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ORIGINAL ARTICLE

Estimation of Breeding Values of Sires Using Different Phase Traits in Murrah Buffaloes

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ABSTRACT
The present investigation was conducted on records of 2959 buffaloes, progeny of 219 sires over a period of 24 years(1992-2015) maintained at Buffalo research centre (BRC), LUVAS, Hisar and Animal farm ICAR-CIRB, Hisar, Four sire evaluation procedures such as ordinary least squares (OLS), regressed least squares (RLS), Derivative free restricted estimated maximum likelihood (DFREML) and best linear unbiased prediction (BLUP) were used to estimate breeding value of sires based on Lactation Length(LL), Dry Period(DP), Service period(SP) and Calving Interval(CI). The results indicated that sire number 2592 had the highest merit computed by OLS (367.62), 2910 by BLUP (349.94), 2308 by DFREML (349.57) and by RLS sire 1875 ranked number one based on LL and while in case of DP sire number 5218 had the highest merit computed by all the four methods. In addition, for SP and CI sire number 3117 had the highest merit computed by different methods.
Keywords: *Breeding values, OLS, RLS, BLUP and DFREML*

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INTRODUCTION

Buffaloes have spread over almost all parts of country with varying population density, the majority (72%) being concentrated in North and Western states.The local defined breeds are stable as well; these can survive the varieties of feed/fodder/shortage, extreme of temperature and / or prevalence of diseases. Preference for buffaloes has continued to increase due to higher fat and SNF content of milk. India is fortunate in terms of largest buffalo population, buffalo germplasm diversity (13 recognized plus17distinct population groups) and the world renowned buffalo breeds: Murrah, Nili-Ravi, Banni, Jaffrabadiand Mehsana. PresentlyIndiapossessesabout108.70 million buffaloes [1]. The aim of animal breeder is to select the genetically superior bull to bring out genetic improvement in the productive as well as reproductive performance of the herd. Therefore, suitable selection criterion which gives best discrimination among sires should be formulated to evaluate sires on the basis of performance of their daughters considering both production and reproduction performance traits. To improve the efficiency and accuracy of sire evaluation programmes many sire indices has been developed such as by using the procedures of Least-squares (LS), Regressed Least-Squares (RLS), Best Linear Unbiased Prediction (BLUP) and Derivative Free Restricted Maximum Likelihood Method (DFREML). The literature is dotted with conflicting reports [2-4] on comparative evaluation of various sire evaluation techniques in dairy animals. Therefore, an effort has been made to estimate breeding values for various performance traits by different procedures for phase traits.

MATERIAL AND METHODS

The data for present investigation was collected from history cum pedigree sheets maintained at Buffalo Research Centre (BRC), LalaLajpat Rai University of Veterinary and Animal Sciences and Central Institute for Research On Murrah Buffaloes (CIRB) Hisar over a period of 24 years from 1992 to 2015. Assuming that there is not much variation in adjacent years, entire period of twenty four years will be divided into 6 periods 1992-1995(period 1); 1996-1999(period2); 2000-2003(period 3); 2004-2007(period 4); 2008-2011(period 5); 2012-2015(period 6).Each year will be divided into four seasons; summer, rainy, autumn and winter.The performance traits considered were: lactation milk yield (LY); lactation peak yield (PY); lactation milk yield per day of lactation length (MLL = LY/LL); lactation milk yield per day of calving interval (MCI = LY/CI) and lactation milk yield per day of age at second calving (MSC = LY/AFC+CI). Breeding value of sires for different performance traits (LY, PY, MLL, MCI and MSC) were computed separately by using different sire evaluation procedures: DFREML(derivative free restricted estimated maximum likelihood), Ordinary Least Squares (OLS), Regressed Least Squares (RLS) and Best Linear Unbiased Predictor (BLUP). In order to overcome non-orthogonality of the data,least Squares and maximum likelihood computer programme of Harvey [9] using Henderson method III [10] was used to estimate the effect of various tangible factors on different performance traits under study. The following mathematical model was deduced to explain the underlying biology of the traits included in the study:

Where

$$Y_{ijkl} = \mu + s_i + h_j + c_k + b_1(X_{ijkl} - \bar{X}) + b_2(X_{ijkl} - \bar{X})^2 + e_{ijkl} \quad (1)$$

Where

Y_{ijkl}	=	l^{th}observation on the progeny of the i^{th}sire in j^{th} period and k^{th} season of calving
μ	=	overall population mean
s_i	=	random effect of i^{th} sire
h_j	=	fixed effect of j^{th} period of calving
c_k	=	fixed effect of k^{th} season of calving
b_1 & b_2	=	partial regression coefficient of age at first calving, linear and quadratic, respectively on the traits
X_{ijkl}	=	age at first calving comparing to Y_{ijkl} observations
$\bar{X}$	=	mean for age at first calving
e_{ijkl}	=	random error associated with each and every observation assumed to be normally and independently distributed with mean zero and variance σ_e^2 NID (0, σ^2_e).

RESULTS AND DISCUSSION

The breeding values of sires were computed using the Simple Daughter Average (DFREML), Ordinary Least Square (OLS), Regressed Least Square (RLS) and Best Linear Unbiased Prediction (BLUP) procedures for different phase traits.

The result for LL revealed that sire number 2592 had the highest merit computed by OLS (367.62), 2910 by BLUP (349.94), 2308 by DFREML (349.57) and by RLS sire 1875 ranked number one when breeding value calculated (table 4.20). Four sires out of top ten, shared their ranks by being in top ten irrespective of methods employed for computation of breeding value of sires. Sire number 1491 was found to be of lowest in merit by OLS (247.5), RLS (300.58), BLUP (281.4) and by DFREML (271.4). Four sires out of bottom ten, shared their ranks by being in bottom ten when breeding value was calculated by either of four methods included in the study (table 1).

The result for DP revealed that sire number 5218 had the highest merit computed by OLS (104.57), 206 by BLUP (120.06) and by DFREML (145.06) and 993 by RLS (133.99) ranked number one when breeding value calculated (table 2). Four sires out of top ten, shared their ranks by being in top ten irrespective of methods employed for computation of breeding value of sires. Sire number 2497 was found to be of lowest in merit by OLS (207.55), RLS (161.92), BLUP (195.62) and by DFREML (189.25). Six sires out of bottom ten, shared their ranks by being in bottom ten when breeding value was calculated by either of four methods included in the study (table 2).

The result for SP revealed that sire number 3117 had the highest merit computed by OLS (92.25), RLS (130.48), BLUP (119.96) and DFREML (128.32) and ranked number one when breeding value calculated (table 3). Only three sires out of top ten, shared their ranks by being in top ten irrespective of methods employed for computation of breeding value of sires. Sire number 2592 was found to be of lowest in merit by OLS (228.5), RLS (167.27), BLUP (212.11) and by DFREML(208.11). Six sires out of bottom ten, shared their ranks by being in bottom ten when breeding value was calculated by either of four methods included in the study (table 4.22).

The result for CI revealed that sire number 3117 had the highest merit computed by OLS (402.25), RLS (440.48), BLUP (429.96) and DFREML (444.58) and ranked number one when breeding value calculated (table 4). Only three sires out of top ten, shared their ranks by being in top ten irrespective of methods employed for computation of breeding value of sires. Sire number 2592 was found to be of lowest in merit by OLS (538.5), RLS (477.27), BLUP (522.11) and by DFREML (516.76). Five sires out of bottom ten, shared their ranks by being in bottom ten when breeding value was calculated by either of four methods included in the study.

The breeding value of sires estimated using four sire evaluation procedures for various economic traits indicated that most of top and bottom ranking sires share their ranks in the topmost and lowermost positions, respectively irrespective of methods of breeding value estimation used and economic traits considered. Higher degree of similarity of ranking from these methods indicated that these methods can be used for evaluation of sires. The results reported by [4-8] in Murrah buffaloes also supported the present investigation.

Ordinary least square procedure showed nearly perfect normal distribution for LL, DP, SP and CI. From the results it can be inferred that all four methods can be used as they rank different sires almost equally with little variation. Kumar and Gandhi [12] supported the results pertinent to coefficient of determination. Banik [3] on the other hand found higher value in Derivative Free Restricted Estimated Maximum Likelihood Method followed by Contemporary Comparison and Least-squares Method. Moreover, the estimates of coefficient of variation were used as criterion (the method that would least alter the coefficient of variation as compared to unadjusted data was considered more stable) to compare the constancy of sire evaluation methods for various traits. Banik [3] suggested BLUP as the most stable method. when standard deviation was considered, RLS was found to be more accurate in case of all production and phase traits except for PY and 305MY(3) for which DFREML was more appropriate. When sire ranking is taken as criterion then either of the two methods (OLS and RLS) selects exactly the same bull and consequently will result in same genetic gain. Choice among methods also to a greater extent depends upon computational difficulty and relative accuracy. RLS is more difficult computationally than BLUP because of the size of the matrix that must be inverted to get the inverse elements needed for computation of RLS estimates. Contrarily, OLS and BLUP are easy to compute since the least-squares and mixed model equations are well suited to the iterative solution and consequently inversion is not required. On a theoretical basis, the BLUP is the best and has minimum prediction error variance provided that true variance of random effects is known. In addition, the estimate obtained from use of mixed model methodology (BLUP) has smaller mean squares error than least squares estimator. Therefore, it is suggested that BLUP procedure should be used in a situation where correct ratio of residual variance to sire variance is known and, use of OLS is suggested in a situation where correct ratio of residual variance to sire variance is unknown. DFREML avoid negative component of variance as well as remove bias in maximum likelihood function.

Maalick *et. al*. [15] reported that comparison of different method of sire evaluation based on single trait for FLMY only shows that the DFREML model for single trait should be preferred over the BLUP, LSM and simple daughter average methods for evaluating the sire breeding value. However, if a sire-breeding value is to be computed from multi traits then BLUPF90 model may be preferred over DFREML model. Singh and Singh [16] also reported estimated breeding values of sires for first lactation traits by LSM, BLUP and DFREML and estimated breeding value of sires by LSM was adjudged as the most efficient and accurate. The error variance of breeding values of sires were estimated and used in computing the relative efficiency of different sire evaluation methods. The sire evaluation method, which estimated the breeding values of sires with the least error variance, was taken as the best and most efficient method. Maalick et. al. [15] reported that, the DFREML-I with univariate model using single trait i.e. FLMY was having the lowest error variance and highest relative efficiency compared to other seven methods used in the study and accordingly, it was adjudged the most efficient sire evaluation method. He further concluded that BLUP-I with univariate model using single trait as FLMY and BLUP-II with multivariate model using two traits as FLMY and FLP were found to have similar relative efficiency of 96.88% to that of DFREML-1 and were placed as second best methods followed by BLUP-III with multivariate model using three traits as FLMY, FLP and AFC with a relative efficiency of 96.02 %, which ranked third with respect to the relative efficiency among the methods used. Arora [17], Jain [18] and Jain and Sadan [19] reported the BLUP method under multitrait animal model incorporating FLMY with other trait to be more efficient and accurate for sire evaluation in different breeds of cattle and buffalo.

CONCLUSION

From the present investigation we estimate the breeding values of sire using different phase traits and four different methods. Critical analysis of breeding values of sires ranked by different methods leads to final conclusion that all four methods they almost ranked different sires equally means a sire which is ranked higher by any one method is also ranked superior by all other methods. this in addition, also help us to infer that all four methods were almost similar in breeding value estimation. We can conclude that selecting which method to use for estimation of breeding value depend also on the computational ease in a particular study and resources available.

Table 1 Estimated sire merit and their rankings for Lactation Length (LL) by different methods of sire evaluation

RANK	OLS			RLS			BLUP			DFREML		
	sire no.	No. obs.	B.V.	sire no.	No. obs.	B.V.	sire no.	No. obs.	B.V.	sire no.	No. obs.	B.V.
1	**2592**	6	367.62	1875	10	318.46	2910	10	349.94	2308	6	349.57
2	**1875**	10	362.57	2592	6	315.44	2308	6	347.27	1666	7	354.24
3	**2308**	6	360.66	2910	10	314.73	1666	7	344.24	2910	10	359.94
4	**1666**	7	351.23	2308	6	314.58	4393	8	340.57	3631	8	329.85
5	1555	6	347.66	1666	7	314.18	3631	8	339.85	4393	8	356.57
6	2910	10	343	2363	17	313.31	4807	5	339.54	2592	6	347.21
7	11	6	340.21	1555	6	312.97	1875	10	337.83	2592	6	356.21
8	1451	5	336.57	4393	8	312.31	2592	6	335.21	4807	5	349.54
9	1796	7	336.46	2184	12	312.14	1165	11	335.14	18	14	287.32
10	4807	5	336.08	1796	7	312.09	1555	6	335.05	1875	10	347.83
81	905	8	280.8	3294	5	303.96	18	14	297.32	1165	11	345.14
82	759	7	279.83	905	8	303.75	2583	11	295.32	1555	6	356.05
83	502	6	276.43	3462	5	303.7	3098	8	294.62	18	14	287.32
84	3949	7	276.33	3949	7	303.58	1341	11	293.59	592	6	286.87
85	**3117**	7	271.81	2709	13	303.51	592	6	292.87	2583	11	274.32
86	2062	5	269.83	93	16	303.08	3949	7	292.49	3098	8	286.62
87	**3294**	5	269	3117	7	302.94	3294	5	292.41	2062	5	284.38
88	3462	5	266.5	3108	23	301.89	2062	5	292.38	3117	7	268.05
89	**3098**	8	264.75	3098	8	301.21	3117	7	291.05	3294	5	277.41
90	**1491**	6	247.5	1491	6	300.58	1491	6	281.4	1491	6	271.4

Table 2 Estimated sire merit and their rankings for Dry Period (DP) by different methods of sire evaluation

RANK	OLS			RLS			BLUP			DFREML		
	sire no.	No. obs.	B.V.	sire no.	No. obs.	B.V.	sire no.	No. obs.	B.V.	sire no.	No. obs.	B.V.
1	5218	5	104.57	993	9	133.99	206	6	120.06	206	6	145.06
2	1903	6	112	5218	5	134.19	2321	12	128.07	2308	6	134.69
3	**2308**	6	113	3127	10	134.32	2308	6	129.69	2321	12	124.17
4	**1964**	6	114.69	1903	6	134.84	993	9	132.1	993	9	136.21
5	**993**	9	117.16	2308	6	135.11	3127	10	133.55	1964	6	141.57
6	3631	8	119.2	3631	8	135.32	1964	6	135.67	3949	7	138.45
7	**3127**	10	119.74	1964	6	135.55	3949	7	136.35	1964	6	136.57
8	3949	7	121.9	3567	11	136.1	5520	8	136.44	3127	10	138.55
9	3462	5	122.05	2921	9	136.24	2921	9	136.7	502	6	142.02
10	3294	5	122.2	1153	29	136.44	502	6	139.02	2921	9	145.7
81	5112	5	162	1727	6	149.77	2910	10	167.82	2184	12	157.01
82	**1084**	8	167.39	2592	6	150.29	1727	6	169.4	4245	7	178.61
83	2363	17	168.25	5516	11	150.3	2184	12	169.98	2910	10	162.82
84	1727	6	168.86	1084	8	150.83	3924	7	170.1	1319	13	138.01
85	**2184**	12	169.51	2184	12	154.01	4245	7	173.61	3924	7	160.1
86	**1319**	13	170.62	1319	13	155.01	1084	8	176.16	11	6	172.68
87	2592	6	170.87	2363	17	155.66	1319	13	176.99	1084	8	176.16
88	**3125**	7	187.44	3125	7	156.02	3125	7	184.25	2479	7	186.62
89	**11**	6	197.84	11	6	157.37	11	6	186.68	4245	7	176.61
90	**2479**	7	207.55	2479	7	161.92	2479	7	195.62	3125	7	189.25

Table 3 Estimated sire merit and their rankings for Service Period (SP) by different methods of sire evaluation

RANK	OLS			RLS			BLUP			DFREML		
	sire no.	No. obs.	B.V.	sire no.	No. obs.	B.V.	sire no.	No. obs.	B.V.	sire no.	No. obs.	B.V.
1	**3117**	7	92.25	3117	7	130.48	206	6	119.96	3117	7	128.32
2	5218	5	93.28	3949	7	130.92	3949	7	123.58	3949	7	132.38
3	**3949**	7	93.8	5218	5	134.06	3117	7	124.42	502	6	142.33
4	**3462**	5	95.55	3462	5	134.55	502	6	132.23	1796	7	144.78
5	1491	6	106.35	759	7	135.09	503	6	132.23	502	6	140.23
6	759	7	108.61	18	14	135.43	1796	7	134.88	18	14	131.10
7	502	6	108.93	3098	8	135.46	4395	5	135.49	3462	5	142.84
8	1964	6	113	1491	6	135.63	18	14	139	4395	5	142.49
9	3098	8	113.1	3631	8	135.89	3462	5	140.94	503	6	142.43
10	1796	7	113.61	3108	23	136.11	5054	5	141.41	5054	5	148.41
81	4244	5	178.47	2910	10	154.93	1084	8	184.86	2479	7	178.34
82	**1319**	13	181.37	1727	6	155.32	1875	10	187.31	3125	7	195.33
83	1727	6	184.66	3125	7	157.15	2479	7	188.34	1875	10	192.31
84	2184	12	184.81	2479	7	160.47	4807	5	189.53	1084	8	186.86
85	**3125**	7	187	1319	13	160.58	3125	7	190.33	1875	10	182.31
86	2363	17	190.2	2184	12	161.27	2910	10	192.59	1319	13	186.34
87	**2479**	7	198.77	1875	10	164.75	4244	5	193.01	2592	6	197.86
88	**1875**	10	199.21	11	6	165.88	1319	13	193.34	2910	10	194.59
89	**11**	6	226.68	2592	6	166.34	2592	6	196.86	2592	6	198.86
90	**2592**	6	228.5	2363	17	167.27	11	6	212.11	11	6	208.11

Table 4 Estimated sire merit and their rankings for Calving Interval (CI) by different methods of sire evaluation

RANK	OLS			RLS			BLUP			DFREML		
	sire no.	No. obs.	B.V.	sire no.	No. obs.	B.V.	sire no.	No. obs.	B.V.	sire no.	No. obs.	B.V.
1	**3117**	7	402.25	3117	7	440.48	206	6	429.96	3949	7	444.58
2	5218	5	403.28	3949	7	440.92	3949	7	433.58	502	6	451.23
3	**3949**	7	403.8	5218	5	444.06	3117	7	434.42	206	6	425.66
4	**3462**	5	405.55	3462	5	444.55	2321	12	440.82	1796	7	453.78
5	1491	6	416.35	759	7	445.09	502	6	442.23	3117	7	438.32
6	759	7	418.61	18	14	445.43	1796	7	444.88	2321	12	447.72
7	502	6	418.93	3098	8	445.46	4395	5	445.49	18	14	452.08
8	1964	6	423	1491	6	445.63	18	14	449	5054	5	462.51
9	3098	8	423.1	3631	8	445.89	3462	5	450.94	4395	5	451.59
10	1796	7	423.61	3108	23	446.11	5054	5	451.41	3462	5	457.84
81	4244	5	488.47	2910	10	464.93	1084	8	494.86	1875	10	487.21
82	**1319**	13	491.37	1727	6	465.32	1875	10	497.31	1319	13	513.44
83	1727	6	494.66	3125	7	467.15	2479	7	498.34	4807	5	509.43
84	2184	12	494.81	2479	7	470.47	4807	5	499.53	1084	8	487.76
85	3125	7	497	1319	13	470.58	3125	7	500.33	2910	10	512.59
86	2363	17	500.2	2184	12	471.27	2910	10	502.59	1319	13	515.34
87	**2479**	7	508.77	1875	10	474.75	4244	5	503.01	2479	7	495.34
88	**1875**	10	509.21	11	6	475.88	1319	13	503.34	3125	7	512.33
89	**11**	6	536.68	2592	6	476.34	2592	6	506.86	11	6	527.20
90	**2592**	6	538.5	2363	17	477.27	11	6	522.11	2592	6	516.76

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REFERENCES
1. BAHS. (2012). Basic Animal Husbandry statistics, Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Government of India. Krishi Bhawan,New Delhi.
2. Bajetha, G. (2006). Selection of sires by using different sires evaluation methods in crossbred cattle. Ph.D. Thesis, G.. Pant University of Agriculture & Technology, Pantnagar, US. Naghar, Uttrakhand.

3. Banik, S. and Gandhi, R.S. (2006), Animal model versus conventional methods of sire evaluation in Sahiwal cattle. Asian Aust. J. Anim. Sci. 19 : 1225-1228.
4. DAHD-GOI-(2011) Department of Animal Husbandry, Dairying and Fisheries. Ministry of Agriculture. Government of India. Krishi Bhawan, New Delhi.
5. DAHD-GOI (2012). Departmetn of Animal Husbandry. Dairying and Fisheries. Ministry of Agriculture. Governmen of India. Krishi Bhawan, New Delhi.
6. Dhaka, S.S., Chaudhary, S.R., Raheja, K.L, Yadav, A.S. and Pander,B.L. (2004). Accuracy of different methods of sire evaluation for production efficiency traits in Sahiwal Cattle. Indian J. Anim. Sci. 74 : 296-298.
7. FAO. (2008). Food and Agriculture Orgtanization. Production Year Book. 2008, Rome, Italy.
8. Gaur, G.K., Tripathi, V.N., Mukherjee, S. and Choudhary, V.K. (2001). Efficiency fo sire evaluation procedures in Frieswal cattle. Indian J. Vet. Res., 10 : 1-6.
9. Harvey, W.R. (1990). User's guide for LSMLMW, mixed model least-squares and maximum likelihood computer program, OhioState Univ., Columbus, Mimeo.
10. Henderson, C.R. (1973). Sire evaluation and genetic trends. Proc. Anim. Breed and Genetics Symposium in honour of Dr. J.L. Lush, pp. 10-14. American Soc. Anim. Sci. Assoc. Champaign, Illinois.
11. Henderson, C.R. (1978). Undesirable Properties of regressed least square prediction of breeding values. J. Dairy. Sco. 61: 114-120.
12. Kamaldeep, Yadav, A.S., Dhaka, S.S., Magotra, Ankit, Malik, Anika (2015). Efficiency of sire evaluation methods by using phase and stayability traits to improve milk yield of Murrah buffaloes. Indian J. Anim. Res.Print ISSN: 0367-6722/ online ISSN: 0976-0555. DOI: 10.18805/ijar.5707.
13. Kumar, A. and Gandhi, R.S. (2010). Comparison of sire evaluation methods for first lactation milk yield and lifetime traits in Sahiwal cattle. Indian J. Anim. Sci., 80 : 1198-1202.
14. 19thlive stockcensus(2012) Available from:http://www.dahd.nic.in /dahd/writereadData/livestock pdf.
15. Mallick, P.K., Ghosh, A.K. and Rajendiran, A.S. (2018). Sire evaluation using animal model versus different conventional methods in Red Sindhi cattle. *Indian J. Anim. Res.*, **52** (1): 1-6.
16. Singh, P .K and Singh, B. P. 1999. Efficacy of different methods in genetic evaluation of Murrah sires. *Indian J. Anim. Sci.,* **69:** 1044-1047.
17. Arora, K.C. (1981). Relative efficiency of different methods of indexing the breeding work of dairy bulls. *Thesis Abstr.* Haryana Agricultural University **9:** 36-37.
18. Jain, A. (1996). Sire evaluation under multiple trait models in Murrah buffaloes. Ph.D. Thesis, National Dairy Research Institute, Deemed University, Karnal, India.
19. Jain, A. and Sadana, D.K. (2000). Sire evaluation using animal model and conventional methods in Murrah buffaloes. *Asian Australasian J. Anim. Sci.* **13:** 1196-200.

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