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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Table of Contents: Volume 8 Number 4 April, 2014

ARTICLES

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Full Length Research Paper

Effect of macroclimatic factors on milk production and reproductive efficiency of Holstein Friesian × Deoni crossbred cows

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This study was undertaken to evaluate the effects of different macro climatic variables on milk production and reproduction efficiency of Holstein Friesian × Deoni crossbred cows. Data of 256 HF × Deoni crossbred cows with 1485 total records of lactation and the meteorological data over a 30-year period (1981 to 2010) were obtained from Marathwada Agricultural University Cattle Cross Breeding Project and the University Meteorological Observatory Weather Station, respectively. The parameters used as indicators of milk production and reproduction performance in this study were lactation milk yield, lactation length, dry period and inter calving period. They were plotted against the monthly climatic variable for regression analysis. It was observed that maximum temperature, maximum humidity, bright sunshine hours and maximum temperature humidity index exhibited negative and significant regression result with lactation milk yield and lactation length. All the considered climatic variables accounted for 28 and 21% direct variation on lactation milk yield and lactation length as verified by the value of coefficients of determination (R^2). In contrast, maximum temperature, maximum humidity, wind speed and maximum temperature humidity index showed positive and significant regression on dry period and inter calving period. All the considered climatic variables accounted for 25 and 23% direct variation on dry period and inter calving period, respectively. The summary of the meteorological data confirmed that there were high values of temperature humidity index for considerable months yearly, which suggested that most crossbred cows were exposed to the negative effects of heat stress. Hence, other productive and reproductive strategies like improving environmental, productive and reproductive management of cows are needed to reduce the adverse effect of heat stress.

Key words: Productive and reproductive traits, milk loss, climatic variables, heat stress.

INTRODUCTION

Climate change has important effects on the role of livestock, both directly and indirectly. The direct effects involve heat exchanges between the animal and its environment that are linked to air temperature, humidity, wind-velocity and thermal radiation. These linkages have bearing on the physiology of the animal and influence

animal performance (e.g., growth, milk production and reproduction) and health. Alterations in the factors arising from climate change like quantity and quality of feed and fodder resources such as pastures, forages, grain and crop residues and the severity and distribution of live-stock diseases and parasites have indirect but significant bearing on the animal productivity (Sere et al., 2008).

Heat stress in dairy cows leads to decline in milk production and fertility. Milk production and reproduction losses during the summer substantially influence the economic potential of dairy cattle.

Heat stress has also been reported to affect the growth rates, feed intake and feed conversion efficiency in dairy cows (Wang et al., 1993; Sindhe et al., 1990). The reproductive function (like conception rates and calving intervals) is also severely affected by heat stress (Drost et al., 1999).

Hyperthermal stress increases body temperature and compromises the uterine environment, thus reducing the likelihood of embryo implantation. It also leads to a high rate of embryonic mortality: this is one of the main causes of poor reproductive performance during summer (Rivera and Hansen, 2001).

Heat stress in dairy cows is caused by a combination of environmental factors (temperature, relative humidity, solar radiation and air movement). Among all environ-mental stressors, the temperature and the relative humi-dity are the major factors, which affect the productive and reproductive performance of dairy cows. The effect of heat stress is caused by high ambient temperature and high relative humidity (Kadzere et al., 2002).

Heat stress poses formidable challenge to the development of livestock sector in India. The anticipated rise in temperature over the entire country resulting from climate change is likely to make worse the heat stress in dairy animals, adversely affecting their productive and reproductive performance, and hence reducing the total area where high yielding dairy cattle are to be econo-mically reared. In addition, when high temperature is associated with decline in rainfall or in increased evapotranspiration, it aggravates the feed and fodder shortage (Sirohi and Michaelowa, 2004).

In most parts of the country, the hot season is relatively long and there is intense radiant energy for an extended period, generally with presence of high relative humidity. The mean summer (April to June) temperature of India ranges from 25 to 45°C in most parts of the country. Crossbred cows, which are high yielders and more economic to farmers, are more susceptible to heat stress as compared to local cows and buffaloes. The proactive management counter measures during heat stress (e.g. providing sprinklers or changing the housing pattern etc.) or animal nutrition strategies to reduce excessive heat loads are often expensive and beyond the means of small and marginal farmers who own most of the livestock (Upadhyay et al., 2009)

A case study reported by Upadhyay et al. (2009) indicated that increased heat stress associated with global climate change, causes distress to dairy animals and possibly affect milk production. They have also been estimated that India loses 1.8 million tonnes of milk production annually, amounting to over 650 million USD due to heat stress in different parts of the country. It is estimated that global warming will further negatively influence milk production more than 15 million tonnes by 2050.

Marathwada Agricultural University has taken a project for improvement of Deoni cattle breed (Figure 1) by cross breeding local Deoni cows with Holstein Friesian. The success of dairy production in general and crossbreeding programmes in particular needs to be monitored regularly by assessing the productive and reproductive perfor-mance under the existing management system.Thus, characterizing the environmental conditions to which these Holstein Friesian × Deoni Crossbred cows (Figure 2) exposed might help to properly anticipate and adapt dairy farming in specific area. The present study was planned and aimed to assess the effect of heat stress on performance of crossbred cows.

MATERIALS AND METHODS

Study area

This study was conducted at Marathwada Agricultural University (MAU), Cattle Cross Breeding Project (CCBP), India. The university is located at an altitude of 407 m a.s.l. It is situated between 17° 35' N and 20° 40' N latitude and between 70° 40' E and 78° 15' E longitude. The climate of the region is semi arid while; on seasonal basis, it oscillates from humid to sub humid in monsoon, sub humid to semiarid during post-monsoon and hot and dry in summer. Thermohydrologically, monsoon season is warm humid, post-monsoon is cold and sub humid, summer is hot and dry along with dry cold winter.

The mean daily maximum temperature varies from 29.1°C in December to 42.5°C in May. The mean daily minimum temperature varies from 6.9°C in December to 25.4°C in May. The relative humidity ranges from 11 to 90%. Normally, the summer is hot and general dryness persists throughout the year except during south-west monsoon. The region is essentially a subtropical one and it comes under assured rainfall zone with an average rainfall of 900 mm spread in about 70 rainy days mostly received from June to September.

Management of animals

The management of animals at CCBP becomes identical with variation due to reasons beyond control. The daily routine

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management activity for lactating animals starts at 8 a.m. After calving, the calves remain with their dams for about 5-7 h. The calves were then weighed, tagged and bucket milk fed twice a day until weaning. All the calves were separated from their dam at birth and weaned at about 3 months of age. The milk recording started after 4th day from calving. The dams remained in barn for the first five days during which they were provided with green fodder, concentrate meal, and transferred to the milking herd afterwards. Cows were hand-milked twice a day, early in the morning (6:00-7:00 am) and late in the afternoon (5:00-6:00 pm) after feeding concentrate mixture regularly. The cows were allowed for grazing infallow land from 9.00 a.m. to 5.00 p.m. on a regular basis. However, in summer season (March-June) the cows were allowed to graze from 9.00 a.m to 12.00 a.m. after that the animals were tied and stall-fed with required quantities of dry and green fodder under the shed.

All animals were routinely checked for any incident of health problem and treatments were given if any abnormality exists. Additionally, animals were regularly vaccinated against major diseases such as FMD, Black Leg and Haemorrhagic Septicaemia. The milking cows were washed and groomed regularly and fed individually. The project used teaser bull for regular heat detection. Upon heat detection, cows were mated naturally to a bull. From conception up to seven months of pregnancy, cows were grazed on natural pasture after which they are were kept indoor and offered roughage and concentrate feed.

For this study, data of 256 Holstein Friesian (HF) × Deoni crossbred cows (1981 to 2010) with 1485 total records of lactation and cows having at least three offspring were selected for analysis. Meteorological data (1981 to 2010) were obtained from the university meteorological observatory weather station. The complete year was divided into 4 seasons. The four seasons are winter (December-February), summer (March-May), monsoon (June-September) and post monsoon (October-November).

Determination of temperature humidity index (THI)

Heat stress is commonly assessed by the THI, because the primary environmental factors that produce heat stress are temperature and humidity. THI is a useful and easy way to assess the risk of heat stress. It is suggested as an indicator of the thermal climatic conditions. This index is widely used in hot areas worldwide to assess the impact of heat stress on dairy cows. An environment is generally considered stressful for cattle when the THI exceeds 72 and when THI is at or above this level, adverse heat stress effects are expected (Johnson, 1987). THI is calculated according to National Research Council (NRC) (1971) recommended equation:

$$THI = 0.72 (Tdb + Twb) + 40.6$$

Tdb = dry bulb temperature (°C); Twb = wet bulb temperature (°C).

Determined THI values were used to identify heat stress seasons and to examine the monthly variation of THI. The classification (None, mild, moderate, severe and danger stress level) reported by Du Preez et al. (1991) was adopted to quantify the intensity of heat stress.

To investigate the effect of macro climatic variables on milk yield and reproductive traits the data were analyzed by using multiple regression model. The main climatic variables were also compiled as monthly minimum and maximum temperature, monthly minimum and maximum relative humidity, monthly wind speed (km/h), monthly sunshine (h) as well as monthly minimum and maximum THI.

Data were analyzed by using the statistical analysis system (SAS, 2002) software programme. The following regression model was utilized to study the effect of different independent variables (climatic factors) on lactation milk yield, lactation length, dry period and inter calving period:

$$Y = a + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_6x_6 + b_7x_7 + uij$$

Where, Y is dependent variable; X is independent variable; a is constant; b is coefficients of X and uij is error term

This multiple regression equation describes an average relationship between dependent and independent variables, which is used to predict the dependent variables. The variability of the model was tested with the help of coefficient of multiple determinations (R^2). The significant of R^2 was tested with 'F' test and the significance of individual partial regression coefficient was tested with student 't' test.

To determine the role of various climatic factors in the variation of milk production and reproductive traits, stepwise regressions were undertaken based on the contribution of different climatic variables. Basically, regression helps to estimate the functional relationship between the independent and dependent variables.

RESULTS AND DISCUSSION

Effect of climatic variables on lactation milk yield and lactation length in winter season

Average LMY and LL was recorded as 2062.72±62.56 kg and 307.84±5.78 days for cows calved in winter season. It is evident from Table 1 that maximum and minimum temperature, maximum and minimum humidity, bright sunshine hours, wind speed and maximum and minimum temperature humidity index showed positive and non-significant regression with LMY and LL in this season. All the considered climatic variables accounted for 18 and 12% variation in LMY and LL.

The value of coefficients of determination (R^2) also revealed non-significant level. This illustrates that winter season is the most favourable season for milk production of these crossbred cows since their maximum milk production were recorded in this season. Similar finding was reported by Barash et al. (1996). Thus, the climatic condition of this season favours the milk production of crossbred cows due to its favourable climate situation and availability of quality fodder. Therefore, it can be inferred that there was no severe heat stress in winter season in the study area.

Effect of climatic variables on dry period and inter calving period in winter season

In winter season, the average DP and ICP was observed as 106.91±3.84 and 404.72±5.94 days, respectively. All the considered climatic variables exhibited positive and non-significant regression with DP and ICP (Table 2). All the considered climatic variables accounted for about 17 and 21% variation in DP and ICP in the season. The R^2 value showed non-significant level statistically, which means the effect of winter season was non-substantial on length of DP and ICP. There was a similar trend between DP and ICP in view of the fact that DP is a component of

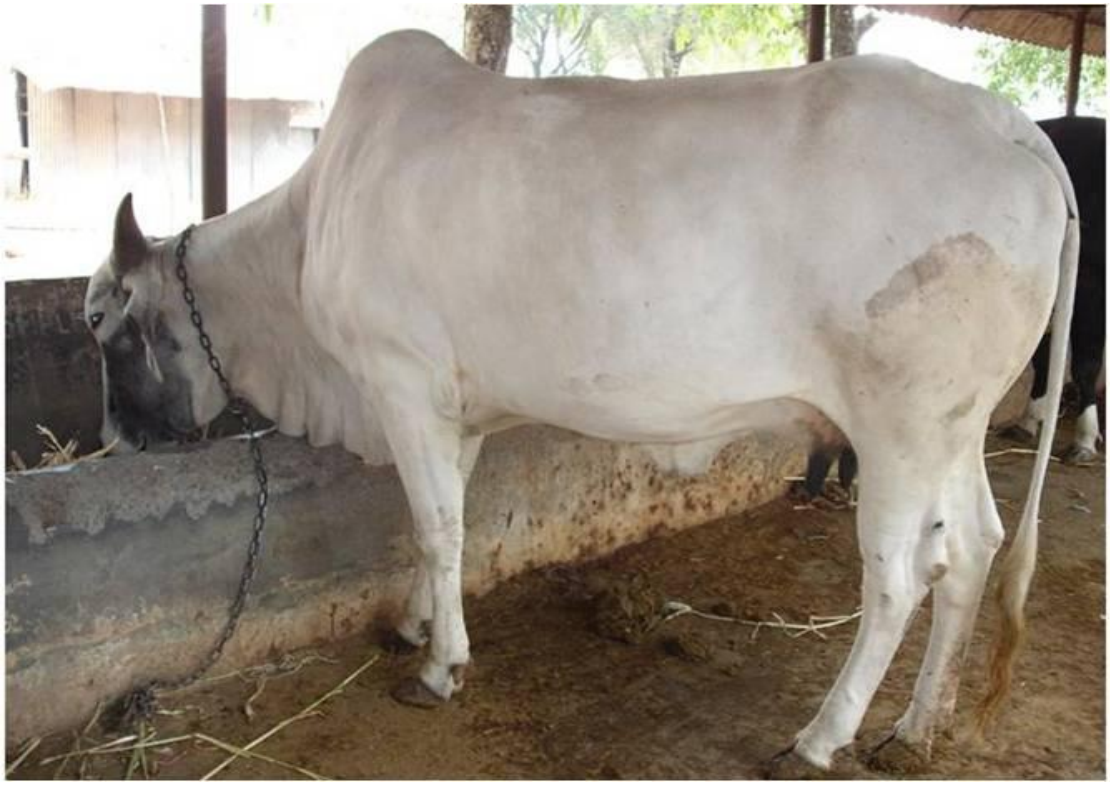

Figure 1. Atypical representative of a Deoni cow at CCBP, MAU.

Figure 2. Atypical representative of HF × Deoni crossbred cow at CCBP, MAU.

Table 1. Regression coefficients for lactation milk yield and lactation length on climatic variables in winter season.

Variable	Mean ± SE	LMY			LL		
		b	SE of (b)	t value	b	SE of (b)	t value
Max T°C	30.74±0.20	151.70	80.87	1.88	0.82	7.18	0.11
Min T°C	11.92±0.22	52.36	58.50	0.90	0.88	5.20	0.17
Max Hum	71.67±0.86	10.14	13.65	0.74	0.57	1.21	0.47
Min Hum	29.38±0.71	23.03	16.99	1.36	0.06	1.51	0.04
BSH	9.97±0.07	183.39	108.11	1.70	6.95	9.60	0.72
WS	4.05±0.13	19.23	63.15	0.30	1.33	5.61	0.24
Max THI	75.30±0.21	60.66	57.16	1.06	0.80	5.08	0.16
Min THI	59.10±0.28	21.36	36.24	0.59	2.90	3.22	0.90
		R^2 = 0.18		F value = 1.68	R^2 = 0.12		F value = 0.52

b = Estimated regression coefficient, LMY = lactation milk yield, LL= lactation length, Max T°C = maximum temperature, Min T°C= minimum temperature, Max Hum= maximum humidity, Min Hum = minimum humidity, BSH=bright sunshine hour, WS =wind speed, Max THI = maximum temperature-humidity index, Min THI=minimum temperature-humidity index.

Table 2. Regression coefficients for dry period and calving interval on climatic variables in winter season.

Variable	DP			ICP		
	b	SE of (b)	t value	b	SE of (b)	t value
Max T°C	0.93	5.53	0.17	1.71	8.87	0.19
Min T°C	4.67	4.00	1.17	5.53	6.42	0.86
Max Hum	1.30	0.93	1.39	0.74	1.50	0.50
Min Hum	2.08	1.16	1.79	2.14	1.86	1.15
BSH	2.36	7.39	0.32	9.35	11.86	0.79
WS	5.22	4.32	1.21	6.51	6.93	0.94
Max THI	4.09	3.91	1.05	3.31	6.27	0.53
Min THI	5.43	2.48	2.19	2.55	3.98	0.64
	R^2 = 0.17		F value = 1.30	R^2 = 0.21		F value = 0.39

b = Estimated regression coefficient, LMY = lactation milk yield, LL= lactation length, Max T°C = maximum temperature, Min T°C= minimum temperature, Max Hum= maximum humidity, Min Hum = minimum humidity, BSH=bright sunshine hour, WS =wind speed, Max THI = maximum temperature-humidity index, Min THI=minimum temperature-humidity index.

ICP. Hence, winter season is the most favourable season for crossbred cows to achieve short DP and ICP as compare to the other seasons.

Effect of climatic variables on lactation milk yield and lactation length in summer season

Average LMY and LL was recorded as 1714.97±47.75 kg and 272.84±5.78 days for cows calved in summer season. It was observed that Max T°C, BSH and Max THI showed negative and significant (P<0.05) regression while; Min T°C, Max Hum and Min THI had negative and non-significant influence with LMY and LL. However, wind speed exhibited positive and non-significant relationship on LMY and LL. This illustrated that an increase in Max T°C, sunshine radiation and Max THI could lead to decrease in LMY and LL due to negative regression (Table 3).

All the considered climatic variables in this study accounted for 33 and 26% variation in LMY and LL, respectively. The R^2 value showed significant level (P<0.05), which means the effect of summer season was substantial on LMY and LL. Thus, it can be inferred that there were significant effect of the selected climatic variables which means the LL was shortened by several days during extreme summer (Table 3). Furthermore, when THI was more than 72, LMY was also affected

Table 3. Regression coefficients for lactation milk yield and lactation length on climatic variables in summer season.

Variable	Mean ± SE	LMY			LL		
		b	SE of (b)	t value	b	SE of (b)	t value
Max T°C	39.37±0.24	-43.65	18.21	-2.40*	-15.35	5.92	-2.59*
Min T°C	21.30±0.34	-52.52	43.63	-1.20	-2.94	6.62	-0.44
Max Hum	46.35±0.86	-2.82	9.82	-0.29	-0.09	1.49	-0.06
Min Hum	19.10±0.53	11.11	16.89	0.66	-0.53	2.56	-0.21
BSH	10.65±0.08	-99.96	37.24	-2.68*	-12.48	5.69	-2.19*
WS	7.04±0.26	3.6	23.74	0.15	0.74	3.6	0.21
Max THI	84.03±0.23	-55.9	26.27	-2.13*	-13.96	5.54	-2.52*
Min THI	71.37±0.45	-53.7	27.36	-1.96	-0.23	4.15	-0.06
		R^2 = 0.32		F value = 2.18*	R^2 = 0.26		F value = 2.05*

b = Estimated regression coefficient, LMY = lactation milk yield, LL= lactation length, Max T°C = maximum temperature, Min T°C= minimum temperature, Max Hum= maximum humidity, Min Hum = minimum humidity, BSH=bright sunshine hour, WS =wind speed, Max THI = maximum temperature-humidity index, Min THI=minimum temperature-humidity index.

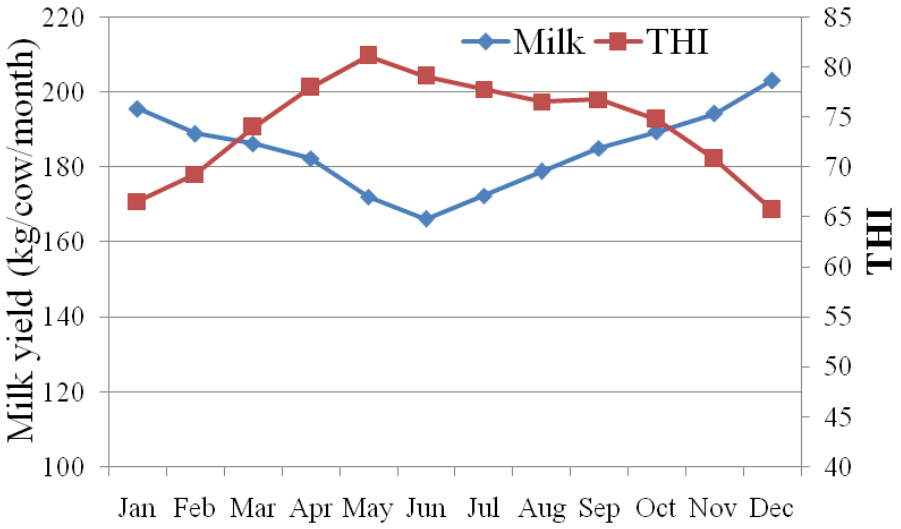

Figure 3. Monthly variation for milk production and THI from 1981-2010.

severely. Similar results were reported by Mandal et al. (2002), Shivprasad and Ramkaran (2002), Bouraoui et al. (2002), West et al. (2003), Daniel et al. (2008) and Singh and Upadhyay (2009).

Figure 3 illustrates the trend for the overall mean THI values and milk yield per cow per month. It indicates that these crossbred cows were exposed to heat stress from March to September with THI values varying between 74 and 81.1 (Table 5) and the lowest milk yield were recorded in these months.

Similar trends were observed for Egypt and Arizona where THI values were higher than 72 for four to six and three to four months, respectively (Johnson, 1985) and a negative relationship between milk production and THI were clearly observed. Indeed, as THI increases from 74 to 81.1 during the summer season, milk yield per cow drops down from 157.2 in March to 133.2 kg/month/cow in June. That is a decrease of about 15% which can be largely explained by the effect of summer heat stress, particularly in May and June when THI values are well above the critical threshold of 72 reported by Johnson (1985) and Dikmen and Hansen (2009) and can be as high as 81.1 in May (Table 5).

Effect of climatic variables on dry period and inter calving period in summer season

The average DP and ICP were observed as 134.42±3.72 and 440.37±6.41 days in summer season, respectively. It

Table 4. Regression coefficients for dry period and calving interval on climatic variables in summer season.

Variable	Dry period			Calving interval		
	b	SE of (b)	t value	b	SE of (b)	t value
Max T°C	12.74	5.84	2.18*	14.4	6.24	2.31*
Min T°C	5.03	3.72	1.35	2.09	5.73	0.36
Max Hum	1.39	1.29	1.08	2.48	1.74	1.43
Min Hum	6.24	2.21	2.82*	6.72	2.99	2.25*
BSH	8.83	5.5	1.61	3.31	10.14	0.33
WS	7.02	3.11	2.26*	5.28	3.2	1.65
Max THI	9.43	4.37	2.16*	15.53	6.96	2.23*
Min THI	2.06	3.58	0.58	2.29	3.84	0.60
	R^2 = 0.27		F value = 2.21*	R^2 = 0.24		F value = 2.13*

b = Estimated regression coefficient, LMY = lactation milk yield, LL= lactation length, Max T°C = maximum temperature, Min T°C= minimum temperature, Max Hum= maximum humidity, Min Hum = minimum humidity, BSH=bright sunshine hour, WS =wind speed, Max THI = maximum temperature-humidity index, Min THI=minimum temperature-humidity index.

Table 5. Summary mean meteorological data from 1981-2010.

Month	Rain	T°C		RH		BSH	WS	THI		
		Max	Min	Max	Min			Max	Min	Mean
Jan	0.32	29.98	11.60	74.85	31.14	9.85	3.92	74.80	58.34	66.57
Feb	0.18	32.86	13.49	63.19	24.48	10.46	4.62	77.37	61.19	69.28
Mar	0.34	36.81	17.44	49.64	19.75	10.59	5.31	81.53	66.41	73.97
Apr	0.21	40.09	21.58	42.27	16.95	10.84	6.36	84.24	71.73	77.99
May	0.63	41.19	24.88	47.15	20.61	10.52	9.47	86.31	75.97	81.14
Jun	5.33	36.35	23.83	72.06	43.42	7.22	10.76	82.44	75.75	79.10
Jul	7.30	32.04	22.48	82.49	58.50	4.74	9.41	80.39	75.20	77.80
Aug	7.62	30.66	22.00	85.03	63.77	4.69	8.25	78.99	74.07	76.53
Sep	5.85	31.82	21.73	84.94	58.13	6.85	5.88	79.76	73.79	76.78
Oct	2.74	32.51	18.42	78.45	43.22	8.99	4.50	79.40	70.19	74.80
Nov	0.75	30.81	13.85	75.85	36.42	9.60	4.24	76.36	65.26	70.81
Dec	0.28	29.36	10.68	76.96	32.51	9.59	3.62	73.72	57.78	65.75

was observed that Max T°C, Min Hum and Max THI exhibited positive and significant (P<0.05) regression whereas; Min T°C, Max Hum, BSH and Min THI revealed positive and non-significant regression with DP and ICP. All the considered climatic variables accounted for 27 and 24% variation in DP and ICP, correspondingly. The R^2 value showed significant level (P<0.05) statistically (Table 4). Thus, it could be inferred that there were significant influence of the selected climatic variables on DP and ICP in this season. This illustrates that an increase in temperature, relative humidity, sunshine radiation and THI, could lead to lengthen DP and ICP by several days (Table 4).

Similar findings were reported by Du Bois and Williams (1980), Weller and Folman (1990), Ray et al. (1992), Bouraoui et al. (2002), Jordan et al. (2002), Mishra and Joshi (2009) and Gaafar et al. (2011). Thus, calving schedules could be adjusted to minimize the adverse effect of heat stress.

Effect of climatic variables on lactation milk yield and lactation length in monsoon season

Average LMY and LL were recorded as 1830.15±40.54 kg and 289.19±6.24 days in monsoon season, respectively. It was observed that Max T°C, Max Hum and Max THI showed negative and significant (P<0.05) regression while BSH and Min THI had negative and non-significant relationship with LMY and LL. However, Min T°C and Min Hum exhibited positive and non-significant regression whereas wind speed revealed positive and significant (P<0.05) regression on LMY. This illustrated that an increase in Max T°C, Max Hum and Max THI could lead

Table 6. Regression coefficients for lactation milk yield and lactation length on climatic variables in monsoon season.

Variable	Mean ± SE	LMY			LL		
		b	SE of (b)	t value	b	SE of (b)	t value
Max T°C	32.27±0.23	-57.37	22.44	-2.56*	-8.87	3.79	-2.34*
Min T°C	22.51±0.12	62.07	39.47	1.57	3.28	4.83	0.68
Max Hum	81.13±0.64	-22.82	10.02	-2.28*	-6.29	2.92	-2.15*
Min Hum	55.96±0.94	6.74	11.34	0.59	0.55	1.67	0.33
BSH	5.87±0.15	-20.79	36.6	-0.57	-12.42	5.41	-2.30*
WS	8.57±0.31	34.86	14.15	2.46*	2.03	2.09	0.97
Max THI	80.39±0.18	-71.43	33.25	-2.15*	-9.47	4.39	-2.16*
Min THI	74.70±0.13	-71.32	52.94	-1.35	-5.61	7.82	-0.72
		R^2 = 0.19		F value = 2.08*	R^2 = 0.17		F value = 2.05*

b = Estimated regression coefficient, LMY = lactation milk yield, LL= lactation length, Max T°C = maximum temperature, Min T°C= minimum temperature, Max Hum= maximum humidity, Min Hum = minimum humidity, BSH=bright sunshine hour, WS =wind speed, Max THI = maximum temperature-humidity index, Min THI=minimum temperature-humidity index.

Table 7. Regression coefficients for dry period and calving interval on climatic variables in monsoon season.

Variable	Dry period			Calving interval		
	b	SE of (b)	t value	b	SE of (b)	t value
Max T°C	7.86	3.35	2.35*	9.98	4.71	2.12*
Min T°C	4.65	4.07	1.14	1.37	5.74	0.24
Max Hum	3.49	1.34	2.60*	4.78	1.89	2.53*
Min Hum	0.76	1.17	0.65	0.21	1.65	0.13
BSH	5.34	3.78	1.41	7.08	5.32	1.33
WS	4.07	1.46	2.79**	2.04	2.06	0.99
Max THI	9.11	4.46	2.04*	9.36	4.29	2.18*
Min THI	3.34	5.46	0.61	8.94	7.69	1.16
	R^2 = 0.20		F value = 3.47**	R^2 = 0.17		F value = 1.85

b = Estimated regression coefficient, LMY = lactation milk yield, LL= lactation length, Max T°C = maximum temperature, Min T°C= minimum temperature, Max Hum= maximum humidity, Min Hum = minimum humidity, BSH=bright sunshine hour, WS =wind speed, Max THI = maximum temperature-humidity index, Min THI=minimum temperature-humidity index.

to decrease LMY and LL due to negative regression (Table 6). Similar findings under Indian condition were reported (Singh and Mishra, 1980; Shinde, 1984; Shindhe et al., 1990; Kulkarni et al., 1998; Mandal et al., 2002; Upadhyay, 2003).

All the considered climatic variables accounted for 19 and 17% variation in LMY and LL, correspondingly. The R^2 value showed significant level (P<0.05) statistically. Thus, it can be inferred that there were significant influence of the selected climatic variables on LMY and LL in monsoon season (Table 6).

Effect of climatic variables on dry period and inter calving interval in monsoon season

Those cows calved in this season achieved an average DP and ICP as 117.58±4.09 and 431.78±5.53 days, respectively. It was observed that Max T°C, Max Hum and Max THI exhibited positive and significant (P<0.05) regression whereas, Min T°C, Min Hum, BSH and Min THI revealed positive and non-significant regression with DP and ICP. In contrast, WS showed positive and significant (P<0.05) regression with DP (Table 7).

Table 8. Regression coefficients for lactation milk yield and lactation length with climatic variables in post-monsoon season.

Variable	Mean ± SE	LMY			LL		
		b	SE of (b)	t value	b	SE of (b)	t value
Max T°C	31.67±0.17	119.5	108.7	1.10	28.61	10.95	2.61
Min T°C	16.38±0.38	14.83	54.2	0.27	3.99	5.46	0.73
Max Hum	77.15±0.71	14.71	18.77	0.78	1.18	1.89	0.62
Min Hum	39.82±1.17	3.52	16.08	0.22	1.48	1.62	0.91
BSH	9.30±0.12	130.17	109.8	1.19	8.38	11.07	0.76
WS	4.37±0.16	19.6	59.09	0.33	4.93	5.95	0.83
Max THI	77.88±0.23	-190.16	84.72	-2.24*	-27.69	8.53	-3.25**
Min THI	67.72±0.45	11.35	32.26	0.35	2.4	3.25	0.74
		R^2 = 0.14 F value = 1.08			R^2 = 0.12	F value = 1.52	

b = Estimated regression coefficient, LMY = lactation milk yield, LL= lactation length, Max T°C = maximum temperature, Min T°C= minimum temperature, Max Hum= maximum humidity, Min Hum = minimum humidity, BSH=bright sunshine hour, WS = wind speed, Max THI = maximum temperature-humidity index, Min THI=minimum temperature-humidity index.

Table 9. Regression coefficients for dry period and calving interval on climatic variables in post-monsoon season.

Variable	DP			ICP		
	b	SE of (b)	t value	b	SE of (b)	t value
Max T°C	16.74	9.31	1.80	9.87	15	0.66
Min T°C	2.73	4.64	0.59	1.27	7.48	0.17
Max Hum	2.53	1.61	1.57	1.34	2.59	0.52
Min Hum	0.35	1.38	0.25	1.13	2.22	0.51
BSH	8.58	9.41	0.91	0.2	15.16	0.01
WS	9.58	5.06	1.89	15.51	8.16	1.90
Max THI	16.92	7.26	2.33	7.77	11.69	0.66
Min THI	4.53	2.76	1.64	4.13	4.45	0.93
	R^2 = 0.23		F value = 1.30	R^2 = 0.21		F value = 0.99

b = Estimated regression coefficient, LMY = lactation milk yield, LL= lactation length, Max T°C = maximum temperature, Min T°C= minimum temperature, Max Hum= maximum humidity, Min Hum = minimum humidity, BSH=bright sunshine hour, WS =wind speed, Max THI = maximum temperature-humidity index, Min THI=minimum temperature-humidity index.

All the considered climatic variables accounted for 20 and 17% variation in DP and ICP. The R^2 value showed significant level (P<0.01) for DP but not for ICP statistically. Thus, it could be inferred that there were significant effect of the selected climatic variables on DP, which means the DP could be lengthen by several days due to heat stress (Table 7).

Effect of climatic variables on lactation milk yield and lactation length in post-monsoon season

Average LMY and LL were recorded as 1970.93±54.46 kg and 304.4±7.94 days in post-monsoon season (Table 8). It was observed that all the selected climatic variables showed positive and non-significant regression while; Max THI had negative and significant relationship with LMY (P<0.05) and LL (P<0.01) in this season. All the considered climatic variables accounted for 14 and 12% variation in LMY and LL. The R^2 value also showed non-significant level statistically (Table 8). The LL recorded in this season was close to the recommended LL of 305-days commonly accepted as a standard. Thus, climatic condition in post-monsoon season is conducive for milk production due to favourable climate and availability of quality fodder.

Effect of climatic variables on dry period and inter calving period in post-monsoon season

Average DP and ICP were recorded as 96.55±5.72 and 389.72±5.94 days, respectively for cows calved in post-monsoon season. It was observed that all of the climatic variables showed positive and non-significant regression with DP and ICP (Table 9). All the climatic variables con-

sidered accounted for 23 and 21% variation in DP and ICP, respectively (Table 9). The (R^2) value showed non-significant level statistically.

Conclusions

This research indicates that crossbred cows were sensitive for seasonal changes on their milk production and reproduction potential. With an increase in values of climatic variables, a decline in milk production and reproduction efficiency performance was manifested for considerable months of the year due to heat stress. The summary of meteorological data confirmed that there was high value of THI in seven months (March-September) yearly, which suggested that most crossbred cows were exposed to the negative effects of heat stress, where it will be difficult for them to thrive and maintain their production and reproduction potential. Therefore, additional productive and reproductive strategies like improving environmental, productive and reproductive management of cows are needed to reduce the adverse effect of heat stress.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Status of organophosphate and carbamate resistance in *Anopheles gambiae sensu lato* from the Sudano Guinean area in the central part of Benin, West Africa

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***Anopheles gambiae*, which is the main malaria vector in Benin has developed high level of resistance to pyrethroid insecticides. This raises serious concerns to the future use of long- lasting insecticidal nets (LLIN) and indoor residual spraying (IRS). It is therefore important to seek better and effective resistance management strategies which will use organophosphates or carbamates as alternatives against pyrethroid resistant malaria vectors in the field. Larvae and pupae of *A. gambiae s.l.* mosquitoes were collected from the breeding sites in Dassa-Zoume and Zogbodomey districts. WHO susceptibility tests were conducted on unfed female mosquitoes aged 2-5 days old. WHO bioassays were performed with impregnated papers with fenitrothion 1%, pirimiphos-methyl 0.25%, and bendiocarb 0.1%. Polymerase chain reaction (PCR) techniques were used to detect species and *Ace-1* mutations. *A. gambiae* Dassa-Zoume populations were susceptible to bendiocarb 0.1% with mortality rate of 99%. *A. gambiae* Zogbodomey populations were susceptible to pirimiphos-methyl 0.25% and fenitrothion 1% with mortality rates of 98.96 and 99%, respectively. PCR assay revealed that 100% of mosquitoes tested were *A. gambiae s.s.* The frequencies of *Ace-1R* mutation in *A. gambiae* Dassa-Zoume and Zogbodomey were 0%. Carbamates (bendiocarb) and organophosphates (fenitrothion and pirimiphos-methyl) have maintained their efficiency against *A. gambiae* Dassa-Zoume and Zogbodomey populations. Carbamates (bendiocarb) and organophosphates (fenitrothion and pirimiphos-methyl) have proven to be powerful alternatives against pyrethroid resistant malaria vectors such as *A. gambiae* Dassa-Zoume and Zogbodomey populations. The use of any of these three compounds in the centre Benin would be successful in malaria vector control.**

Key words: *Anopheles gambiae*, *Ace-1*, resistance, fenitrothion, pirimiphos-methyl, bendiocarb, Benin.

INTRODUCTION

In Africa, vector control is very dependent on a single class of insecticides, the pyrethroids. The dramatic increase in reports of pyrethroid resistance in malaria vectors (Santolamazza et al., 2008; Coleman et al., 2006) over the past decade is therefore a great cause for concern. Pyrethroid-impregnated nets are now widely used to reduce malaria morbidity and mortality in tropical countries. Unfortunately, in West Africa, resistance to pyrethroids is widespread in *Anopheles gambiae s.s.* populations (Chandre et al., 1999), the major malaria

vector in sub-Saharan Africa. Current status of pyrethroid resistance in malaria vectors was recently studied in Benin (Djègbé et al., 2011; Aïzoun et al. 2013a; Aïzoun et al. 2013b).

Resistance management strategies are mainly based on the rational use of the compounds already available, especially in public health because the number of insecticides is very limited. An alternative strategy to maintain the global effectiveness of insecticide-treated nets should be the use of other insecticides such as carbamates (C) and organophosphates (OP). Carbamate and OP are the main alternatives for indoor residual spraying or larval treatments against mosquitoes in the case of pyrethroid resistance. These insecticides inactivate acetylcholinesterase (AChE). Acetylcholinesterase (AChE) is a synaptic enzyme that hydrolyzes the neurotransmitter acetylcholine to terminate nerve impulses. It is also involved in the development of the nervous system in vertebrates and invertebrates (Grisaru et al., 1999; Cousin et al., 2005). Organophosphates and carbamates (OP and C) insecticides are competitive inhibitors that irreversibly inhibit the AChE enzyme, blocking nervous transmission and leading to the death of the insect. So, acetylcholinesterase is a key enzyme in the nervous system, terminating nerve impulses by catalysing the hydrolysis of the neurotransmitter acetylcholine. It (AChE) is the major target for organophosphate (OP) and carbamate insecticides, which inhibit enzyme activity by covalently phosphorylating or carbamylating the serine residue within the active site gorge (Corbett, 1974).

Quantitative and qualitative changes in AChE confer resistance to insecticides (Fournier, 2005). Across all insect species there are two very distinct types of target site resistance, conferring (i) high carbamates and low OP resistance, or (ii) high OP with either equivalent or low carbamate resistance (Russell et al., 2004). In *A. gambiae* species, AChE1 insensitivity is due to the same Gly-to-Ser substitution at position 119 (Mas-soulié et al., 1992). *A. gambiae s.s.* displays resistance to organophosphates and carbamates due to a single amino-acid substitution in the AChE1 catalytic site G119S (Weill et al., 2003).

The Benin National Malaria Control Programme has implemented indoor residual spraying (IRS) campaign under the financial support of the PMI (President's Malaria Initiative) using bendiocarb in the north of the country since 2011 and pyrimiphos-methyl since 2013. Bendiocarb was also the product previously used to control *A. gambiae s.l.* populations from Oueme department in southern Benin (2008-2010). Permethrin was the insecticide used on OlysetNets that were distributed free by the NMCP in July 2011 across the entire country whereas deltamethrin was the insecticide used on Permanets 2.0 that were distributed free by the NMCP in Oueme department in October 2008 and May 2009 in the framework of President's Malaria Initiative of the U.S. Government in Oueme department. In addition, Aïzoun et al. (2013c) suggested that further studies are needed to show the current distribution of the *Ace-1R* mutation in other localities in the south-north transect Benin, which localities will be different from those already studied in the north and south of the country. According to Akogbéto et al. (2011), the IRS campaign in the department of Oueme was an initial experience and the plan was to implement IRS strategy in other parts of Benin if initial results were encouraging.

The current study was proposed to assess the resistance status of malaria vectors from Dassa-Zoume to bendiocarb and from Zogbodomey to fenitrothion and pyrimiphos-methyl in order to check if the insensitive acetylcholinesterase (*ace-1R*) detected in the north of the country (Aïkpon et al., 2013; Aïzoun et al., 2013c) was already widespread in *A. gambiae s.l.* from the central part of the country.

METHODOLOGY

Study area

The study was carried out in some localities; following a south-north transect, Benin. Two contrasting localities of Benin were selected for mosquito collection on the basis of variation in agricultural production, use of insecticides and/or ecological settings (Figure 1). The localities were: Lema, a rice growing area located in Dassa-Zoume district in Collines department, in the central part of the country and Cana, a cereal (maize, ground-nut and so on) growing area located in Zogbodomey district in Zou department, in the central part of the country too. The rice farm of Lema is located in the centre of Dassa-Zoume district. It is a small rice growing area about 5 ha with only one rice growing per year (July to November). According to some farmers of Lema, there has been no use of insecticide in this rice growing area (except fertilizers, weed-killers and threadworm-killers) (Akogbeto et al., 2005). The choice of the study sites took into account the economic activities of populations, their usual protection practices against mosquito bites, and peasant practices to control farming pests. These factors have a direct impact on the development of insecticide resistance in the local mosquito vectors. The central part of the country is characterized by a Sudano Guinean climate with two rainy seasons (April-July and September-November) with an average rainfall of 1,000 mm per year.

Mosquito sampling

A. gambiae s.l. mosquitoes were collected from April-July 2012 during the first rainy season in Zogbodomey district more precisely in Cana locality and in Dassa-Zoume district more precisely in Lema locality, both located in the central part of the

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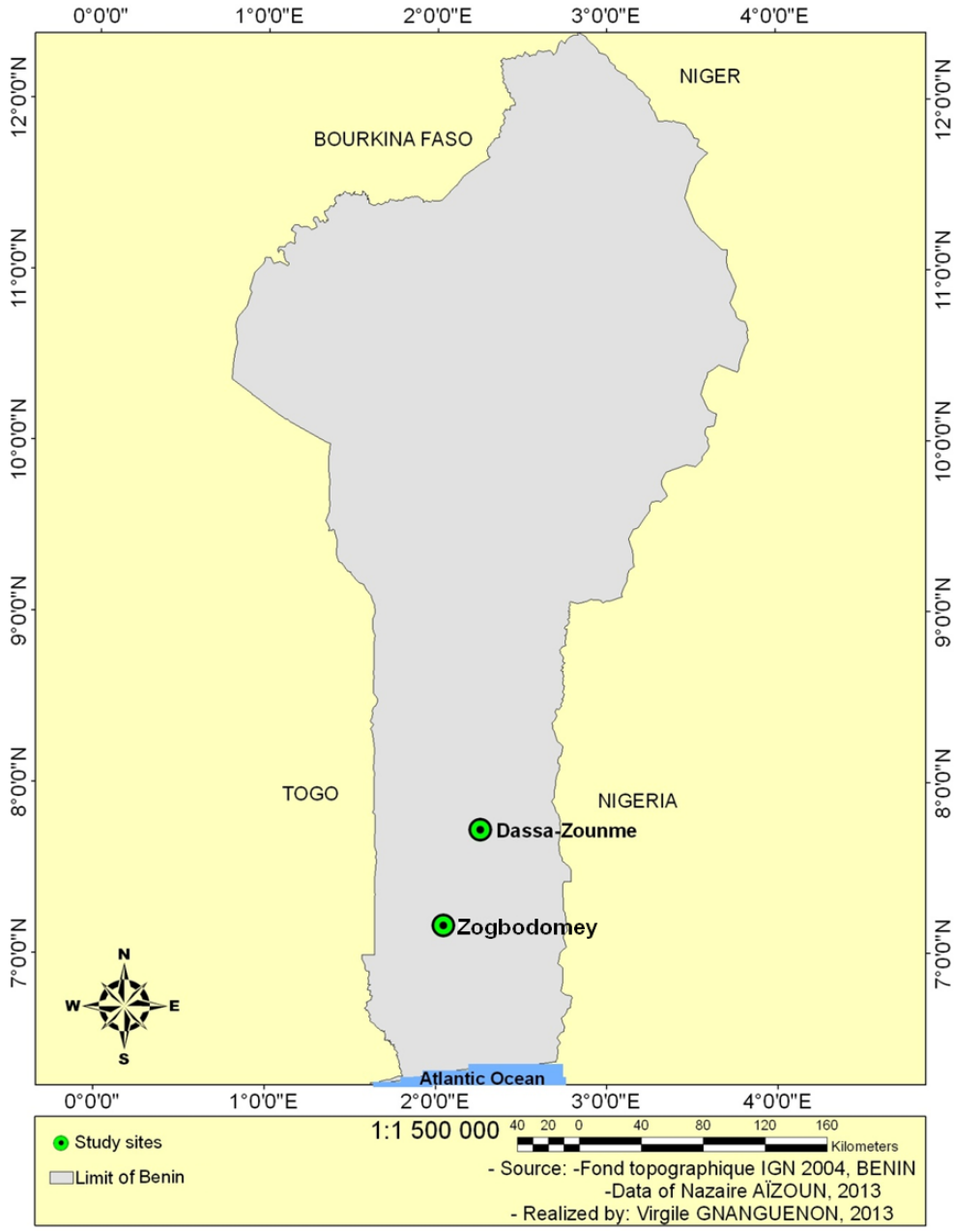

Figure 1. Map of the study area.

country. Larvae and pupae were collected in Cana locality within both padding and village using the dipping method on several breeding sites (brick pits, pools, marshes, streams, ditches, pits dug for plastering traditional huts, puddles of water, water pockets caused by the passage of cattle and gutters).

Anopheles pre-imaginal stages (L1 to L4 instars) were also collected via ladles within rice farms from Lema. Since the farms are irrigated, breeding sites are present throughout the year and we therefore assumed that the larvae collected in the study period were representative of the population that could be found during other periods of the year. Larvae collected from multiple breeding sites were pooled together then re-distributed evenly in development trays containing tap water. Larvae were provided access to powdered TetraFin® fish food, and were reared to adults under insectary conditions of 25+/-2°C and 70 to 80% relative humidity at Centre de Recherche Entomologique de Cotonou (CREC) located in Akpakpa, in Cotonou district. Larvae and pupae collected in Cana locality were also reared to adults under insectary conditions at CREC. *A. gambiae* Kisumu, a reference susceptible strain was used as a control for the bioassay tests. We used Kisumu more precisely to confirm the quality of treated or impregnated papers and then to calculate the resistance ratio. Susceptibility tests were done following WHO protocol on unfed females mosquitoes aged 2-five days old reared from larval and pupal collections. All susceptibility tests were conducted in the CREC laboratory at 25+/-2°C and 70 to 80% relative humidity.

Table 1. Percentage of dead *Anopheles gambiae* observed after 1 h exposure to WHO papers impregnated with bendiocarb in Dassa-Zoume district and with pirimiphos- methyl and fenitrothion in Zogbodomey district.

Population	Insecticide	Number tested	Mortality (%)	Resistance status
Kisumu (Control)	Fenitrothion	102	100	S
	Pyrimiphos-methyl	101	100	S
	Bendiocarb	101	100	S
Dassa-Zoume	Bendiocarb	99	99	S
Zogbodomey	Fenitrothion	100	99	S
	Pyrimiphos-methyl	94	98.96	S

Testing insecticide susceptibility

Females *A. gambiae* aged 2 to 5 days old were exposed to WHO diagnostic dosage of bendiocarb 0.1%, pirimiphos-methyl 0.25% and fenitrothion 1% according to the WHO protocol (WHO, 1998). Thus, an aspirator was used to introduce 20 to 25 unfed female mosquitoes into five WHO holding tubes (four tests and one control) that contained untreated papers. They were then gently blown into the exposure tubes containing the insecticide impregnated papers. After one-hour exposure, mosquitoes were transferred back into holding tubes and provided with cotton wool moistened with a 10% honey solution. The number of mosquitoes "knocked down" at 60 min and mortalities at 24 h were recorded following the WHO protocol (WHO, 1998). Dead and surviving mosquitoes were separately stored in individual tubes with silicagel and preserved at -20°C in the laboratory, for further molecular characterization. We used bendiocarb and pirimiphos-methyl because of the indoor residual spraying (IRS) campaign with these two compounds in progress in the north of the country. Fenitrothion, an insecticide of same class as pirimiphos-methyl, was used to check if there was cross-resistance to these two compounds in the central part of Benin.

PCR detection of species and *Ace-1* mutations

Specimens of *A. gambiae* from the WHO bioassay tests were subjected to the *A. gambiae* species specific PCR assays for species identification (Scott et al., 1993). The PCR-restricted fragment length polymorphism (PCR-RFLP) diagnostic test was used to detect the presence of G119S mutation (*ace.1R* gene) as described by Weill et al. (2003). Mosquito genomic DNA was amplified using the primers Ex3AGdir 5′GATCGTGGACACCGTGTTCG3′ and Ex3AGrev 5′AGGATGGCCCGCTGGAACAG3′according (Weill et al., 2003). One microlitre of total DNA extracted from a single mosquito was used as a template in a 25 µl PCR reaction containing Taq DNA polymerase buffer, 0.2 mM dNTP and 10 pmol of each primer. The PCR conditions were 94°C for 5 min and then 35 cycles of (94°C for 30 s, 54°C for 30 s and 72°C for 30 s) with a final 5 min extension at 72°C. Fifteen microlitres of PCR product were digested with 5U of AluI restriction enzyme (Promega) in a final volume of 25 µl. The PCR fragments were fractionated on a 2% agarose gel stained with ethidium bromide and visualized under UV light.

Data analysis

The resistance status of mosquito samples was determined according to the latest WHO criteria (WHO, 2013) as follows:

1. Mortality rates between 98-100% indicate full susceptibility
2. Mortality rates between 90-97% require further investigation
3. Mortality rates < 90%, the population is considered resistant to the tested insecticides.

Abbott's modified formula was not used in this study for the correction of mortality rates in test-tubes because the mortality rates in all control tubes was less than 5% (Abbott, 1987).

To compare the status of insecticide resistance, Fisher's exact test was carried out to determine if there was any significant difference between mortality rates of populations of *A. gambiae s.s.* of districts using Statistica 6.0. Allelic frequencies of G119S mutation were analysed using the version 1.2 of Genepop (Raymond and Rousset, 1995). To assess if the mutation frequencies were identical across populations, the test of genotypic differentiation was performed (Goudet et al., 1996).

The mortality times or lethal times for 50 and 95% of tested mosquitoes (LT_{50} and LT_{95}) were estimated using SPSS version 16.0 (SPSS Inc., Chicago, IL). The resistance ratio (RR_{50}) was determined relative to the Kisumu susceptible strain. This was obtained by dividing the LT_{50} of wild strain to the LT_{50} of the susceptible strain. The software R-2.15.2. (R Development Core Team, 2011) was used for the statistical analysis.

RESULTS

Susceptibility of *A. gambiae s.l.* populations to pirimiphos-methyl, fenitrothion and bendiocarb

Table 1 shows that Kisumu strain (control) confirmed its susceptibility status as a reference strain. We used Kisumu to confirm the quality of treated or impregnated papers and then to calculate the resistance ratio. The 24 h mortality recording shows that female mosquitoes of *A. gambiae* Kisumu which were exposed to WHO papers impregnated with bendiocarb 0.1%, pirimiphos-methyl 0.25%, and fenitrothion 1% were susceptible to these products with the mortality rates of 100%. Regarding *A. gambiae* Dassa-Zoume populations, they were also susceptible to bendiocarb 0.1% with the mortality rate of 99%. *A. gambiae* Zogbodomey populations were susceptible to pirimiphos-methyl 0.25% and fenitrothion 1% with the mortality rates of 98.96 and 99% respectively (Table 1).

Table 2. Resistance ratio of RR_{50} and RR_{95} with regard to *Anopheles gambiae* Dassa-Zoume and Kisumu populations susceptibility to bendiocarb.

Insecticide	LT50 Dassa-Zoume	LT50 Kisumu	RR50	LT95 Dassa-Zoume	LT95Kisumu	RR95
Bendiocarb	29.040	9.755	2.97	45.402	25.360	1.79

Table 3. Resistance ratio of RR_{50} and RR_{95} with regard to *Anopheles gambiae* Zogbodomey and Kisumu populations susceptibility to fenitrothion.

Insecticide	LT50 Zogbodomey	LT50 Kisumu	RR50	LT95 Zogbodomey	LT95Kisumu	RR95
Fenitrothion	68.840	183.512	0.375	86.449	309.229	0.27

Table 4. Resistance ratio of RR_{50} and RR_{95} with regard to *Anopheles gambiae* Zogbodomey and Kisumu populations susceptibility to pyrimiphos-methyl.

Insecticide	LT50 Zogbodomey	LT50 Kisumu	RR50	LT95 Zogbodomey	LT95Kisumu	RR95
Pyrimiphos-methyl	26.437	10.894	2.42	46.419	19.986	2.32

Table 5. *Ace-1* mutation frequency in *A. gambiae* populations issue from WHO bioassays tests.

Locality	Number tested	Species Ag	*Ace-1* mutation			
			RR	RS	SS	F(*Ace-1*)
Dassa-Zoume	47	47	0	0	47	0
Zogbodomey	49	49	0	0	49	0

Determination of resistance ratio (RR)

The resistance ratio (RR_{50}) of the wild populations of *A. gambiae s.l.* from Dassa-Zoume with regard to bendiocarb and from Zogbodomey with regard to pyrimiphos-methyl were higher than 1 (Tables 2 and 4). But the RR_{50} of the wild population of *A. gambiae s.l.* from Zogbodomey with regard to fenitrothion was lower than 1 (Table 3). The lethal time or mortality time (LT_{50}) of *A. gambiae s.l.* from Dassa-Zoume with regard to bendiocarb was 29.040 versus 9.755 min for *A. gambiae s.l.* Kisumu susceptible reference strain. The resistance ratio (RR_{50}) was 2.97. In similar way, the LT_{50} of *A. gambiae s.l.* from Zogbodomey with regard to pyrimiphos-methyl was 26.437 versus 10.894 min for *A. gambiae s.l.* Kisumu susceptible reference strain. The resistance ratio (RR_{50}) was 2.42. The same remark was made with LT_{95} values obtained with these same wild populations of *A. gambiae s.l* using these same insecticides. The resistance ratio (RR_{95}) obtained with *A. gambiae s.l.* from Dassa-Zoume with regard to bendiocarb was 1.79 whereas the resistance ratio (RR_{95}) obtained with *A. gambiae s.l.* from Zogbodomey with regard to pyrimiphos-methyl was 2.32. Conversely, the LT_{50} of *A. gambiae s.l.* from Zogbodomey with regard to fenitrothion was 68.840 versus 183.512 min for *A. gambiae s.l.* Kisumu susceptible reference strain. The resistance ratio (RR_{50}) was 0.375. The same remark was made with LT_{95} value obtained with this same wild population of *A. gambiae s.l* using this same insecticide. The resistance ratio (RR_{95}) obtained with *A. gambiae s.l.* from Zogbodomey with regard to fenitrothion was 0.27.

Species of *Anopheles gambiae* and *Ace-1* genotype

PCR revealed 100% of mosquitoes tested were *A. gambiae s.s.* The frequencies of *Ace-1R* in *A. gambiae* Dassa-Zoume and Zogbodomey were 0% (Table 5).

DISCUSSION

Anopheles gambiae, which is the main vector for malaria in Benin has developed high level of resistance to pyrethroid insecticides. This raises serious concerns to future use of long-lasting insecticidal nets (LLIN) and indoor residual spraying (IRS). In this context, one of the

pathways available for malaria vector control was the use of alternative classes of insecticides with different mode of action and which will be different for pyrethroids. The Benin National Malaria Control Programme distributes regularly pyrethroid-impregnated nets to the households. The IRS with carbamates (bendiocarb) was done in Atlantic department (Ouidah, Kpomassè and Tori districts) (2007-2009) in southern Benin and then in Oueme department (Adjohoun, Dangbo, Missérété and Sèmè districts) (2008-2010) in southern Benin. Both pyrethroid and carbamate compounds are currently in use in the country through recent free distribution of OlysetNets by the NMCP in July 2011 and through the (IRS) campaign under the financial support of the President's Malaria Initiative (PMI) using bendiocarb in the north of the country since 2011 and using pyrimiphos-methyl since 2013. The use of both compounds is in progress in the country in order to control *A. gambiae s.l.* populations, malaria vectors using integrated control.

In the current study, the LT_{50} and LT_{95} values obtained with *A. gambiae s.l.* Kisumu susceptible reference strain were lower than those recorded with the wild populations of *A. gambiae s.l.* from Dassa-Zoume. Therefore, *A. gambiae s.l.* Dassa-Zoume populations took more time to die when they were exposed to bendiocarb comparatively to Kisumu susceptible strain. The same remark was made with the wild populations of *A. gambiae s.l* from Zogbodomey with regard to pyrimiphos-methyl. However, a converse situation was observed with *A. gambiae s.l.* populations from Zogbodomey with regard to fenitrothion. In fact, the LT_{50} and LT_{95} values obtained with *A. gambiae s.l.* Kisumu susceptible reference strain were higher than those recorded with the wild populations of *A. gambiae s.l.* from Zogbodomey. So, the slow effect or action which characterizes organophosphates was observed with *A. gambiae s.l.* from Zogbodomey after their exposure to fenitrothion. But, this action or effect was not observed with these same *A. gambiae s.l.* populations when they were exposed to pyrimiphos-methyl, another organophosphate compound.

A. gambiae s.l. Dassa-Zoume populations were susceptible to bendiocarb. The susceptibility of *A. gambiae* to bendiocarb may be explained by the absence of individual homozygous RR in the central part of the country. This absence of resistance to bendiocarb has previously been documented in southern Benin (Akogbeto et al., 2010, Padonou et al., 2012). In addition, no insecticide product was generally used in rice growing area of Lema to control pests (Akogbeto et al., 2005). Aïzoun et al. (2013c) also recently found that *A. gambiae* Kandi and Malanville populations were still susceptible to bendiocarb in the northern Benin. Even if, certain *A. gambiae s.l.* populations from northern Benin, such as *A. gambiae s.l.* populations from Kouandé, Matéri, Natitingou, Péhunco and Tanguiéta were already resistant to bendiocarb (Aïkpon et al., 2013), those from the central part of the country were still susceptible to this product. So, bendiocarb has emerged as a promising insecticide for the control of vector populations that are resistant to pyrethroids (Akogbeto et al., 2011). According to Padonou et al. (2011), a possible alternative in the case of pyrethroid resistance in *A. gambiae*, is the use of bendiocarb, to which it was observed a good sensitivity. In addition, these authors mentioned that in case this product is held there is hope that malaria transmission will be reduced drastically.

A. gambiae s.l. Zogbodomey populations were susceptible to both fenitrothion and pyrimiphos-methyl, organophosphate compounds. The susceptibility of *A. gambiae* to these two products may also be explained by the absence of individual homozygous RR in the central part of the country. In addition, no insecticide product was generally used in cereal growing area of Cana to control pests. A recent study carried out by Aïzoun et al. (2013c) also showed that *A. gambiae* Kandi and Seme populations were still susceptible to fenitrothion in the northern and southern Benin respectively. According to Akogbeto and Yakoubou (1999), the difference between carbamate, organophosphate and pyrethroid insecticides can be explained by the emergence and widespread resistance of *A. gambiae s.l.* to pyrethroids.

In the current study, all *A. gambiae* specimens issued from WHO bioassays, were homozygous susceptible individuals. There were no homozygous resistant and heterozygote individuals. These results might be related to high fitness cost of the *ace-1R* mutation, resulting in death of the homozygous resistant mosquitoes (Weill et al., 2004, Asidi et al., 2005, Djogbenou et al., 2010). In *A. gambiae s.s.* populations, the *ace-1* mutation has been associated with a high fitness cost as the frequency of the *ace-1* mutation in mosquito populations declines rapidly after a few generations in the absence of selection pressure from organophosphates or carbamates insecticides (Labbé et al., 2007). In addition, no *ace-1* mutation has been found in *A. gambiae* Tchaourou and Savè populations susceptible to carbamates and organophosphates, both from the north-central Benin (data not shown).

There are no previous published studies on the resistance status of *A. gambiae* populations from Dassa-Zoume and Zogbodomey to carbamates and OPs until 2013. Our study was the first conducted for this purpose. Therefore, these populations of *A. gambiae* need to be monitored for insecticide resistance in this area.

Conclusion

Carbamates (bendiocarb) and organophosphates (fenitrothion and pirimiphos-methyl) have maintained their efficiency against *A. gambiae* Dassa-Zoume and Zogbodomey populations. The good efficiency of these three compounds against *A. gambiae* populations from the central part of Benin is clearly demonstrated in the

current study. The use of any of these three compounds in this part of the country would be successful for malaria control in this area.

Conflict of Interests

The author(s) have not declared any conflict of interests.

Ethical approval

This study was approved by the Ministry of Health and the Center for Entomological Research of Cotonou.

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Productive performance and carcass characteristics of lori-bakhtiari finishing lambs supplemented with sodium bicarbonate or magnesium oxide

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Effect of sodium bicarbonate (SB) and magnesium oxide (MgO) in high concentrate fed Lori-Bakhtiari lambs was studied on productive performance and carcass characteristics. Eighteen weaned (90 days old) Lori-Bakhtiari male lambs divided into three equal groups were fed basal diet. Each group received a basal diet for 75 days with one of the following three treatments: (1) no additives (NA); (2) 0.05% magnesium oxide and (3) 0.2% sodium bicarbonate. Lambs were individually confined to 1.5 m^2 metabolic cages. Cold and hot carcass weight (kg) and hot dressing (%) were higher in group receiving MgO than the SB group ($p<0.05$). There were no significant different ($p>0.05$) in visceral fat contents (kidney, rumen mesenteric) in lambs of control and treated groups. Body weight (kg) and average daily gain (ADG) was higher in group receiving MgO than the SB group ($p<0.05$). No effect of MgO or SB in the diet was observed on weight of liver, lungs, blood and lie. Heart weight was greater ($p<0.05$) for lambs consumed diets supplemented with MgO. The results showed that the use of 0.05% magnesium oxide in the diet can increase dry matter intake, weight gain and improvement is weight and percent carcass than the control group and sodium bicarbonate.

Key words: Hot dressing, skin, lungs, lamb, carcass.

INTRODUCTION

The concentrate feeds are important components of the diet of ruminant animals. The lambs maintained on high-concentrate feed for maximizing gain usually exhibit rumen acidosis and lower fiber digestibility (Snyder et al., 1983; Krehbiel et al., 1995; Santra et al., 2003) due to changing rumen environment including pH and rumen microbial population. The cost per unit of energy of feedlot diet is lower with high-grain diets than with forage-based diets (Huntington, 1997). However, high-grain diets fed to lambs can cause digestive disturbances related to ruminal acidosis. Rumen acidosis has been defined as biochemical and physiological stresses

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Table 1. Ingredients (dry matter basis) and chemical composition of the basal diet.

Ingredient	Percentage
Wheat	14
Barley	33.8
Corn	3
Soybean meal	3.1
Cotton seed meal	3.8
Wheat bran	33
Rice bran	4
Anzymite	1.6
Mineral and vitamin per mix[1]	2
NaCl	0.3
[3]Ca	0.80
[3]P	0.6
Chemical composition (%)	
Crude protein	14.16
Neutral detergent fiber	36.4
Acid detergent fiber	20.6
Ash	6.3
Metabolizable Energy[2] (Mcal/kg)	2.6

[1]Supplies per kg of feed: 4.9 mg of Zn, 4.05 mg of Mn,0.45 mg of Cu, 0.075 mg of I, 0.1 mg of Se, 2.500 IU Vitamin A, 400 mg of Vitamin D, 2.5 IU Vitamin E. [2]Calculated metabolized energy. [3]Di calcium phosphate (DCP).

caused by a rapid production and absorption of organic acids in the rumen, which may cause severe damage to rumen papillae, in some cases severe ulceration of rumen wall (Britton and Stock, 1987). Acidosis can cause keratinization of rumen epithelium and various secondary disorders observed in feedlots animals, such as laminitis, polioencephalomalacia, rumenitis and liver abscesses (Huber, 1976; Owens et al., 1998). To prevent acidosis in feedlot cattle and lambs, feeding of fibrous material, in enough quantities and in a particle size that would stimulate rumination, is recommended to stimulate saliva production, counteracting a drastic reduction of ruminal pH. Additives or products that buffer rumen environment may prevent acidosis and improve the productive performance of feedlot animals that consume high-grain diets (NRC, 2001; Wallace and Newbold, 1993). Sodium bicarbonate and magnesium oxide are the additives most commonly used against acidosis. These additives can be included (0.5-2.5%) in diets (Coskun, 1998). The growth of animal as influenced by dietary buffer is studied by various workers (James and Whohlt, 1985; Nishino, 1994; Wondra et al., 1995), but little information is available on carcass quality (Mandebvu and Galbraith, 1999). Therefore, the objective of the present study was to observe the effect of magnesium oxide or sodium bicarbonate supplementation, in high concentrate fed lambs, on carcass characteristics and productive performance of Lori-Bakhtiari finishing lambs.

MATERIALS AND METHODS

Experimental location, animals, diets and sampling procedures

The experiment was conducted at Lori-Bakhtiari sheep breeding and culture center, shahrekord, iran. Eighteen Lori-Bakhtiariweaned (90 days old) lambs were divided into three equal groups, 1) Control group (No Additive), 2) Control group + 0.05% magnesium oxide (MgO) and 3) Control group + 0.2% sodium bicarbonate (SB). The lambs of control group were maintained on a complete diet containing 40:60, forage: concentrate ratio for 75 days. Sodium bicarbonate and magnesium oxide were added in the feed offer preparation in the required proportion. Ingredients and chemical composition of basal diet is presented in Table 1. All the lambs were individually fed throughout the study. Lambs were confined to individual metabolic cages (1.5 m^2) equipped with water and feed troughs. Diets were formulated to be isonitrogenous and contain 14.6% crude protein. Diets were offered three times daily at 8:00, 13:00 and 17:00 h. Feed offered was based on the intake of the previous day plus an additional 10% in order to reduce selection of feed components. This trial lasted for 75 days; including a 15-days adaptation period and 60-days data collection period. After adapting to the metabolic cages and experimental diets, lambs was weighed on provide baseline weights. At the end of the experiment, the lambs were transported to a slaughter house, where they were weighed before sacrifice. Hot carcass weight and dressing percentage (carcass weight as a percent of live weight) were recorded. Weights of skin, kidney, lungs, blood, heart, lie, legs and head were recorded.

Statistical analysis

Data were analyzed with an analysis of variance of a completely randomized design. Treatment effects on DM intake, weight body (0-30, 30-60 and 0-60 days), weight gain, feed efficiencies, slaughter weight, hot and cold carcass weight, hot and cold dressing percentages, weight of visceral organs, were analyzed using the GLM procedure of a completely randomized design (SAS, 2009). Initial live weight was used as a variable and covariance analysis was conducted on live weight gain. The significant treatment means were compared by Duncan's multiple range test (Duncan, 1995).

RESULTS

Feed efficiency, DM intake, Body weight (0-30, 30-60 and 0-60 days), live weight gain

Feed efficiency (Table 2) of lambs was influenced by SB ($p<0.05$). More feed was required per unit of gain when MgO was added to the ration ($p<0.05$). Feed efficiency expressed as kg feed/kg daily weight gain averaged 7.03 for lambs fed ration with 0.2% sodium bicarbonate. It appears that since the lambs were not at maturity, most of the feed energy may have been used primarily for muscular synthesis, and very little for fat deposition, throughout the 60- days study. Treatments means of DM intake (kg), body weight (0-30, 30-60 and 0-60 days) and body weight gain (kg/day) of lambs are presented in Table 2. Initial body weight (kg) of lambs did not differ ($p>0.05$) among treatments (Table 1). Effect of period (0-30 versus 30-60 and 0-60 days) on body weight was not

Table 2. Feed intake, body weight, weight gain and feed efficiency of lambs fed finishing diets with Magnesium Oxide (MgO) Sodium Bicarbonate (SB) during the 60-days trial

Item	NA	MgO	SB
Initial body weight(kg)	39.31 ± 0.74[a]	40.03 ± 1.37[a]	40.38 ± 0.86[a]
Body weight (kg)			
0-30 days	46.14 ± 4.63[a]	46.06 ± 5.62[a]	41.96 ± 3.24[a]
30-60 days	49.56 ± 4.11[a]	51.18 ± 9.27[a]	48.25 ± 3.66[a]
0-60 days	53.76 ± 0.65[b]	56.48 ± 2.43[a]	54.45 ± 1.75[ab]
Average daily gain (g/day)	199 ± 0.01[b]	247 ± 0.14[a]	206 ± 0.01[b]
Feed intake (kg)	141.06 ± 0.28[a]	146.13 ± 1.57[a]	128.64 ± 1.13[b]
Feed efficiency (intake/gain)	9.43 ± 0.28[a]	7.83 ± 0.53[b]	7.03 ± 1.34[b]

[a-b]Values in the same row without a common superscript letter are significantly different (P<0.05).

Table 3. Hot and carcass weight and dressing percentage of lambs fed finishing diets with magnesium oxide and sodium bicarbonate.

Carcass trait	NA	MgO	SB
Slaughter weight (kg)	52.46 ± 0.28[c]	58.35 ± 0.83[a]	55.41 ± 1.31[b]
Hot carcass weight (kg)	26.06 ± 0.87[c]	30.38 ± 0.63[a]	28.71 ± 1.34[b]
Chilled carcass weight (kg)	25.03 ± 1.18[c]	29.36 ± 0.53[a]	27.76 ± 1.29[b]
Hot dressing (%)	49.67 ± 1.65[b]	52.07 ± 0.99[a]	51.79 ± 1.29[a]
Chilled dressing (%)	48.34 ± 3.04[a]	50.68 ± 1.41[a]	50.08 ± 1.19[a]

[a-b]Values in the same row without a common superscript letter are significantly different (P<0.05).

significant (p>0.05). DM intake was different among treatments (p<0.05) during the entire 60- days period. Dry matter intake was 141.06±0.28, 146.13±1.57 and 128.64±1.13 (kg) for NA, MgO and SB treatments. Body weightof lambs during 30-60 and 0-60 days was different (p<0.05) among treatments, so that during days 30-60 and 0-60, body weight was significantly greater (p<0.05) for treatments with MgO. Weight gain of lambs was change (p<0.05) among treatments. Average weight gain was 199±0.01, 247±0.14 and 206±0.01 g/d for NA, MgO and SB treatments.

Carcass weights and dressing percentage

The effects of MgO and SB in ration on carcass weight and dressing percentage are presented in Table 3. Weight (kg) of lambs slaughtered was different among treatments (p<0.05). Weights were 52.46±0.28, 58.35±0.83 and 55.41±1.31 kg for NA, MgO and SB, respectively. Hot and cold carcass weight were affected (p<0.05) by treatments in the diet. Hot carcass weights of 26.06±0.87, 30.38±0.63 and 28.71±1.34 kg and cold carcass weights of 25.03±1.18, 29.36±0.53 and 27.76±1.29 kg were for NA, MgO and SB, respectively. Hot dressing percentages affected (p<0.05) by treatments in the diet. Hot dressing percentages were 49.67±1.65, 52.07±0.99 and 51.79±1.29% for NA, MgO and SB. Chilled dressing percentages were not affected (p>0.05) by treatments.

Fat distribution (weight in gram)

The depot (non- carcass) fat distribution in both control and treated lambs are presented in Table 4. There was no significant difference in visceral fat contents kidney fat, mesenteric fat and rumen fat control and treated groups (p>0.05). Scrotal fat (g) were affected by treatments (p<0.05). Scrotal fat weights of 24.66±14.01, 44.83±17.27 and 36.5±3.61 g for NA, MgO and SB, respectively.

Visceral organ weight

Weights of skin (kg), heart (g), legs (kg) and head (kg) were affected (p<0.05) by MgO and SB in the ration (p<0.05). Experimental results showed no significant difference on liver weight, lungs, blood and lie (p>0.05).

Table 4. Fat distribution (weight in gr) of lambs fed fattening diets with magnesium oxide (MgO) and sodium bicarbonate (SB).

Trait	NA	MgO	SB
Scrotal around fat (g)	24.66 ± 14.01[b]	44.83 ± 17.27[a]	36.5 ± 3.61[ab]
kidney around fat (g)	81.33 ± 20.59[a]	90.16 ± 15.35[a]	94 ± 3.84[a]
Rumen around fat (g)	0.347 ± 0.173[a]	0.604 ± 0.258[a]	0.486 ± 0.137[a]
Mesentric around fat (g)	46.20 ± 9.58[a]	46.21 ± 6.65[a]	41.75 ± 12.3[a]

[a-b]Values in the same row without a common superscript letter are significantly different (P<0.05).

Table 5. Weight of visceral organs of lambs fed finishing diets with magnesium oxide and sodium bicarbonate.

Item	NA	MgO	SB
Skin (kg)	3.845 ± 0.21[b]	4.483 ± 0.26[a]	4.375 ± 0.37[a]
Liver (kg)	0.873 ± 0.04[a]	0.797 ± 0.22[a]	0.777 ± 0.06[a]
lungs (kg)	0.562 ± 0.03[a]	0.553 ± 0.09[a]	0.556 ± 0.06[a]
Blood (kg)	2.618 ± 0.25[a]	2.726 ± 0.21[a]	2.611 ± 0.25[a]
Heart (kg)	0.209 ± 0.01[b]	0.227 ± 0.01[a]	0.226 ± 0.02[a]
Lie (kg)	0.09 ± 0.003[a]	0.08 ± 0.006[a]	0.07 ± 0.009[a]
Foots (kg)	1.20 ± 0.08[b]	1.32 ± 0.07[a]	1.25 ± 0.09[ab]
Head (kg)	2.62 ± 0.12[b]	2.73 ± 0.09[a]	2.58 ± 0.05[b]

[a-b]Values in the same row without a common superscript letter are significantly different (P<0.05).

DISCUSSION

Dry matter intake, body weight (0-30, 30-60, 0-60 days), average daily gain and feed efficiency

Dry matter intake (DMI), body weight (30- 60 and 0-60 days) and daily weight gain was increase in fed lambs with MgO supplement (p<0.05). Supplementation of MgO in the diet of animals is known to increase the number of total ruminal as well as cellulolytic bacteria which could have contributed to better cellulose digestibility (Koul et al., 1998). In addition, in agreement with the results of the present study Linda and Wohlt (1985) also reported that the addition 0.18% MgO in diet finishing lambs improved the dry matter intake of lambs. Body weight 30-60 and 0-60 days vs. 0-30 increased linearly (p<0.05) with increasing MgO supplementation. However, Fisher and Mackay (1983) reported that the addition of MgO improved weight gain in animals feed diet low in protein, with no benefit with high protein diets. The addition of MgO in the diet did not improve feed efficiency of lambs in this study. Feed efficiency of lambs fed with SB diet improved in comparative with control group. Nicholson and Cuningham (1961) observed that the addition of 2 to 6% SB to ruminant ration improve the feedlot calves performance.

Carcass weight and dressing percent

Pre-slaughter weight was higher in MgO supplement group than in the control group. Hot and chilled carcass weight and hot dressing percentages were affected by the addition MgO and SB to the diets of lambs. Higher dressing percentage in MgO and SB in the present study resulted mainly from pre-slaughter weight differences as well as the proportion of gastro intestinal tract and its content with respect to live weight. Neither Fimbres et al. (2002) nor Petit et al. (1997) found differences in carcass dressing percentage of lambs fed diets with various levels of forage and concentrate.

Fat distribution (weight in gram)

The depot (non- carcass) fat distribution in both control and treated lambs are presented in Table 4. There was no significant difference in visceral fat contents (kidney, rument and mesenteric fat) in lambs of control and treated groups. No significant effect in visceral fat content was also observed by Mandebvu and Galbraith (1999) in young male lambs supplemented with sodium bicarbonate. Subcutaneous fat was poorly developed in control and treated groups and may be due to breed-specific characteristics. It is established that the adapted tropical breed in order to facilitate thermolysis by cutaneous evaporative codling, deposits more fat in the viscera rather than in the subcutaneous region.

Effect on the size of internal organs

In our study, weights of liver, lungs, blood, lie did not differ (p>0.05) between the treatment and control groups (Table 5). In a study by Fimbres et al. (2002) with feedlot lambs, no difference in visceral organ weight was observed. In both our study and that of Fimbres et al. (2002), feed was offered adlibitum. The reduction in visceral organ mass seemed to be partly responsible for lowered maintenance energy requirements. Large proportion of animal maintenance energy requirements can be attributed to the visceral organs, especially the liver and the gastro-intestinal treat (GIT), and seem to be

associated with the high rates of protein synthesis and degradation in these tissues (Ferrel and Jenkins, 1985).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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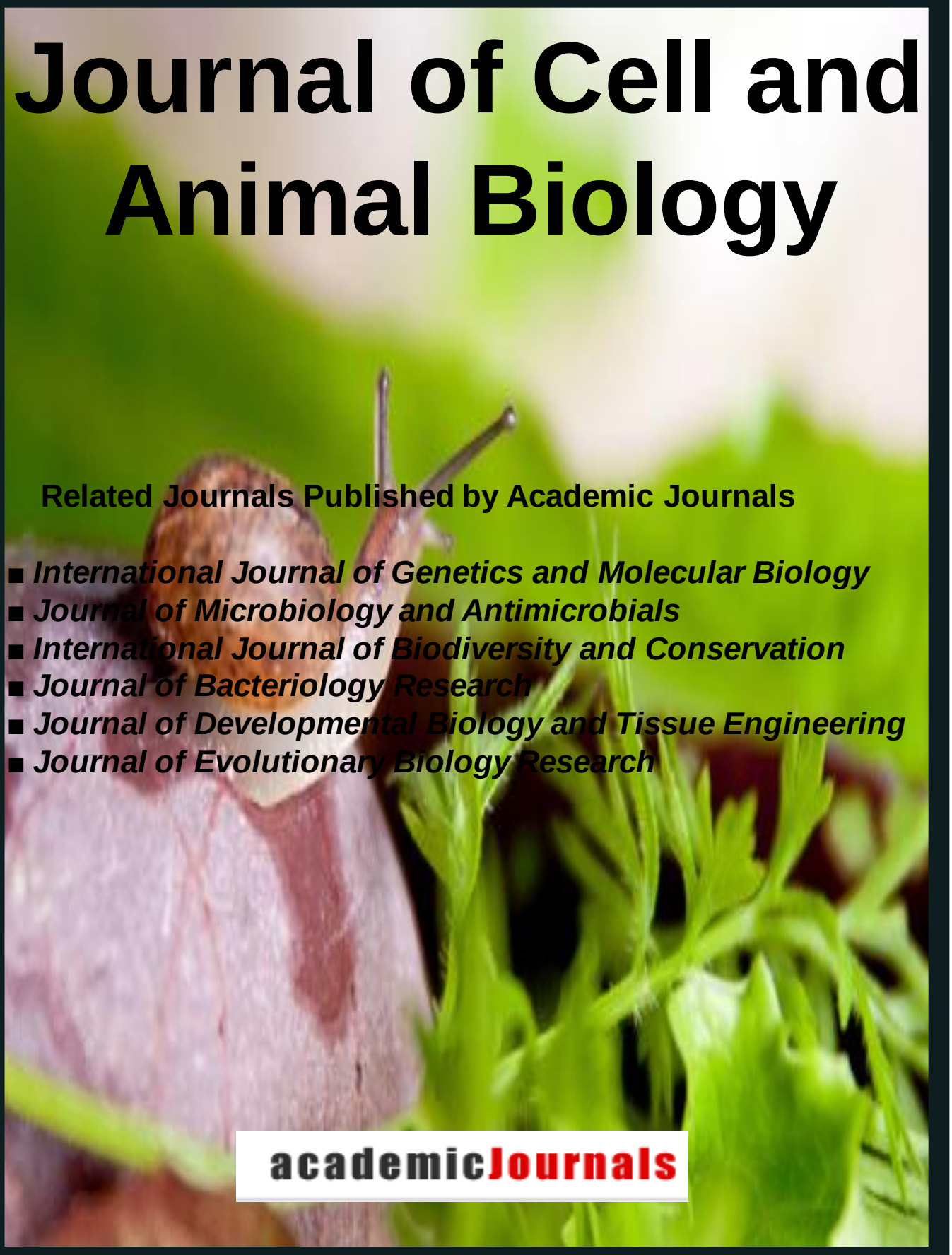

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