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The Impact of Ventilation Rate on Behavioral Activities and Welfare of Egyptian Buffalo Calves in the Nile Delta

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ABSTRACT

In the Nile delta of Egypt, small farmers raise their animals inside closed pens with bad ventilation without considering the health conditions. The objective of this work was to study the impact of 1) different ventilation rates on barn air condition to determine the optimum ventilation rate (VR) inside the barn. 2) poor and optimum VR on Egyptian buffalo calves' welfare. This study was carried out at the research unit for animal behavior, Faculty of Agriculture, Menoufia University, Egypt. Ten Egyptian buffalo calves aged about 5-6 months and the average weight of 154 ± 17 kg, tied in closed pens with concrete floor were used. The calves were allocated in two similar groups, each 5 animals in a similar pen, one as control with limited natural ventilation and the other (treatment) supplied by mechanical ventilation. Four treatments A, B, C, and D (by using 2, 4, 6, and 8 fans, respectively) were applied to determine the optimum VR as an initial experiment (object. 1). The optimum VR was achieved by Treat. (D) in which the lowest temperature of 18.43 °C, the relative humidity of 59.33%, THI of 55.56, and CO_2 concentration at 401.87 ppm. Calves' performances (growth rate, CBC and behavior) and environmental variables (T, RH, and CO2 con.) were recorded for 60 days in control and Treat. (D) (object. 2). The results showed decreased values of T and RH inside the treatment pen than control (18.43 °C and 56.65 % vs. 23.49 °C and 62.86 %, respectively).

Keywords: *ventilation rate, calves welfare, animal behavior and CO_2 concentration.*

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1. INTRODUCTION

The buffaloes (*Bubalus bubalis*) are originally Asian animals and have mainly been distributed in tropical and subtropical Asia **(Abd ellah *et al.,* 2013)**. The buffaloes have been introduced into Egypt from approximately the middle of the seventh century and mainly distributed in the Nile valley and delta region. Buffalo is a multipurpose animal, besides being a better source of milk, it is a source of meat as well **(Aggarwal *et al.,* 2016**). Egyptian buffaloes contribute to about 14 and 5% of the world buffalo's meat and milk, respectively. Egyptian population of buffaloes is 3,375,727 heads in 2017 **(FAO, 2017).** Adequate calf house ventilation is vital to increase calf comfort and promote the growth of healthy calves. The primary functions of ventilation are to eliminate noxious gases, maintain optimum temperature and relative humidity levels, and decrease airborne dust and pathogen concentration. Poor ventilation inside the animal's pens affects not only the animal but also the human. According to **Senthilselvan *et al.* (1997)**, an unbalanced environment leads to the incidence of respiratory disorders in people working in swine barns. Natural ventilation within the farms is more efficient and cheaper than mechanical ventilation. In the Nile delta of Egypt with limited agricultural land, small farmers tend to raise their animals inside closed pens within the villages and suburbs of cities without considering the health conditions. The bad ventilation barns can be overcome by reducing the density of housing and providing mechanical ventilation to help the natural one. But in the delta of Egypt, the farmers often resort to increase the density of housing to maximize the use of the area inside closed barns with limited ventilation openings. The bad ventilation is often available inside these barns around the animals. Monitoring environmental variables including gas concentrations, relative humidity, and environmental temperature are crucial to effectively control the physical environment inside barns where animals are reared **(Mendes *et al.*, 2013)**. Temperature and relative humidity may influence air quality by affecting the survival and proliferation of some pathogens that may cause performance impairment, animal welfare problems, and disease **(Wathes *et al.*, 1998; Yalçin *et al.*, 1997)**. The total CO_2 production includes CO_2 emitted from the manure and CO_2 produced by the animals **(Pedersen *et al.*, 2008)**. The number of scientific evidence of adverse effect on farm animals when the concentration of carbon dioxide is 3000 ppm or higher; this also applies to dairy cows and although CO_2 is not poisonous at elevated levels (>3,000 ppm), it adversely affects animal as less oxygen is present **(Valerii *et al.,* 2016)**. Behavioral changes are the most manifest symptoms of poor welfare **(Candiani *et al.*, 2008)**. **Borderas *et al.* (2008)** found many behavioral changes that occur during an infectious illness, including reduced rumination, grooming, feeding, and activity in dairy calves. **Anderson (2000)** reported that animals in unsuitable facilities increased standing and less lying. The blood profile is an important indicator of an individual's welfare, the knowledge of hematological/biochemical indices is useful in distinguishing the normal state from the state of stress, which can be nutritional, environmental or physical **(Aggarwal *et al.*, 2016)**. The animal's blood profile significantly changes under stress **(Banerjee, 2008; Thankachan, 2007)**. The objective of this work was to study the impact of 1) different ventilation rates (mechanical means) on indoor air of barn to determine the optimum ventilation rate (VR) inside the barn, 2) poor and optimum ventilation on the welfare of buffalo calves under Egyptian conditions in the Nile Delta.

2. MATERIALS AND METHODS

2.1. Study location

This experiment was carried out at the research unit for animal behavior, belonging to the Faculty of Agriculture, Menoufia University, Menoufia, Egypt. All the experimental procedures were approved by the Menoufia University Committee of Animal Ethics and complied with Egyptian guidelines for animal welfare.

2.2. Animals and management

Ten Egyptian buffalo calves tied in the closed housing system with concrete floor were used in this study. The animals aged about 5-6 months and had an average weight of 154 ± 17 kg at the beginning of the experiment. Pens were cleaned twice daily (9 am and 3 pm) using water rush; after that calves were fed twice daily (10 am and 4 pm) with the Egyptian clover (*Trifolium alexandrinum*), concentrate mixture, and rice straw that formulated to meet **NRC (2001)** requirements. Water was available ad-lib from automatic drinkers. The natural lighting was adequate to monitor the animals at the day, while industrial dim lighting was used at night.

2.3. Experimental Design

The experiment was conducted in 11 weeks from 10 February to 28 April 2019. The ten calves were allocated in two similar groups, each group with 5 animals was housed in a similar pen with the same ventilation opening and the same dimensions of 3×4×4 m^3 for length, width, and height respectively. One of these pens was used as control with closed east ventilation opening and opened west ventilation opening (1×1 m^2). The other pen (treatment) was supplied by mechanical ventilation from the eastern side and opened west ventilation as listed previously.

Four ventilation rates were applied by using 2, 4, 6, and 8 fans for an optimum ventilation rate, CO_2 concentration in the indoor was approximately equal to the outdoor as an initial experiment. The pen was equipped with a neutral-pressure mechanical ventilation system consisting of one inlet and one outlet unit with fan. The fans' diameter and velocity were 20 cm and 1.5 ms^{-1}, respectively. Each treatment lasted 2 days to measure the following indoor and outdoor environmental variables: environmental temperature (T), relative humidity (RH), and CO_2 concentration.

The optimum ventilation rate in the initial experiment was achieved by using 8 fans as mechanical ventilation in the treatment pen. Calves' performance (growth rate, CBC, and behavior) and environmental variables (T [°C], RH [%], and CO_2 concentration [ppm]) were measured in control and treatment pens. The experimental model was a randomized complete block design with 5 replicates per each treatment.

2.4. Measurements

2.4.1. Environmental conditions measurements

Temperature, relative humidity, and CO_2 concentration were measured by CO_2/T/RH/ data logger HT-2000. The collected data were recorded every 2 minutes all over the 24 hours throughout the experiment, as shown in Figure 1.

Figure 1. CO_2/Temp/RH/ data logger HT-2000

The temperature humidity index (THI) was computed according to the National Research Council formula (NRC 1971), as the following equation 1.

$$THI= (1.8 \times T_{db} + 32) – (0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} – 26) \tag{1}$$

Where:
T_{db}=dry bulb temperature, °C
RH=Relative humidity %

2.4.2. Estimation of the ventilation rate

The direct method to determine the ventilation rate (Q) is the fluid continuity equation (Equation 2) to pen ventilation rates and Equation 3 to determine the air exchange rate (AER) **(Joo *et al.*, 2014)**. Ventilation flow in the outlet pipe was measured by measuring velocity in ms^{-1} using Protmex Digital Anemometer (Ms6252a) that records 2 measurements per hour. The ventilation rate on an hourly basis was calculated as the average of those measurements.

$$Q = 3600\ v\ A \tag{2}$$

$$AER_{Direct}= Q/V \tag{3}$$

Where: Q is ventilation rate (m^3s^{-1}); v is the average perpendicular air velocity to the face of the opening (ms^{-1}); A is the opening area (m^2), for a given barn inlet or outlet; AER_{Direct} is air exchange rate by direct method (h^{-1}); and V is the pen volume (m^{-3}).

Estimation of the emission rate of CO_2 (ER) from livestock buildings was an important target. The emission rate is defined as the amount of the pollutant CO_2 concentration and the ventilation rate, both of which should be measured accurately and reliably. A perfect method would be to measure the ER in each of concentration differences between exhaust and incoming air using the equation 4 **(Joo *et al.*, 2014)**:

$$ER = Q\frac{293}{T}\,(C_e - C_i) \times 10^6 \qquad 4$$

where, ER is CO_2 emission rate (m^3h^{-1}), Q is ventilation rate at room temperature and pressure (m^3h^{-1}), T is the air temperature in the room exhaust air (K), C_e is gas concentration in the room exhaust air (ppm), and C_i is gas concentration in the incoming air (ppm).

2.4.3. Calves daily gain and behavior

The calves were weighed individually every weekend by digital livestock scale to determine the daily gain for all calves housed in each pen.

All animals in compared pens were monitored at the last three days of the experiment to declare their behavior and/or daily activity. In the continued 72 hours, a video was recorded for each experimented animal using a complete digital behavioral observation unit consisting of 4 digital observation cameras, a digital storage unit, and a control unit. Continuous observation methods were used to record the activity patterns of each animal during the observation periods, which included:

a. Frequency and a total period of lying behavior: Lie was defined as all legs relaxed with the underside in contact with the floor **(Weimer 2012).**

b. Frequency and a total period of eating behavior: defined as feeding in mouth, chewing, or head down in the manger close to the feed **(Alzahal *et al.*, 2006).**

c. Frequency and a total period of ruminating behavior: defined as the time when the animals were not eating and were re-chewing the bolus **(Mezzalira *et al.*, 2012).**

2.4.4. Hematological examination

At the end of the experiment, the blood samples were collected from all calves' jugular vein into evacuated collection tubes containing EDTA and used for hematological examination. Samples were sent directly after collection in ice tank to the research laboratory (Department of Animal production, Faculty of agriculture, Menoufia University, Egypt). Hematological examination was directly performed within 0.5-1 h after collection by using Medonic Veterinary Hematology analyzer (Medonic CA 620, Sweeden).

The measured hematological analytes were Complete Blood Count (CBC), Differential Leukocyte Count, and Blood Indices. CBC was Hemoglobin (HGB), Red Blood Cells (RBC) and total leukocyte (WBCs) counts. The counted differential leukocytes included lymphocytes, neutrophils, monocytes, eosinophils and basophils. Hematocrit (HCT), M.C.V, M.C.H, M.C.H.C, and Platelet (PLT) count were measured as blood indices.

2.5. Statistical analysis

The experiment was performed using a completely randomized design with 5 treatments in the initial experiment and 2 treatments with 5 replicates of each as the second experiment. The Kolmogorov Smirnov test was performed to test the normality of the data before the statistical analysis. Experimental data were subjected to One-way ANOVA using IBM SPSS software v.22 (SPSS Inc., Chicago, IL, US) as a completely randomized design. The experimental animal was used as an experimental unit for analyzing the experimental data. The results were expressed as means ± SEM.

3. RESULTS

3.1. Initial experiment

The overall average Temperature (°C), Relative humidity (%), THI, CO_2 concentration (ppm) and CO_2 emission rates (m^3h^{-1}) are shown in Table 1 at control and all treatment groups, Treatment A, B, C, and D (using 2, 4, 6, and 8 fans, respectively). The ventilation rate achieved by Treatments A, B, C, and D were 0.094, 0.188, 0.283, and 0.337 m^3s^{-1} respectively. Furthermore, air exchange rates were 7.07, 14.13, 21.2, and 28.26 h^{-1}, respectively. Figure 1 shows the AER calculated using the direct method. It shows a strong correlation (R2=1) between ventilation rate and AER based on the direct method. When the ventilation rate was 0.094 m^3s^{-1}, the AER increase was 7.07 h^{-1}. The same correlation between the AER and ventilation rate, shown in Figure 2 was also found in the work of **Wu *et al.* (2012)**.

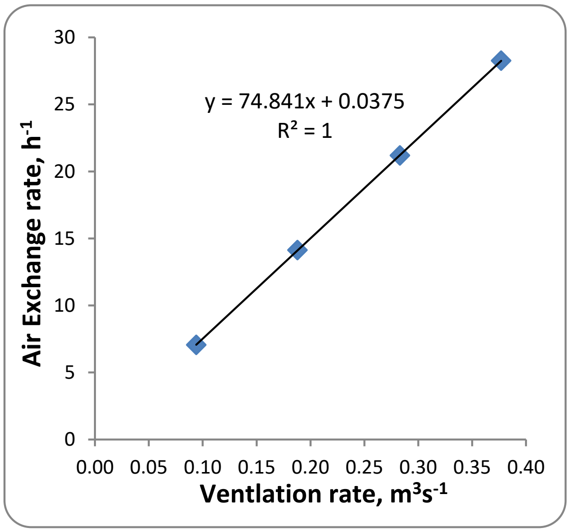

Figure 2. AER calculated based on the direct method.

The mean values showed significant differences in the overall ventilation rates of the treatments and control groups. The results showed that the minimum values of temperature, RH, THI, CO_2 concentration, and CO_2 emission rate were achieved at the fourth ventilation rates (Treat. D), in which the average values were 18.43±0.027, 59.33±0.059, 63.56±0.037, 401.87±0.77, and 1.18±0.0095 as the previous order. While the maximum values were achieved with the control group, which were 23.49±0.018, 55.56±0.076, 64.95±0.027, 792.35±5.56, and 0 for temperature, RH, THI, CO_2 concentration, and CO_2 emission rate, respectively. Table 1 illustrates the decrease in CO_2 concentration inside the animals' barn by increasing the ventilation rate. The CO_2 concentration values were 401.87±0.77, 532.59±1.20, 645.2±1.46, 897.76±2.44, and 1382.35±5.56 at all ventilation rate and control, respectively. Therefore, the fourth ventilation rate of 0.337 m^3s^{-1} was the optimum treatment where the CO_2 concentrations inside and outside the animal barn were almost equal. These facts may be caused by increasing the air exchange rate. Increasing ventilation rate on the other hand, has proven to be one of the most effective methods for reducing air pollutant concentrations in animal barns. These results agree with **Hamon *et al.* (2012)**.

Table 1. The effect of ventilation rates on Temperature, Relative humidity, THI, CO_2 production rates, and CO_2 concentration inside claves' barns.

	Control	Treat. A	Treat. B	Treat. C	Treat. D
Temperature (°C)	23.49±0.018^{a}	22.59±0.012^{b}	21.27±0.015^{c}	20.11±0.025^{d}	18.43±0.027^{e}
RH (%)	69.36±0.14^{a}	66.46±0.077^{b}	64.59±0.11^{c}	62.78±0.076^{d}	59.33±0.059^{e}
THI	64.95±0.027^{a}	64.202±0.019^{b}	62.89±0.021^{c}	58.22±0.038^{d}	55.56±0.037^{e}
CO_2 concentration (ppm)	1382.35±5.56^{a}	897.76±2.44^{b}	645.2±1.46^{c}	532.59±1.20^{d}	401.87±0.77^{e}
CO_2 Emission rate (m^3/h)	0^{a}	2.28±0.0056^{b}	2.07±0.008^{c}	1.82±0.009^{d}	1.23±0.0095^{e}

a, b, c, d, and e means within each row with different superscript differ significantly P≤0.01.

Control (without mechanical ventilation), Treat. A (VR=0.094 m^3s^{-1}), Treat. B (VR=0.188 m^3s^{-1}), Treat. C (VR=0.283 m^3s^{-1}), and Treat. D (VR=0.337 m^3s^{-1}).

3.2. Main experiment

Figure 3 and Figure 4 illustrate temperatures and relative humidity deference during the experiment period inside the ventilated and control pens. The results showed decreased values of T and RH inside ventilated pen than control at all experiment periods. The average T and RH were 18.43 °C and 56.65% for ventilated vs. 23.49 °C and 62.86% in the control barns, respectively; while average T and RH outside barns (surrounding environment) were 18 °C and 50%. In addition, it could be noticed that temperature variation ranged from 2.1 to 3 °C all over the day due to temperature difference during night and day.

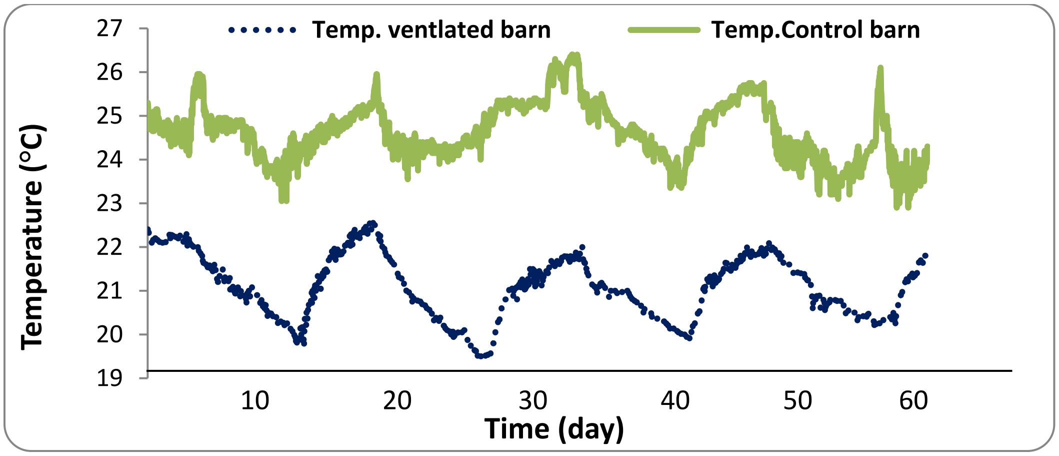

Figure 3. Temperature differences inside ventilated and control barns during the treatment period.

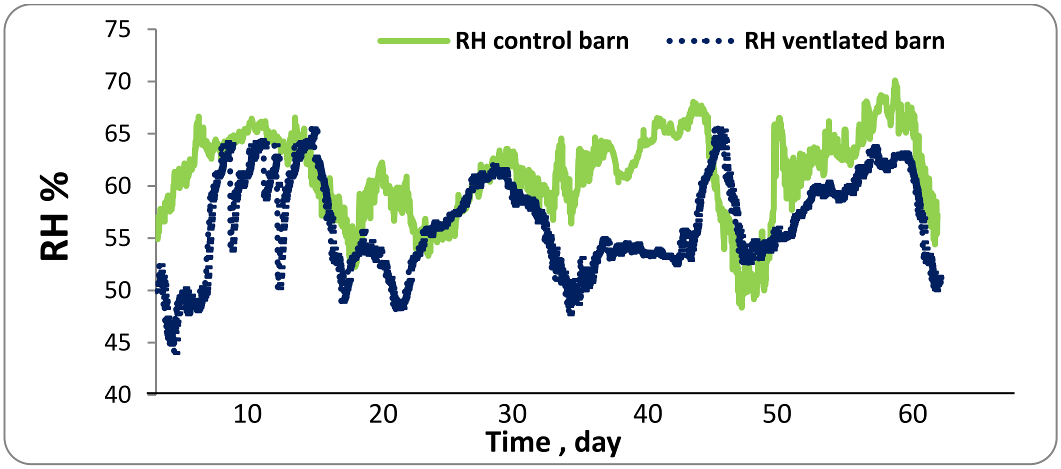

Figure 4. RH difference inside the ventilated and control barns during the experiment period.

The results of CO_2 concentration (ppm) are shown in Figure 4. The data were recorded during the experiment period (60 days). According to figure 4, there were significant differences in CO_2 concentrations (ppm) for ventilated and control barn. The CO_2 concentration ranged from 365 to 432 ppm and from 1074 to 1858 ppm for ventilated and control barns, respectively. It is clearly seen that the CO_2 concentration varied considerably during the measurement period in the control barn more than the ventilated barn. These results ensure the efficiency of mechanical ventilation tools on removing CO_2 produced by the animals and emitted from the manure.

Figure (6) shows differences in CO_2 emission rates (m3/h) in the ventilated and control barns. From the results, it can be noticed that the CO_2 emission rate ranged from 0.66 to 2.25 m^3/h for the ventilated barn, while it was equal to zero in the control barn because there was no ventilation rate; therefore, the air exchange rate was almost zero. This experiment data can be helpful to evaluate the measurements of gases emission to identify optimum mechanical ventilation for livestock buildings.

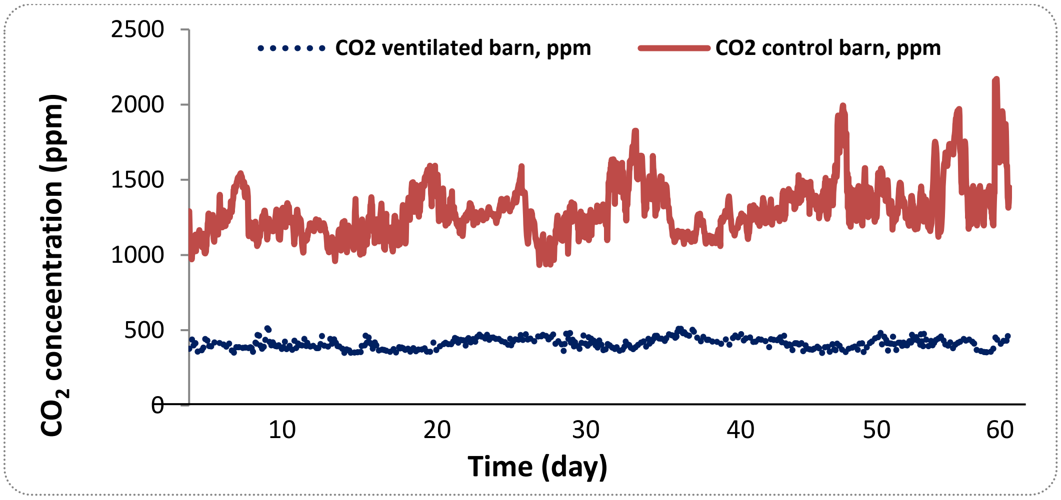

Figure 5. Variation of CO_2 concentrations (ppm) for ventilated and control barns during the experiment period.

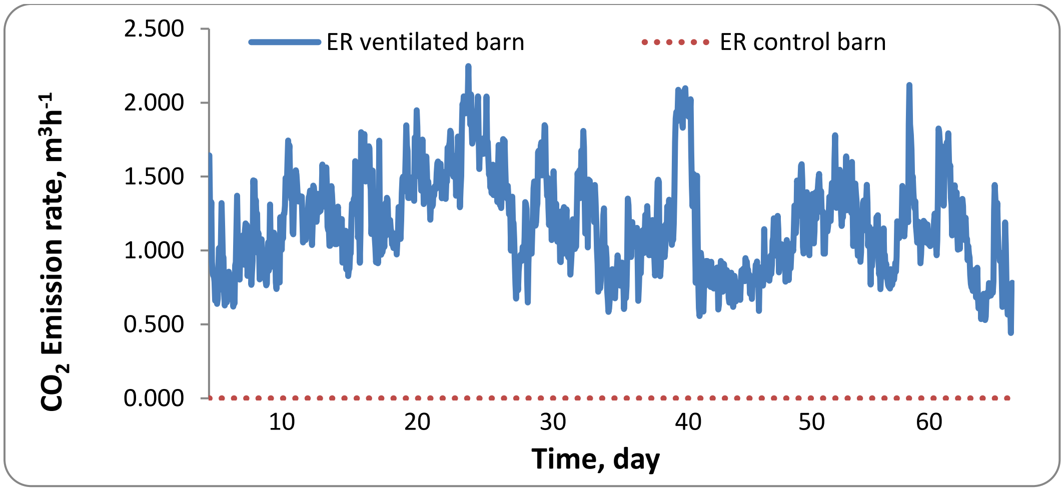

Figure 6. CO_2 emission rate deference inside ventilated and control barns during the experiment period.

The results of Egyptian buffalo calves daily weight gain, lying, eating, and ruminating behaviors with and without mechanical ventilation are presented in Table (2). It could be seen that the daily weight gain of calves, which were in the pen with no mechanical ventilation (Control) was almost the same but slightly lower than the others with mechanical ventilation (Treatment), which were 1.05 and 1.13 kg/day, respectively, but this difference did not reach to significant levels. This result showed that the Egyptian buffalo calves' daily gain did not affected by bad ventilation (1382.35±5.56 ppm of CO_2). The behavioral changes are the most manifest symptoms of poor welfare **(Candiani *et al.*, 2008)**. Calves in the control group without mechanical ventilation lied down more frequently (8.25 ± 0.94 times/day) than the others in the other groups (6.75 ± 0.77 times/day) but the difference was not significant. However, the time spent in lying position throughout the day significantly ($P \leq 0.01$) decreased from 863.5 ± 19.82 minutes/day in the control group to 781 ± 18.70 minutes/day in the treatment group (Table 2).

Table 2: Egyptian buffalo calves' daily weight gain, lying, eating, and ruminating behaviors (means ±SE) with and without mechanical ventilation.

		Control (without mechanical ventilation)	**Treatment (mechanical ventilation)**	**Sig.**
Daily weight gain (kg/day)		1.05 ± 0.47	1.13 ± 0.62	NS
Lying behavior	Lying frequency (time/day)	8.25 ± 0.94	6.75 ± 0.77	NS
	Lying period (min./day)	863.5 ± 19.82	781 ± 18.70	0.01
	Lying period (% /day)	59.96%	54.23%	——
Eating behavior	Eating frequency (time/day)	16.25 ± 0.90	10.00 ± 0.65	0.01
	Eating period (min./day)	420.75 ± 5.51	322.25 ± 9.46	0.05
	Eating period (% /day)	29.22%	22.38%	——
Ruminating behavior	Ruminating frequency (time/day)	14.75 ± 1.44	16.25 ± 1.37	NS
	Ruminating period (min./day)	494.25 ± 20.94	513.50 ± 16.91	NS
	Ruminating period (% /day)	34.32%	35.66%	——

These results are in agreement with **Hernandez-Mendo *et al.* (2007)** who showed that cows actually spent more time standing (and thus less time lying down) when kept on pasture. In contrast, **Anderson (2000)** reported that animals in unsuitable facilities increased standing and less lying. The treatment had a highly significant (P≤0.01) effect on eating frequency and a significant (P≤0.05) effect on the eating period. The Calves in the treatment group spent less period and less frequent times in eating (322.25 min/day and 10.00 time/day, resp.) than the control group (420.75 min. /day and 16.25 time/day, resp.). On the other hand, the results of ruminating behavior had an opposite style (Table 2). Despite the treatment significantly affected ruminating behavior neither period nor frequency, calves in the treatment group spent longer and more frequently period in ruminating (513.50 min/day and 16.25 times/day resp.) than the control group (494.25 min/day and 14.75 times/day resp.). **Herskin *et al.* (2004)** studied the effects of acute stressors on the behavior of dairy cows. They noted decreased rumination for all stressors. Also, **Borderas *et al.* (2008)** found many behavioral changes in dairy calves during an infectious illness including reduced rumination, feeding, grooming, and activity. High eating time and a low ruminating period in the control group may indicate anxiety and a feeling of discomfort.

The blood profile of an individual is an important indicator of an individual's welfare **(Aggarwal *et al.,* 2016)**. The results of the CBC and differential leucocyte count are shown in Table 3. The analyses showed that there was not any significant difference between the studied groups. In spite of that, hemoglobin and Red Blood Cells counts slightly increased with mechanical ventilation (11.45 g/dl and 6.58 µl resp.). While Total leukocyte count decreased; this was 7.47 µl in the treatment group versus 7.82 µl in the control group. The results of differential leucocytes count in Table 3 obviously show that lymphocytes and monocytes percentage in the treatment group (46.13 % and 11.02 % resp.) were more than the control group (44.75 % and 10.20 %, resp.). However, neutrophils percentage decreased in the treatment group (42.76 %) than the control group (45.02%). In the blood of calves of the comparative groups, there were no significant differences in blood indices (Table 3). HCT and PLT slightly increased with mechanical ventilation (32.11% and 182.22 µl, resp.) compared to the other groups (30.48% and 178.20 µl, resp). However, the values of MCV, MCH, and MCHC in the control group (49.26 fl, 17.81 pg, and 36.16 g/dl, resp.) were more than the treatment group (49.12 fl, 17.53 pg, and 35.71 g/ dl, resp.).

Table 3: Egyptian buffalo calves' blood profile, CBC, differential leucocytes count, and blood indices (means ±SE) with and without mechanical ventilation.

		Control (without mechanical ventilation)	**Treatment (mechanical ventilation)**	**Sig.**
CBC	Hemoglobin (HGB) g/dl	11.04±0.50	11.45±0.26	Ns
	Red Blood Cells Count (RBC) (µl)	6.23±0.34	6.58±0.28	Ns

	Total leukocyte count (WBCs) (µl)	7.82±0.71	7.47±0.82	Ns
Differential leucocytes count	Lymphocytes (%)	44.75±2.34	46.13±2.84	Ns
	Neutrophils (%)	45.02±2.66	42.76±2.66	Ns
	Monocytes (%)	10.20±0.78	11.02±1.47	Ns
	Eosinophils (%)	0	0	
	Basophils (%)	0	0	
Blood Indices	(hematocrit) HCT (%)	30.48±1.34	32.11±0.74	Ns
	MCV (fl)	49.26±1.36	49.12±1.22	Ns
	MCH (pg)	17.81±0.45	17.53±0.55	Ns
	M.CHC (g/dl)	36.16±0.24	35.71±0.82	Ns
	Platelet (PLT) (µl)	178.20±26.65	182.22±34.86	Ns

The knowledge of hematological/biochemical indices is useful in distinguishing the normal state from the stress state, which can be nutritional, environmental or physical **(Aggarwal *et al.,* 2016)**. The animal's blood profile significantly changed under stress **(Aggarwal *et al.,* 2016; Banerjee 2008; Thankachan 2007)**. However, in this study, the blood profiles of the Egyptian buffalo calves that exposed for continued 60 days of unsuitable ventilation (bad vent., without mechanical vent., 1382.35±5.56 ppm of CO_2) was not significantly different from the other calves under good ventilation (with mechanical vent., 401.87±0.77 ppm of CO_2). These results indicate the ability of Egyptian buffalo calves to be high and rapid to adapt endurance and face stressful factors.

CONCLUSION

It can be concluded that mechanical ventilation tools are efficient in removing CO_2 produced by the animals and emitted from the manure. The use of 8 fans (each with 4 inlets, 4 outlets, 20 cm in diameter, and velocity of 1.5 ms^{-1}) sufficiently affects the ventilation of a pen with the dimensions of 3×4×4 m^3 for length, width, and height, respectively. Even though the Egyptian buffalo calves' behavior was affected by unsuitable ventilation for continued 60 days, their daily weight gain and blood profiles did not significantly differ. These results indicate the ability of Egyptian buffalo calves to be high and rapid to adapt endurance and face stressful factors.

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