum antibiotics (Ceftriaxone plus Sulbactum), anti-inflammatory drugs (Flunixin meglumine), herbal ecbolics, calcium boro-gluconate, normal saline, metronidazole and multivitamins. The treatment was recommended further for seven days.

Fetal pelvis was deformed and the deformation of the pelvis is a variable skeletal anomaly that results from the spinal inversion and compression between the inverted spine and the caudal bones of the skull (Bugalia *et al.*, 1982; Roberts, 1971). The lungs of fetus were hypoplastic which also have been reported in other cases (Dennis and Meyer, 1965; Fatimah *et al.*, 1981). |Hepatomegaly was observed and similar case was also reported by Laughton *et al.* (2005). Fore and hind limbs were ankylosed and it was difficult to straighten them. Cervical intervertebral joints were in ankylosed state. A more or less similar characteristic monstrosity in crossbred cattle (Jana and Ghosh, 2001; Singh *et al.*, 2010) and buffalo (Chandraprasad *et al.*, 2010) was observed when dystocia was relieved by caesarean section.

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DYSTOCIA DUE TO FETAL SKELETAL DEFECTS IN A MURRAH GRADED BUFFALO

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ABSTRACT

Present case report deals with a rare case of dystocia due to foetal scoliosis (lateral curvature of lumbar region of vertebral column), dwarfism (short body length) and arthrogryposis (deformed joints of limbs) and its successful management through pervaginum.

Keywords: *Bubalus bubalis*, buffalo, scoliosis, arthrogryposis, Murrah graded buffalo, dystocia

INTRODUCTION

Congenital anomalies and less frequently, multiple congenital anomalies, are reported to be encountered in domestic animals that are present at birth, which in turn may cause obstetrical problems (Arthur *et al*., 2001). These Congenital abnormalities of foetus with structure and function are present at birth is relatively less frequent in bovines (Rahman *et al*., 2006). Anomalies occurring due to congenital defects often lead to dystocia. Limb joint deformaties like arthrogryposis is caused by an autosomal recessive gene with complete penetrance in the homozygous state (Goonewardene and Berg, 1976). Arthrogryposis is a rare congenital musculoskeletal anomaly and is seen in all breeds of cattle, with greater incidence in Angus and Charolais breeds (Abbot *et al*., 1986; Windsor, 2011). The affected calves exhibit joints fixed in abnormal positions and frequently have scoliosis and kyphosis (Shupe *et al*., 1967; Keeler, 1974). The musculoskeletal defects of the foetus like rigid and fixed limbs in abnormal posture often lead to dystocia (Aiello, 2000; Katiyar *et al*., 2015). Scoliosis is a condition characterized by abnormal dorsal curvature of a spine and dwarfism is characterized by shortened length of body and limbs (Vegad and Swamy, 2010). The incidence of congenital defects in calves ranges from 2 to 3.5% (Aiello, 2000) of which, musculoskeletal defects account for 24% (Leipold *et al*., 1983).

CASE HISTORY AND CLINICAL OBSERVATIONS

A pleuriparous Murrah graded buffalo aged about 6 years was presented in clinics with a history of full term gestation. Animal was showing signs of straining for last 12 h and first water bag was ruptured 6 h back. Limbs were visible in vulva when case was presented in clinics. Animal was previously handled by quakes for 2 to 3 h with unsuccessful attempts. Per vaginal examination revealed a fetus with abnormal dorsal curvature

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and was presented in posterior presentation with dorso-iliac position. Fetus was matured with short body length and was packed in pelvic cavity. Acute dorso-lateral curvature of vertebral column was preventing the extraction of the fetus. Cervix was completely dilated with no lubrication.

TREATMENT AND DISCUSSION

The buffalo was pre-medicated with inj. Dexamethasone 40 mg, i.m total dose (Zidex; 20 mL; Laborate Pharma Ltd.). Lubrication of birth canal was achieved with heavy Liquid Paraffin (approximately 2 litres) which was followed by snaring of hind limbs at fetlock joint. As the fetus was in dorso-iliac position, it was rotated to dorso-sacral position by holding hind limbs. Fetus was pulled in downward and lateral direction just to create additional gap between pelvic bone of buffalo and scoliotic back of calf. A dead male calf was extracted after forced extraction. The buffalo was treated with Inj. Intacef 4.0 gm (Intas Pharma. Ltd.), Inj. Meloxicam 0.2 mg/kg body wt. i.m (Intas Pharma Ltd.) for 5 days. The fluid therapy was done with inj. Ringer's Lactate (5 litres) and inj. Normal saline (5 litres) by i.v. route along with supportive therapy for 5 days. Animal Showed uneventful recovery and was discharged after 5 days.

Gross examination of the fetus revealed an abnormal dorso-lateral curvature of spine (Scoliosis) and bending of joints at knee and fetlock joint (Arthrogryposis) (Figure 1). Overall shortened length of both forelimbs and body showed dwarfism. Generally defects of vertebral column such as Kyphosis and scoliosis are observed in ruminants (Rahman *et al*., 2006) and are responsible for dystocia in these animals (Katiyar *et al*., 2015) as observed in our case. However other skeletal defects of extremities for example torticollis and arthrogryposis can also cause dystocia in animals as affected fetuses occupy more space in pelvic cavity and are difficult to manage (Mahajan *et al*., 2006; Singh *et al*., 2008).

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RECTAL PROLAPSE IN CROSSBRED BUFFALO (*BUBALUS BUBALIS*) CALF

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ABSTRACT

A 2 years old male buffalo calf was presented with a history of prolapsed mass hanging from of anal region from last 2 weeks with signs of straining and difficulty to defecate. Another signs included inflammatory signs on rectal wall, soiling with dirt and congested mucous membrane. The animal was treated under posterior epidural anesthesia using lignocaine hydrochloride 2%. The prolapsed mass was manually replaced and purse string suture was applied. Broad spectrum antibiotics and analgesics were administered post-operatively. Animal recovered uneventfully 8th post treatment days.

Keywords: *Bubalus bubalis*, buffalo, rectal prolapse, crossbred, buffalo calf

INTRODUCTION

Many affections of digestive system found buffaloes due to indigestion and sometimes worm infestation. Prolapse of rectum one of them it involves eversion of the rectum beyond the level of the anus. Rectum prolapse involves rectal mucosa along rectal wall. It may be incomplete type where rectal mucosa is everted, or complete, in which all rectal layers are protruded. Sometimes, the rectum prolapse may associated with intussusceptions of the large intestine i.e. rectum, colon or even small intestine Slatter (2003). The prolapse of the rectum occurs not only in buffaloes also in other animals like pigs, ruminants, horses and carnivores (Anderson and Meisner, 2008). Symptoms and corrections depend on the degree of damage to the mucosal layers. Generally, manual reposition of anatomical position with support of external application of suture helps faster recovery of the prolapsed. If condition persists without any signs of recovery needs amputation of the prolapse is indicated or when perforating injuries or necrosis of the mucosal layers are present (Kersjes *et al.*, 1985). This paper presents a case of prolapse of rectum and its management in a male buffalo calf.

MATERIALS AND METHODS

History

A 2 years old male buffalo calf presented with the complaint of large protruded mass through the perineum region. As per owner anamnesis this condition was presented since 2 weeks and previously the condition was treated by local paravets with manual manipulation with application of various medicines. Treatment showed no significant changes and fruitful results. Owner was unsatisfied with the previous treatment

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and wanted permanent remedies. The owner came again for the treatment with lots of hope. Other history revealed anorexia. constipation, frequent straining and difficulty in defecation.

Clinical observations

The animal was examined to find out different sings for proper diagnosis of the condition. Clinical signs included dry, rough skin, pale mucous membrane along with large bulged mass protruded through anal opening (Figure 1).

Diagnosis

For the confirmation of the condition diagnosis was made by the help of owner history and clinical observations showed by animal. Differential diagnosis was also for made for comparison with similar findings like intussusceptions etc. Finally on the basis of all the observations the case was diagnosed as prolapsed of rectum. As the case was delayed presented, older and emergency need, decided to perform manual manipulation supported with surgical procedures.

RESULT AND DISCUSSIONS

Treatment was initiated with fluid therapy injection DNS (1000 ml, i/v) was given to improve the condition from dehydration. Animal secured properly and desensitization was achieved by posterior epidural anaesthesia using 15 ml of 2% lignocaine hydrochloride. The visible dirt materials of prolapsed mass were removed manually with gloved hand. The mass was washed with the help of 2% potassium permagnate solution

Figure 1. Showing the large protruded mass of rectum.Due to external exposures the mass was injured, tense, oedematous, swollen and contaminated with mud. Animal showed weak pulse, irregular respiration due to stress and depression. On manual manipulation by hand the protruded mass was repositioned in to their normal anatomical position but when the hand was removed the mass was again returned back in to as usual condition.

(1:1000 dilutions). The mass was repositioned in to the normal anatomical position manually after lubrication with liquid paraffin. To prevent the recurrence the additionally retention suture (purse string suture) were applied over the wall with cotton thread after impregnation of povidone iodine antiseptic solution (Figure 2).

Povidone iodine followed by Silver nitrate ointment was applied over the external wounds created during suturing. Post-operatively, antibiotic inection Ceftrixone 20 mg/kg, Antispasmotic agent Dicyclomine acetate, 10 ml and anti-inflammatory Meloxicam 0.2 mg/kg body weight intramuscularly were given on same and subsequently next four days. Cotton thread was removed after 8th days after complete healing. The animals showed uneventful recovery without signs of any complications and recurrence of the case.

Prolapse of rectum usually found in young animals due to with severe diarrhea and tenesmus. Any delay in treatments may lead to oedema, ischaemia, laceration, haemorrhages and shock, resulting in prognosis as poor to hopeless (Pande and Pande, 2002; Rubin, 2013). Long term condition creates intra abdominal pressure due to bloat, proctitis, diarrhorea, act of parturition and constipation (Tyagi and Singh, 2010). Similar finding was also observed on the present study. Sometimes it is genetically inherited trait is another factor that predisposes calves to develop rectal prolapse (David and Matt, 2008). Lignocaine hydrochloride 2% epidural was used by Singh and Jain (2013) as in the present case. The similar treatment was adopted for rectal prolapse reposition and application of a purse string suture as described by the Jean and Anderson (2006); Borobia-Belsne (2006); Madhu *et al*. (2014). Several techniques of prolapsed rectum amputation have been described and accepted like submucosal resection (Johnson, 1943), rectal ring method, stair step amputation (Fubini and Duchrame, 2004; Weaver *et al*., 2005), delorme's operation (Delorme, 1900) and popular trans abdominal procedures namely Ivalon® sponge (Morgan *et al*., 1972), Marlex

Figure 2. Purse string suture over the wall.

mesh (Keighley *et al*., 1983), Ripstein (Ripstein, 1972), perineal surgical repair (Nay and Blair, 1972), prophylactic colcopexy (Sherding, 1996) and extended abdominal rectopexy (Mann and Hoffman, 1988). It was concluded that this type of cases need early diagnosis and needful treatment because sometimes it may be life threatening.

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OVARIAN CYST IN MILKING SWAMP BUFFALO: A CASE STUDY

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ABSTRACT

A postpartum swamp buffalo with a high body condition score (4.5/5) that did not show any signs of heat after calving (days open= 122 days) was submitted for reproductive examination. The buffalo was milked every morning and suckled after that the whole day. On examination, an ovarian cyst of about 3 cm diameter was palpated rectally in the left ovary, which was confirmed by transrectal ultrasonography and by measuring the plasma progesterone level 7 days later. The case was treated with a common treatment regimen of gonadotropin releasing hormone followed by prostaglandin F2 alpha 7 days later. The buffalo returned to estrus and was mated with a bull 16 days after the onset of treatment. This case study suggests that a regular postpartum reproductive examination should be performed for a timely diagnosis of problems, and that treatment with a standard hormonal protocol can resolve cases of ovarian cyst in swamp buffaloes.

Keywords: *Bubalus bubalis*, buffalo, ovarian cyst, swamp buffalo, gonadotropin releasing hormone, prostaglandin F2 alpha

INTRODUCTION

Cystic ovarian follicle (COF) is a major reproductive problem, causing infertility and economic loss in buffaloes (Vanholder *et al*., 2006). The percent of slaughtered buffalo in India found to have COF was 9.5% (Saxena *et al*., 2006). The main categories of ovarian cysts are follicular cyst and luteal cyst (Vanholder *et al*., 2006). Follicular cysts are primarily observed in the early postpartum period (Vanholder *et al*., 2006). Follicular cysts resemble enlarged follicles, generally defined as varying in size greater than 2.5 cm in diameter, that persist for at least 10 days in the absence of a corpus luteum (Garverick, 1997). The most noticeable signs of follicular cysts are irregular estrus intervals, reduced milk production, persistent bulling behavior or nymphomania and anestrus (Vanholder *et al*., 2006). The cyst's surface during rectal palpation is smooth, slightly elevated, and fluctuating (Farin *et al*., 1990). Follicular cysts are usually thin-wall and secrete little progesterone (Brito *et al*., 2004; Noseir and Sosa, 2015). In the later stages of follicular cysts lutinization will occur and they will turn into luteal cyst (Brito *et al*., 2004). Luteal cysts have thicker walls (more than follicular cysts) produce more progesterone (level ≥1 ng/ml)

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and are associated with anestrus behavior (Brito *et al*., 2004). Rectal palpation reveals a flaccid uterus, firm, protruding on the surface of the ovary (Farin *et al*., 1993). The development of ovarian cysts in buffalo has been associated in the early postpartum period with hormonal, metabolic, nutritional and environmental imbalances (Garverick, 1997).

Intensive farming system methods have recently begun to be used in swamp buffalo farms in Thailand. The animals are fed prepared concentrates both for nourishment and to prepare the animals for buffalo contests or milking – rather than the free grazing system used in the past. Artificial insemination has also been used in the last 10 years. Many infertility issues have been detected, one of which is ovarian cysts which cause a long calving interval and reduce the lifetime productivity of the animal. There are no publications on ovarian cysts, however, in swamp buffalo. This case report could provide useful information and basic guidelines on how to approach the ovarian cyst problem in swamp buffalo.

CASE DESCRIPTION

The present study was carried out by a veterinarian team in a swamp buffalo farm in Prachinburi in the Eastern part of Thailand during a 4 month period from June 23rd, 2018 to August 19th, 2018. A postpartum routine checking of the case study's swamp buffalo was done. The buffalo was 5 years old, second parity with the last calving on 1 March 2018 (days open= 122 days) and the animal had shown no estrous signs since calving. This buffalo's milk output was less than other buffalo in the farm. The average milk yield from March 30, 2018 – to June 29, 2018 was 1.3 kg/day. The buffalo was fed 16% protein concentrate 6 kg/day once a day, roughage 2 times a day with fresh grass and rice straw, cleaned tab water was presented ad libitum. Both roughage and concentrate were stored in a clean and dry house. The buffalo and her calf were kept in an individual 25 m^2 pen with an insect protective net, rubber reclining pads and 15 minutes of sprinkle water every hour - throughout the day.

PHYSICAL AND REPRODUCTIVE EXAMINATIONS

Evaluation of vital signs: all were normal (temperature 101.8 °F, heart rate 52 bpm, pulse rate 60 bpm, respiration rate 72 bpm, CRT <2 seconds, pink mucous membrane). General physical examination showed a body condition score of 4.5/5, good hair coat, normal hydration status, feces score 2/5. External reproductive organs examination presented normal vulva conformation, pink-red vulva, clear mucous vaginal discharge, vaginoscopy score 0/3 (no pus) and shiny with normal moisture.

Internal reproductive organs were examined by rectal palpation which presented a closed cervix, 3 cm in diameter and 7 cm in length. Uterine tone was graded 1/3 (slightly flaccid) and exhibited a symmetry of uterine horns. A large size follicle of about 3 cm in diameter with smooth, fluctuating and elastic surface was found in the left ovary.

DIFFERENTIAL DIAGNOSIS

Ovarian cysts have some similar symptoms to, and are often misdiagnosed as: hydronephrosis, hydrosalphinx, paraovarian cyst,

ectopic pregnancy, ovarian torsion, tubo-ovarian abcesses, ovarian cancer and other conditions such as appendicitis or diverticulitis (Farin *et al.*, 1993).

FURTHER DIAGNOSIS

The reproductive tract of the swamp buffalo was examined by transrectal ultrasonography. The ovaries were scanned with an ultrasound scanner equipped with a 6.5 to -7.5 MHz linear probe (SonoScape®, Italy). The cervix was 2.8 cm in diameter and absent uterine discharge (discharge score=0/3). The right uterine horn wall was 0.7 cm in thickness, the right ovary 3x2 cm in diameter with small spots of anechoic areas that appeared as small follicles. The left uterine horn wall was 0.9 cm in thickness, and the left ovary 3.4x4 cm in diameter with a thin-wall (1 mm), and a 3cm diameter anechoic and hypoechoic area. Blood analysis found macrocytic normochromic with neutrophilia, lymphopenia, monocytosis and stress leukogram. A plasma progesterone concentration of 0.2 ng/ml was measured using a standard assay kit (Chemiluminescent Microparticle Immunoassay, Abbotte Laboratory Limited, USA).

DIAGNOSIS

Tentatively, the case was diagnosed as an ovarian cyst (3 cm in diameter) in the left ovary, which was confirmed by transrectal ultrasonography and a plasma progesterone level of less than 1 ng/ml.

TREATMENT

The standard treatment protocol utilizes GnRH, followed 7 days later with prostaglandin F2 alpha ($PGF_{2\alpha}$). This protocol appears to induce ovulation in a high percentage of anovulation cases (Noseir and Sosa, 2015).

This protocol was selected for treating this case. As shown in Figure 1, on day -12 (June 23, 2018) the initial diagnosis was done (palpation and ultrasound). On day – 5, (June 30, 2018) blood collection for a complete blood count (CBC) and blood chemistry analysis was performed. On day -2 (July 3, 2018), blood collection to check the progesterone level was done. On day 0 (July 5, 2018), the buffalo was injected with 5 ml GnRH (0.02 mg buserelin acetate, Receptal®, Intervet International GmbH, Germany). Seven days later (July 12, 2018) blood collection to check the progesterone level and an ultrasound for corpus luteum detection were done, followed by an injection of 5 ml $PGF_{2\alpha}$ (25 mg dinoprost tromethamine, Lutalyse®, Pfizer Inc, USA). Finally on day 23 (July 18, 2018) the buffalo was successfully mated (Figure 1).

PROGNOSIS

After treatment with this protocol, the ovarian cyst in the swamp buffalo was resolved in 10 days (Figure 2). A normal, fertile estrus can be expected in 15–30 days. Likewise, successful treatment will increase the rate of pregnancy.

DISCUSSION

Ovarian cysts are one of the most important ovarian disorders in buffalo. This buffalo showed

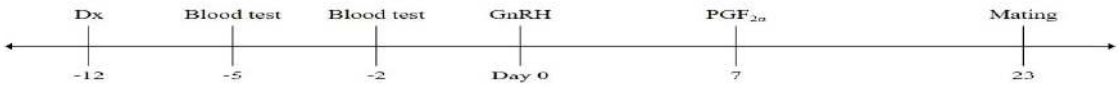

Figure 1. Follicular cyst treatment protocol.

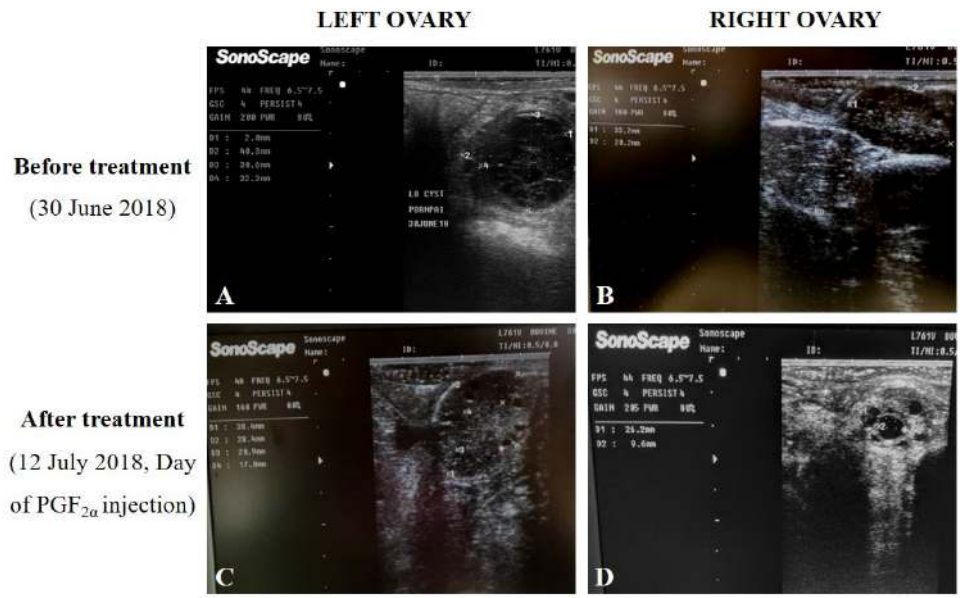

Figure 2. The ovarian structures before and after treatment were monitored by ultrasonography;

(A) The diameter of the left ovary is 4x2 cm the diameter of the ovarian cyst is 3.2x3 cm with a thin- wall (1 mm).

(B) The diameter of the right ovary 3.3x2 cm with small and medium follicles.

(C) The diameter of the left ovary is 3.8x2.8 cm, the diameter of a corpus luteum is 2x1.7 cm.

(D) The diameter of the right ovary is 2.2x1.4 cm with small, medium follicle.

a slightly fat (4.5/5) body condition score which could suggest that nutritional imbalances and metabolic disorder factors induced the ovarian cyst issue, which is consistent with the blood test results that showed a stress leukogram (Garverick, 1997). Our study presented a case of an ovarian cyst in a swamp buffalo as confirmed by transrectal ultrasonography and plasma progesterone level detection as its differential diagnostic methods (Medan et al., 2004). This ovarian cyst could have been a follicular cyst based on the evidence. The cow presented a milk production rate which was lower than normal in swamp buffalo (2.0±0.9 kg/cow/day) (Chaikhun et al., 2012). Transrectal ultrasound of these structures was compatible with a large follicle with a thin wall and a fluid and content-filled cavity. These findings were in agreement with Brito et al. (2004) as was the concentration of plasma progesterone 0.2-0.4 ng/ml (Lin *et al.*, 1993). The ovarian cyst may have been in the process of transforming from a follicular cyst to a luteal cyst which may be why the cow presented anestrus. The results of the present study demonstrate that an ovarian cyst can be treated effectively using a single injection of GnRH followed by $PGF_{2\alpha}$ (Garverick, 1997). The efficacy of this treatment of follicular cysts was confirmed by the luteinization of the follicular cyst and an increase in the concentration of progesterone (Medan *et al.*, 2004). In this case study on day 7 after treatment examination showed the corpus luteum in the left ovary similar to a previous report (Nanda *et al.,* 1988). The hormonal protocol in this case has been used successfully for the treatment of follicular cysts in buffalo with a success rate of 60 to 70%, similar to the rate reported in cows treated with GnRH (Ribadu, 1991; Garverick, 1997). A report has suggested that a successful mating program may reduce recurrences by establishing pregnancy as soon as possible (Brito *et al.*, 2004). Therefore, regular estrous detection and mating either by bull or artificial insemination were suggested to the farmer and the buffalo was mated by bull 16 days after treatment. Postpartum reproductive function should be monitored at least 45 days after calving for an ovarian resumption evaluation (Peter *et al.*, 2009). This could help detect any reproductive issues and assist in the creation of an efficient mating plan in buffalo farms.

In conclusion, the predisposing factors in the present study were nutritional imbalances and metabolic disorders. The follicular cyst in this case was confirmed by transrectal ultrasonography and the concentration of plasma progesterone. The standard treatment with GnRH and $PGF_{2\alpha}$ injections was found to be effective in treating the follicular cyst in this swamp buffalo. However, stress management and proper dietary management should be monitored in this case to prevent reoccurrence.

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RECTAL STRICTURE DUE TO CIRCUMFERENTIAL FIBROMA IN BUFFALOES

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ABSTRACT

The present clinical study describes successful surgical management of a rectal stricture due to circumferential fibroma (fibrous ring) in two recently calved adult buffaloes. Both buffaloes had a chronic history of straining while defecation with no rectal prolapse. At presentation, these buffaloes were passing scanty and pasty feces. Per rectal examination revealed rectal stricture due to demarkable circumferential fibrous ring which was later histopathologically diagnosed as fibroma in the sub mucosa of the rectum. Surgical resection of the fibrous ring was done under caudal epidural anesthesia. Both buffaloes recovered uneventfully and no recurrence of this condition was observed on long term follow up of 4 years and six months, respectively.

Keywords: *Bubalus bubalis*, buffalo, bovine, fecal obstruction, fibrous ring, fibroma, straining, surgery

INTRODUCTION

Stricture is a narrowing of the lumen due to cicatrical tissue. Injury to the rectum due to foreign bodies or trauma, neoplasia or fat necrosis impinging on or within the lumen or from defects associated with rectal and vaginal strictures may result in rectal strictures in cattle (Kahn and Line, 2010). Various tumors such as scirrhous adenocarcinoma (Suzuki and Ohshima, 1993), fibroma (Bose *et al*., 1981) and poorly differentiated carcinoid (Michishita *et al*., 2007) of the rectum have been reported in cattle. The recto-vaginal stricture in cattle may also be congenital (Radostits *et al*., 2007). Acquired rectal stricture in the feeder pigs of 2 to 3 months has also been reported due to *Salmonella typhimurium*; though, it has been suggested that there was an inherited component in the etiology. The rectal stricture in pigs has been reported to be 2 to 5 cm anterior to the anus and can be felt with digital palpation (Jackson and Cockcroft, 2002; Radostits *et al*., 2007).

The treatment of rectal stricture in small animals is usually balloon dilation combined with intra lesional injections of long acting corticosteroids such as triamcinolone. But in large animals it usually includes resection of the strictured area using rectal pull through technique (Kahn and Line, 2010). Occurrence and surgical management of caudal rectal tumors have also been described in buffaloes (Mosbah and Naggar, 2012) but associated rectal stricture which could have led

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to difficult defecation has not been mentioned. The present case report describes two cases of buffalo having rectal strictures due to fibrous ring, later diagnosed to be a fibroma, which were successfully surgically treated by resection of the stricture area.

CASE HISTORY AND PRESENTATION

Two recently calved, non-gravid buffaloes were presented to the department of Veterinary Surgery and Radiology at different times with the chronic history of straining while defecation of feces. The detailed signalment, history and clinical presentation of these buffaloes has been given in Table 1.

Per rectal examination revealed rectal lumen stricture at a depth of about 10 cm from the anus in both the cases. The stricture could be pulled out of anus using finger (Figure 2). Only one finger opening in buffalo 2 and three fingers in buffalo 1 was present in the stricture region. A circumferential fibrous ring in the rectal mucosa was felt causing the stricture. Considering the findings, resection of the stricture ring was planned under caudal epidural anesthesia.

TREATMENT AND DISCUSSION

The surgical intervention was done under caudal epidural anesthesia by injecting 5 ml of inj. lignocaine Hcl 2% into the sacro-coccygeal space. The onset of anesthesia was observed within 10 minutes. The surgical site was cleaned and prepared aseptically. By pulling the stricture out of the anus with the help of finger, two stay sutures were applied using silk No. 2 at 3 and 9 o' clock position of the stricture (Figure 3). The sutures were grasped with artery forceps and pulled, so that the stricture site could be visualized and made stable during surgical maneuver. A circumferential incision was made on the rectal mucosa over the fibrous ring. The fibrous tissue causing stricture was dissected out (Figure 4). Four horizontal mattress sutures were applied at four quadrants to oppose the mucosal incision using catgut no. 1 in buffalo I; however, no sutures were applied in buffalo II. After surgery, the rectal lumen was large enough (Figure 5) to pass full hand. The resected tissue was subjected to histopathological examination. Post operatively inj. Ampicillin-Cloxacillin 10 mg/kg and inj Gentamicin 2 mg/kg, twice daily, intramuscularly, were administered for 5 and 3 days, respectively along with inj. Meloxicam 0.2 mg/kg, once daily, for 3 days. Daily application of soframycin cream and lignocaine jelly was advised at the surgery site inside the rectum for 5 to 7 days. It was also advised to pass full hand into the rectum, 2 to 3 times daily for 10 days to act as a dilator.

Histopathologically, well circumscribed nodules of dense fibrous tissue with occasional spindle cells having scant cytoplasm and elongate nuclei with evenly distributed fine chromatin were seen on H & E stain. The collagenous fibers were repetitive and were arranged in interwoven fascicles. On the basis of these findings, the condition was diagnosed as fibroma (Figure 6). Telephonic follow up at regular intervals up to 4 years in buffalo I and 6 months in buffalo II revealed these to be healthy, with good reproductive status and were passing normal feces without straining.

Rectal stricture has been reported as a congenital condition in cattle along with vaginal involvement (Radostits *et al.*, 2007) though other reasons like foreign body, neoplasia or fat necrosis in the rectum have also been reported to be the causes of rectal stricture in cattle (Kahn and Line,

Figure 1. Photograph showing frequent and excessive straining with bulging of almost 15 cm of anal area while defecation of feces.

Figure 2. Photograph showing site of rectal stricture.

Figure 3. Photograph showing rectal stricture with stay sutures at 3 and 9 '0 clock position (yellow arrow).

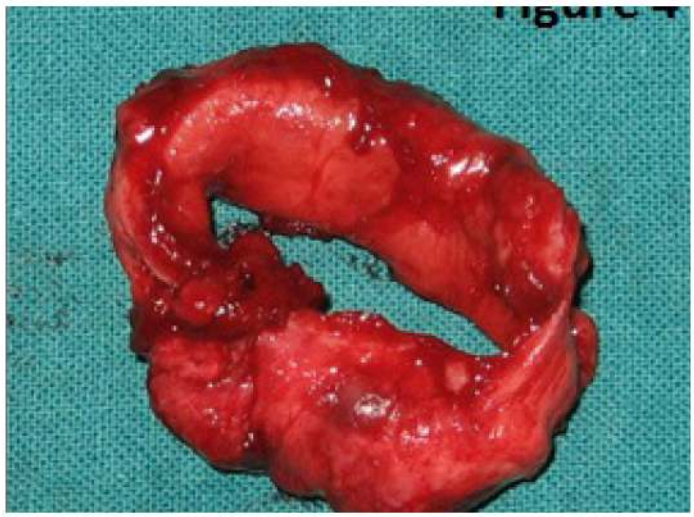

Figure 4. Photograph showing resected fibrous ring.

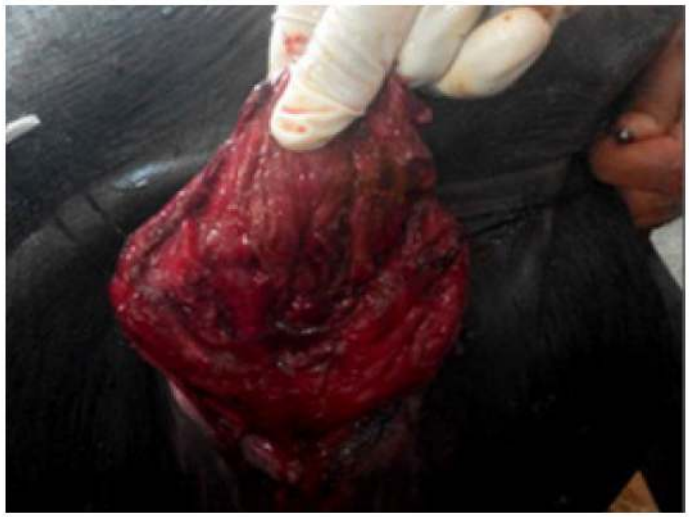

Figure 5. Photograph showing widening of rectal opening after surgery.

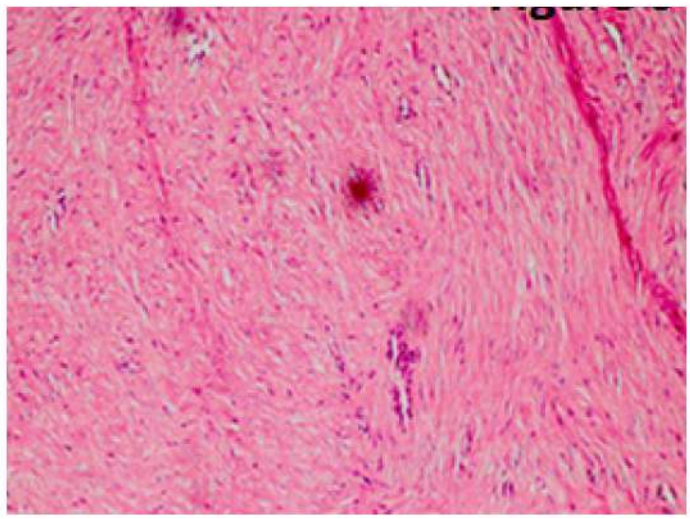

Figure 6. Photograph showing H and E stain of histological sample in 10x showing typical features of fibroma.

2010). The pigs suffering from rectal stricture have been reported with clinical signs of progressive abdominal distension, inappetence, emaciation, dehydration and watery to pasty feces (Jackson and Cockcroft, 2002; Radostits *et al.*, 2007). However, the buffaloes in the present study had no apparent abdominal distension, though straining was persistent. Para rectal abscess (Sangwan *et al.*, 2008) or cyst (Sangwan *et al*., 2011) in bullock and buffalo, respectively are also been reported as the cause of frequent straining while defecation of feces. Per rectal examination/ digital palpation along with certain biochemical tests and needle centesis are sometimes required to differentiate these conditions (Jackson and Cockcroft, 2002; Radostits *et al*., 2007; Sangwan *et al.*, 2008; Sangwan *et al*., 2011). Caudal epidural anesthesia has been reported to be sufficient for perianal region (Sangwan *et al*., 2008; Sangwan *et al*., 2011). In pigs the stricture can be felt digitally at 2 to 5 cm anterior to the anus (Radostits *et al.*, 2007 and Jackson and Cockcroft, 2002), while in the present cases, it was felt almost 10 cm anterior to the anus. Incision at the dorsal and lateral aspect of the stricture or complete resection of the stricture area using pull through technique has been suggested in large animals (Kahn and Line, 2010) but no such report in buffaloes could be traceable. In the present report complete removal of the fibrous ring causing stricture was found to successful in both the buffaloes on long term follow up.

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Table 1. Clinical signs and signalment of the buffaloes with rectal stricture.

Parameter	Buffalo 1	Buffalo 2
Age (year)	6	5
Weight (Kg)	550	350
Physiological status	calved 6 weeks back	calved 8 weeks back
History of dystocia	None	None
Chronicity of illness	3 weeks	More than 4 week old
Straining while defecation	Moderate straining with less bulging of anus	Severe straining with bulging of almost 15 cm of anal area (Figure1)
Feces	Small quantity of pasty flat feces	Very small quantity of pasty feces were passed
Feed and water status	Normal	Partial anorexia

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…….liquid nitrogen vapour freezing technique (Verma *et al*., 1975)

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