A Cohort Study of Reproductive Performance, Associated Infections and Management Factors in Zebu Cows from Smallholder Farms in Malawi

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Abstract Smallholder Zebu cattle farming is of prime importance for the livelihood of Malawians, and optimising cattle reproductive efficiency represents one way to increase food production. The objective of this study was to determine the reproductive performance and associated factors in a cohort of Malawi Zebu cows (n = 100) across 36 smallholder farms for a 2-year period. The birth of a live calf was observed in 78 dams and stillbirths/late abortions in 5 cows, 14 cows were censored, and 3 cows did not calve throughout the study period.

Median time from calving to resumption of luteal activity was 61 days. Mean calving interval was 457 days with 32% of the variation residing at herd level. A significant association was found between time from calving to conception (open days) and the presence of a breeding bull in a herd (156 versus 235 days) and between multiparous and primiparous cows (170 versus 220 days). Cows were screened three times, at 6-month interval, for antibodies against selected infectious agents. None were positive for *Brucella* spp., six were positive for bovine viral diarrhoea virus and two for *N. caninum* antibodies. No blood smears were positive for *Babesia* spp. or *Theileria* spp., but three were positive for *Trypanosoma* spp. Conclusively, this study indicated a low prevalence of the studied infections and a

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B. R. Singh et al. (eds.), *Climate Impacts on Agricultural and Natural Resource Sustainability in Africa*, https://doi.org/10.1007/978-3-030-37537-9_13

large potential for increased reproductive efficiency in Zebu cattle of this area. Particularly, the keeping of a herd-own bull, adequate breeding management and care of first parity cows seem to be key factors to increase reproductive efficiency and thereby food production from Malawi Zebu.

1 Introduction

The African population of 1.2 billion people is expected to double by 2050 (Groth and May 2017) which will require significant improvements in food production. In addition, climate changes represent new challenges in crop and livestock production. The Food and Agricultural Organization (FAO) reports that 14.5% of greenhouse gas emissions are caused by livestock, and intensification of the production is the most efficient way of reducing carbon footprint from this sector (Gerber et al. 2013). In sub-Saharan African countries, severe dry weather has affected the large grazing areas leading to less grazing, and livestock struggle to maintain body condition resulting in compromised reproductive efficiency (Kanuya et al. 2006b; Matiko et al. 2008). During the dry season, rivers remain the only source of available water for the majority of cattle herds, and cattle need to walk longer distances to get water, which could also potentially influence their reproductive performance.

Rural Malawians' (80% of total population) existence is largely dependent on smallholder agriculture, and Malawian Zebu cattle farming contributes significantly to food production (Dixon et al. 2004). Optimising cattle reproductive efficiency represents one way to increase milk and meat production (Kiwuwa et al. 1983). Previous studies from other countries on indigenous Zebu cattle reproduction have emphasised the importance of having a breeding bull at the farm, which minimises the incidence of transmissible venereal diseases among herds (Fernandez et al. 1993; Rekwot et al. 2004; Young et al. 2014).

Improvements in Malawi Zebu breeding programs have been called for, and management of bulls has been identified as a major limiting factor (Nandolo et al. 2015; Rekwot et al. 2000). In a study of Tanzanian Zebu cows, Matiko et al. (2008) reported a significant effect of parity (> second parity) on post-partum resumption of ovarian cyclicity. It may, therefore, be speculated that first parity Zebu cows, maintained in a pastoral system with no breeding management, are mated too early for optimal reproductive performance.

Infectious disease agents like bovine viral diarrhoea virus (BVDV), *Brucella* spp. and *Neospora caninum* have been identified to exert direct effects on the reproductive organs, whereas protozoan parasites such as *Theileria parva*, *Babesia bovis*, *Babesia bigemina* and *Trypanosoma* spp. have an indirect effect on reproductive performance through compromised general health (Mukasa-Mugerwa 1989; Sekoni 1994). High prevalence of BVDV and *Brucella* spp. in Tanzania (Mathew et al. 2017) and *N. caninum* in Ethiopia (Asmare 2014) has been reported to exert a negative impact on Zebu cattle reproductive performance in these regions. The occurrence of these infections and their association with reproductive disorders is unknown in Malawi Zebu cattle.

The objective of this study was to investigate reproductive performance and its association with selected factors in Malawi Zebu to gain knowledge on potential intensification. Specific objectives were (i) to determine key reproductive performance indicators (time from calving to onset of regular luteal function, calving interval and calf mortality), (ii) to determine the relative impact of herd and cow level factors on calving interval by variance components analyses, (iii) to study associations between likelihood of pregnancy and availability of breeding bulls and parity of the cows and (iv) to determine the prevalence of selected infectious agents known to have an impact on reproductive performance.

2 Materials and Methods

A cohort study of Malawi Zebu cows was conducted in northern Malawi. The cohort was followed by veterinary examinations, four times during a 2-year period from September 2015 to October 2017.

Malawi is divided into 187 Extension Planning Areas (EPA), which are further subdivided into agricultural sections comprising 5–15 villages (Kundhlande et al. 2014). This study was conducted across seven agricultural sections (Bolero, Bata, Chozoli, Chikwawa, Chirambo, Mjuma and Jalira) of Bolero EPA, Rumphi District. Bolero EPA was selected because smallholder livestock farming is the predominant agrarian practice in this area (MZADD 2009).

Three farmer meetings were conducted in Bolero EPA before the start of the study. The selection of cattle farms was based on the presence of newly calved cows in the herd (cows that calved after August 15, 2015, and four subsequent weeks onwards). Cows from these herds were selected based on the calving information provided by the farmers in these three meetings.

A single-visit-multiple-subject survey technique (ILCA 1990) was used to obtain smallholder cattle farm data including herd size, availability of breeding bulls as well as parity, pregnancy and reproductive status of cows. Initially, 109 clinically healthy Malawi Zebu cows from 39 smallholder farms were selected and ear tagged. Later on, three farmers with nine cows withdrew before April 2016, leaving a cohort of 100 cows belonging to 36 farms, which were followed throughout the study. In this study, female calves older than 8 months were defined as heifers until first calving took place.

In addition to the permanent presence of a field assistant during the study period, four experienced large animal veterinarians (Faculty of Veterinary Medicine, Department of Production Animal Clinical Sciences, Norwegian University of Life Sciences) conducted cattle rectal examinations for reproductive status and pregnancy diagnosis at four time points at approximately 6-month intervals: April 2016, October 2016, April 2017 and October 2017. Pregnancy diagnosis was conducted based on the following criteria:

- Membrane slip + asymmetry (>35 days pregnant).
- Balloting of the foetus (>70 days).
- Fremitus in one of the uterine arteries (>105 days).
- Fremitus in both uterine arteries (>180 days).
- Large parts of the foetus apparent close to the pelvic inlet (>240 days).

An early abortion was suspected if cows were not late pregnant or had not calved within 8 months after diagnosis of an early pregnancy (>35 days). The terms *late abortion* and *stillbirth* were used for the foetus of the last trimester or full born calves dead at birth. If a cow with an assumed early abortion later became pregnant, the successful calving of a live calf was included in the assessment of the calving interval. The herd record keeping was regularly maintained by the farmers and monitored by the field assistant. Calving dates were obtained from these herd records, which enabled the calculation of calving intervals. Days from calving to pregnancy were approximated by subtracting 280 days from the next calving date. Observations of calf mortality in live born calves were restricted to the period from the date the calf was born until the end of December 2015.

The animals were treated for ticks in April 2016 and 2017 (Taktic®, Utrecht, The Netherlands) and for helminths in October 2016 and October 2017 (Kepromec®, Deventer, The Netherlands). Treatments were conducted in accordance with the manufacturer's recommendations.

2.1 Milk Sampling

Altogether, 96 cows (four cows omitted due to sampling irregularity) were subjected to milk progesterone analyses. A semi-quantitative assessment of progesterone in milk was performed using a cow-side P4 Rapid test (Ridgeway Science, St Briavels, UK). Progesterone analyses were used to determine the time from calving to onset of luteal activity (OLA) defined as three consecutive positive reactions to elevated milk progesterone values within 21 days. Milk was collected every third to seventh day from October to the end of December 2015.

2.2 Blood Sampling

Blood was sampled on three occasions: October 2015, April 2016 and October 2016 from 108, 99, and 81 cows, respectively. Blood was collected from either the coccygeal vein or the jugular vein into sterile vacutainers and immediately cooled in the field cooler until centrifugation at 1200 × g at room temperature. Sera were pipetted into sterile tubes and kept frozen at approximately −20 °C. The material was shipped on ice to the National Veterinary Institute in Oslo, Norway, and submitted to the Biosafety level 3 laboratories and kept frozen at −20 °C until analyses. EDTA mixed blood was sampled at two occasions (April 2016 and April 2017) and blood smears were prepared on site.

2.3 Laboratory Analyses

All sera were analysed for antibodies against BVDV, *Brucella* spp. and *N. caninum* using enzyme-linked immunosorbent assay (ELISA) from Boehringer Ingelheim Svanova in Uppsala, Sweden. The optical density (OD) was measured in a LabSystems Multiskan EX spectrophotometer (LabX, Ontario, Canada). All OD values were correlated to positive control sera referred to as corrected OD values, and percent positivity (PP) of each sample was calculated following the manufacturer's instructions. Anti-BVDV antibodies were detected using an indirect ELISA commercial kit (SVANOVIR® BVDV-Ab I-ELISA) following the manufacturer's instructions. Serum samples with PP ≥10% were considered positive and those with PP <10% were negative. The presence of antibodies to *Brucella* spp. was analysed using the SVANOVIR® *Brucella*-Ab-I-ELISA antibody test following the manufacturer's instructions. Serum samples with PP ≥40% were considered positive, and samples with PP <40% were considered negative. *N. caninum* specific antibodies were detected using an indirect SVANOVIR® *N. caninum* ISCOM ELISA following manufacturer instructions. Sera were considered positive when PP ≥20%, while samples with PP <20% were considered negative.

The air-dried blood smears were fixed in methanol, stained with Giemsa stain and examined for evaluation of *Theileria* spp., *Babesia* spp. and *Trypanosoma* spp. in microscopy at 100 × magnification by a trained technician from Central Veterinary Laboratory, Lilongwe, Malawi.

3 Statistical Analyses

The statistical software package STATA 14 (StataCorp LLC, Texas, USA) was used in all statistical analyses. Statistical significance was considered with a *P*-value less than 0.05 in all models. Variance components mixed model analyses using the 'xtmixed' procedure were performed allowing REML (restricted maximum likelihood) estimation to discriminate between variance of calving interval at cow, farm and section level. Agricultural sections and farm were included as random effect variables both separately and jointly in three models.

Univariable Kaplan–Meier (K-M) estimators were used to assess relationships between time from calving to pregnancy for the explanatory variables; presence of a breeding bull in the herd (1/0) and first parity versus multiparous cows (1/0). The time variable describing days from calving to pregnancy was calculated for cows that delivered a live calf within the period June 5, 2016, to October 6, 2017. All other cows were censored at the end of the study, at culling or death or when a late abortion/stillbirth had occurred. Statistical significance was assessed both by log likelihood and Wald chi-square statistics. Both approaches were used, as the Wald chi-square test is more sensitive to early observation than the default log-likelihood statistic (Dohoo et al. 2009). Explanatory variables with *P*-values of 0.10 and less for the association with likelihood of conception from the K-M estimators were included in a multivariable Cox proportional hazards model (Cox regression), with shared frailty at farm level.

Parity was included in the Cox model both as an ordinal variable (parity: 1, 2 and >2) and as first parity versus multiparous cows (1/0). Number of bulls in the herd was evaluated both as an ordinal variable (number of bulls: 1, 2 and >2) and to whether a breeding bull was present in the herd or not (1/0). Interaction terms between the bull and parity variables were tested and subsequently omitted for non-significance by backward elimination. As the likelihood ratio test for frailty was not significant, it was also removed from the final models. The proportional hazard assumption was evaluated by plotting the negative logarithm of the hazard function against the logarithm of survival time ($-\ln(H)$ versus $\ln(t)$) separately for the parity and the bull effects. The assumption of independent censoring was explored in sensitivity analyses of complete negative and positive correlations between censoring events and the event of interest. The overall fit of the model was assessed by Cox-Snell residuals and Moreau goodness of fit tests; outliers were evaluated by deviance residuals, whereas the lack of influential points was identified by scaled score residuals (Dohoo et al. 2009).

4 Results

The background information of herds from where the cohort of 100 cows were recruited is given in Table 1. The information regarding parity and milking status of cows was collected during the initial survey meetings.

Table 1 Background information of herds (with respect to agricultural sections) that included the 100 Malawi Zebu cows of the cohort study

	Adult cows					
	Milking	Dry	Heifers	Calves	Bulls	Total
Agricultural sections	(*n*)	(*n*)	(*n*)	(*n*)	(*n*)	(*n*)
Bata	52	28	38	68	10	196
Bolero	7	2	6	11	1	27
Chikwawa	25	13	16	27	6	87
Chirmabo	24	9	20	23	7	83
Chozoli	21	12	16	23	11	83
Jalira	4	3	4	4	1	16
Mjuma	5	2	3	6	1	17
Total	138	69	103	162	37	509

After three farmers had withdrawn, the number of bulls per farm ranged from zero to four, and 30 (out of 100) cows originated from 17 farms with no breeding bull in the herd. The primiparous cows (n = 19) of the cohort study originated from 13 farms.

The mean calving interval in 78 cows that subsequently delivered a live calf was 457.4 days with a standard deviation (SD) of 163.0 days, which corresponds to a mean period from calving to pregnancy of 177.4 days. The variance component analysis of the calving intervals from these 78 cows showed that 32% of the variance in calving interval resided at the herd level, with significant variation in calving interval between herds (P = 0.008), indicating an important contribution of herd management to reproductive outcome. The variance in calving interval between agricultural sections was not significant, indicating minimal geographical differences in reproductive efficiency within the study area.

Results of the reproductive outcomes of the study are reported in Table 2. Late abortion/stillbirth was reported for five cows. No cases of early abortions were reported by the farmers, but long intervals from a positive diagnosis of pregnancy until subsequent calving indicated that five cases of early abortions had occurred. Nine calves died before they reached the age of 3 months, and one calf died at 4 months of age, making the total loss of calves of about 13% (10 out of 78) during the dry period of 2015. This figure increased to 18% (15 out of 83) when late abortions and stillbirths were included.

Milk progesterone tests showed that OLA had already started in 43 cows at the beginning of the milk-sampling period in October 2015. The median value of OLA after calving was 61 days. OLA did not start in 25 out of 100 cows before milk sampling ended in the last week of December.

In herds with a breeding bull, the mean number of days to pregnancy was 156 (SD = 155), and for cows without a bull in the herd, the mean number of days to pregnancy increased to 235 (SD = 175). Figure 1 displays the Kaplan–Meier plot of pregnant cows by days after calving between cows from a herd with a breeding bull and cows in herds that relied on mating during periods of common grazing. The

Table 2 Reproductive status of 100 Malawi Zebu cows from 36 farms obtained at 6-month intervals from calving in August and September 2015 until October 2017. Cows at risk were followed until calving, abortion, death or until the cow had left the farm

Status	Oct. 2015	Apr. 2016	Oct. 2016	Apr. 2017	Oct. 2017	Oct. 2015–2017
Not pregnant	100	35 (1[a])	20 (4[a])	3	2	2
Pregnant	–	56	18	23	1	1
Late abortion/stillbirth	–	–	5	–	–	5
Calved	–	–	47	8	23	78
Dead	–	5	–	2	–	7
Sold/slaughtered	–	4	1	2	–	7
Cows at risk of calving	100	91	38	26	3	–

[a]The number of non-pregnant cows that possibly had experienced early abortions

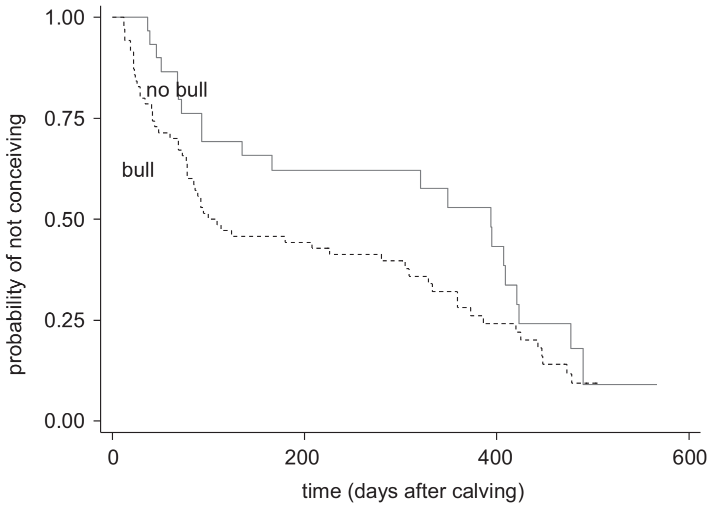

Fig. 1 Kaplan–Meier plot of the proportion of pregnant cows distributed by days after calving between cows from a herd with a breeding bull and cows in herds without a bull

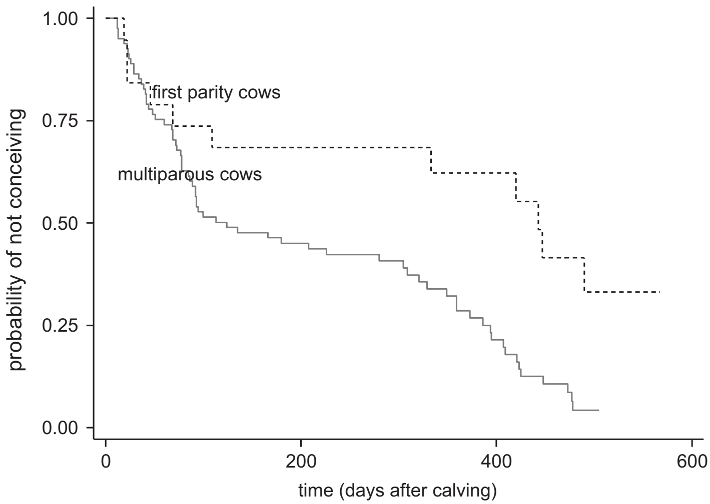

Fig. 2 Kaplan–Meier plot of proportion of pregnant cows distributed by days after calving between first parity and multiparous cows

difference between the two groups was not significant when assessed by the log-rank test for equality of survivor functions ($P = 0.097$) but significant according to the Wald chi-square statistic ($P = 0.032$).

The mean number of days from calving to pregnancy was 220 (SD = 202) for first parity cows and 170 (SD = 156) for multiparous cows. Figure 2 displays the Kaplan–Meier plot of first parity versus multiparous cows by days after calving.

Table 3 Multivariable Cox proportional hazards model of days from calving to pregnancy by the keeping of a bull in the herd (1/0) and first versus older parities of the cows (1/0)

Explanatory variables	Coeff. (*b*)	S.E	*P*	95% Confidence interval
Bull	0.549	0.259	0.034	0.042–1.055
First parity	−1.052	0.338	0.002	−1.715 to −0.390

The difference between first calving and older cows was significant when assessed by the log-rank test for equality of survivor functions ($P = 0.003$) but borderline non-significant according to the Wald chi-square statistic ($P = 0.051$).

The presence of a breeding bull in the herd and the effect of parity were both significantly related to pregnancy success when assessed in a multivariable Cox proportional hazards model of days from calving to pregnancy (Table 3). The hazard rate (HR) of pregnancy in primiparous cows compared to multiparous cows was 0.35 ($e^{-1.0524}$), indicating a significantly reduced likelihood of pregnancy in primiparous cows. The HR for pregnancy in cows from a herd with a breeding bull was 1.73 times higher ($e^{.0547}$) compared to cows in a herd without a bull.

An interaction term between the bull and parity effects was initially included but omitted for non-significance. The ordinal variables of number of bulls and parity were not significantly related to pregnancy rates in the multivariable models. The likelihood ratio of the multivariable Cox proportional hazards model was $\chi^2 = 14.8$ with 2 df ($P = 0.001$).

4.1 *Antibody Results*

Out of 288 serum samples collected during three occasions, no serum was positive in the *Brucella* spp. ELISA in either sampling. Only two cows, one sampled in October 2015 and another cow sampled in April 2016, were seropositive in the *N. caninum* ELISA. Four cows were found positive for BVDV in October 2016, whereas only one BVDV-positive cow was found in each of the samples obtained in October 2015 and April 2016 (Table 4). Of the blood smears, three cows were positive for *Trypanosoma* spp., but all were negative for *Babesia* spp. and *Theileria* spp. at all sampling events.

5 Discussion

This study shows that improved reproductive management and better care of first parity cows are key factors to increase meat and milk production from smallholder Zebu cattle farms of northern Malawi. In accordance with our findings, a recent study from Malawi (Nandolo et al. 2015) also reported that 64.6% farms were without a breeding bull in the herd. Furthermore, that farmers' decision on which bull to

Table 4 Number of seropositive animals for bovine viral diarrhoea virus (BVDV), *Brucella* spp. and *N. caninum* in Malawi Zebu herds

Infectious agents	Positive cows		
	October 2015 ($n = 105$)	April 2016 ($n = 95$)	October 2016 ($n = 81$)
BVDV	1	1	4
Brucella spp.	0	0	0
N. caninum	1	1	0

keep will affect the breeding in that region because cattle are mixed freely during the grazing period. In addition, a relatively high inbreeding rate was also estimated, and in conclusion, the authors suggested to establish community breeding programs (Nandolo et al. 2015). In accordance with the present study, parity has been reported to affect reproductive performance in Tanzanian Zebu cows (Matiko et al. 2008). This study also reported that the number of days open was significantly less in older cows as compared to the younger cows. Herd composition (keeping older cows) is a management strategy that may help in getting more calves and higher milk yield on a yearly basis. Also, avoiding premature mating of heifers may be a valuable strategy for increasing reproductive efficiency in first parity Zebu heifers (Madani et al. 2008).

The observed median days from calving to the onset of luteal function and mean calving interval were shorter than corresponding figures for Tanzanian Zebu cows (Kanuya et al. 2006a), indicating better reproductive performance in this cohort of Malawi Zebu cows.

N. caninum is one of the major causes of reproductive problems and abortions in dairy and beef cattle worldwide (Innes 2007). The two seropositive cows in our study indicate a very low seroprevalence in the study population and no impact on reproductive performance in our included herds. The finding of a *Brucella* spp.–free population in this study is important since human's brucellosis originates from animals. Previous studies from the neighbouring country, Tanzania, have reported differences in seroprevalence of BVDV and *Brucella* spp. in geographically very close areas (Mathew et al. 2015; Mathew et al. 2017; Swai et al. 2005). Further research is necessary to detect if the low occurrence is general or if the study area represents a free area surrounded by populations with higher prevalence. Such knowledge is necessary in order to choose the most cost-effective preventive measures to avoid introduction of these high-impact infections.

In addition, the occurrence of *Trypanosoma* spp., *Babesia* spp. and *Theileria* spp. has showed surprisingly lower prevalence than what has been found in other areas of neighbouring Tanzania. However, the results presented in the current study must be interpreted with care due to a relatively low sensitivity of the methods (Delgado et al. 1998).

The infections studied were chosen because they are widespread and generally cause substantial losses. Since the study population were free, or had very low prevalence, of all of them, it may be viewed as a population with low prevalence of infections. In such case, the reproductive outcome of cows from the best managed farms in this study may demonstrate a bench mark for reproductive efficiency in a low prevalence situation. However, a wide range of other infections could be present. Pathogens such as *Campylobacter fetus*, bovine herpesvirus-1 and *Leptospira* spp. that cause reproductive disorders could be further investigated. However, neither early nor late abortion rates were high in the current investigation. It is therefore plausible to assume that differences in herd management are of major importance for the observed variance in reproductive efficiency between the cows that were followed throughout the 2-year observation period. This is also demonstrated by a significant contribution of the herd effect in the variance component analysis of calving intervals. Furthermore, no variability in calving interval could be explained by agricultural section. This may indicate only minor geographical differences within the study area, with negligible impact on reproductive efficiency.

6 Conclusion

In conclusion, a large potential for increased reproductive efficiency by improving herd management was identified in the current investigation. Improved availability of bulls, better care of primiparous animals and higher calf survival in Malawi Zebu should be prioritised in order to mitigate the effects of climate change and improve farmers' economic situation and food security in the region.

Acknowledgement The authors would like to thank the smallholder farmers of Bolero EPA, Rumphi District, Malawi, who actively participated in this study. We are grateful to Dr. Liveness Jessica Banda and Dr. Fanny Chigwa, Animal Science Department, Lilongwe University of Agriculture and Natural Resources, for their help in laboratory analyses. This study was supported by the project: Capacity Building for Managing Climate Change in Malawi (CABMACC), Norwegian University of Life Sciences, Norway. The corresponding author is very much thankful to Prof. Peter Wynn (Graham Centre for Agricultural Innovation, Charles Sturt University, NSW 2650, Australia) for his thorough guidance and help in all the research activities.

Conflict of Interest The authors declare that they have no conflict of interest.

Funding This study was funded by Capacity Building for Managing Climate Change in Malawi (CABMACC).

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