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The Effects of Gas Changes in the Shelter in the Summer Period on the Milk yield and Dry Material Consumption of Anatolian Water Buffalo (*Bubalus bubalis*)

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ABSTRACT
In this study, the effects of in-shelter gas concentration on milk yield and total dry matter intake (TDMI) of buffaloes during the spring and summer periods were investigated. The research was carried out in a shelter with 20 main Anatolian buffaloes between March and July. Values for temperature, relative humidity, air velocity, and CH_4, NH_3, and CO_2 gases were recorded in the shelter. Data records were collected continuously for 24 hours for 4 days. Milk yield and DMI of buffaloes were also determined. As it was observed, higher air velocity in the house reduces the methane gas levels ($P<0.01$). The regression equation between milk yield and relative humidity was Y= 6.011-0.03RH and showed a negative and low degree correlation. It was illustrated that TDMI varied between 11.00 ± 0.12-13.20 ± 0.06 kg during the summer. The difference observed between months in terms of feed intake was found to be statistically significant ($P<0.05$). Although the milk yield of water buffalo was low in March, it increased in April and May. However, there was a decrease in DMI (0.50 kg/day) and milk yield (264 mL/day) for an increase of + 1 °C in air temperature. The recorded values for CO_2 concentration in the buffalo shelter during the summer period varied between 620-1120 ppm. Considering the obtained results, NH_3 and CO_2 gas levels in the shelter were below the higher limits and can be considered as not dangerous for animal and human health. It was determined that regression equation between feed intake and temperature was Y= 9.901 + 0.089T**, regression coefficient was R^2= 0.19 and correlation coefficient was r= 0.44 ($P<0.001$).

Keywords: Buffalo, Bubalus bubalis; Gas changes, Summer period; Milk yield; Feed intake

1. Introduction

Gas changes occurring in the shelter and indoor environmental conditions, in general, are important parameters that affect animal health and performance. In recent years, many studies have been conducted on examining the effects of indoor gas density and environmental conditions on feed intake and milk yield (Johnson 1985; Du Preez et al. 1990; Brose et al. 1998; Jungbluth et al. 2001; Bouraoui et al. 2002; Snell et al. 2003; Zhang et al. 2005; Zhao et al. 2007; Zhang et al. 2007 and Bjorneberg et al. 2009). It has been reported that in-house temperature and gas changes affect milk yield in cows. As a matter of fact, at high temperatures, the milk yield was decreased by 10-50% (McDowell et al. 1976; West et al. 2003 and Fournel et al. 2017). Fertility is also negatively affected and the conception rate could be decreased by 20-30% (De Rensis and Scaramuzzi 2003).

According to Steevens & Ricketts (1993), feed intake and milk yield of dairy cattle were significantly decreased at temperatures above 27 °C. Appropriate temperatures for buffaloes are within 10-27 °C (Schein & Hafez 1969). At the same time, the relative humidity in the shelter should be between 55-75% (Bickert 2001). Although buffaloes live in tropical areas, they are very sensitive to changing climatic conditions. They can be easily affected by increased temperature and humidity values (Degirmencioglu et al. 2020).

While conducting studies on in-house gas levels, their effects on human and animal health have been taken into account (Maghirang and Manbeck 1993). As previously indicated, when the ammonia concentration in animal shelters exceeds 50-60 ppm, feed intake and production are decreased (Alagoz et al. 1996). Moreover, when the shelter air is dry and relative humidity falls below 40%, the dust from litter and manure mixtures with the air and causes infections in the respiratory tract of animals (Okuroglu & Delibas 1986). In terms of occupational health and safety, in the country legislation, ammonia exposure limit values are 20 ppm for 8 hours (TWA) exposure, 50 ppm for 15 minutes (STEL) exposure, and carbon dioxide is 5000 ppm (Sainsbury 1981). Bayhan (1996) stated that the carbon dioxide concentration in the barn should not exceed 3300 ppm and the ammonia concentration should not exceed 20 ppm.

The present study aiming at the observation of the effects of changes in the environmental conditions inside the shelter on milk yield and dry matter intake in buffaloes. For this purpose, 24-hour continuous measurements were recorded for 4 days in the summer season at the buffalo shelter. Indoor environmental conditions such as NH_3, CH_4, and CO_2 gas concentrations and temperature, wind, and relative humidity were determined in the buffalo barn.

2. Material and Methods

2.1. Study site

Building materials such as sand, briquettes, and cement are used for the construction of shelter walls and bedding. Eternite roof is widely used for the cover of the roofs. There are 20 cm air inlets between the sidewalls and under the eaves.

The chimney height on the roof is 4 m. The length of the stall is 200 cm, the width of the stall is 116 cm, the height of the bar is 100 cm. Feeder path, width, and depth are also determined as 90 cm, 60 cm, and 40 cm respectively. When the barn size of the enterprise was examined; length, width, and height values were obtained as 20 m, 8 m, and 3 m respectively. Two windows are facing each other on the sidewalls of the shelter. The windows are rectangular and 60 cm high. Fertilizers accumulated in the shelter are transferred to the manure pit with a shovel.

The barn ventilation in the enterprise is provided by natural ventilation. The trial is planned in a semi-open barn system with a capacity of 20 milking buffalo cows. The barn stall design is a two-row structure. The buffaloes are taken to the birth chambers between January and February, and the calves (malaks) are kept in a two-month feeding program after birth. AWB (Anatolian Water Buffalo) (5 and 6 years old) at stage 50-60 days of lactation are randomly selected.

According to the 40-year average values in Bursa City, it is stated that the dominant wind direction is southwest and north direction in the first degree and be the south direction in the second degree In the same report, average wind speeds were obtained as 2.1 and 1.5 m/sec, respectively (Anonymous 2016).

This study was carried out on a buffalo farm in Karaoglan village of Mustafakemalpasa district (40º 05' 17'' N, 28º 30' 53'' E) as shown in Figure 1.

Figure 1- Location of Karaoglan District (Google Earth 2021)

2.2. Nutrition regime and milking of buffaloes

Alfalfa was provided at 6:00 a.m., and silage was offered once a day at 7:00 p.m. The buffaloes in the trial had free access to water and pasture. At the end of the study, the individual feed intake of buffalo cows was determined daily, taking into account the reports of Maynet and Gordon (1984). DMI was determined at the end of the sample collection period by weighing the offered diet and remnants from the previous day. Pasture intake cannot be measured since animals had free access to it. The animals were milked twice a day at 6:00 a.m. and 7:00 p.m. The concentrate feed mixture (CFM) consisted of 33% barley, 34% wheat, 31% sunflower meal, 1% marble powder, 0.75% salt and 0.25% vitamin+mineral mix. During the trial, all buffaloes were provided with corn silage (15 kg day^{-1}), alfalfa hay (6 kg day^{-1}), and 0.70 kg of the CFM (per 1.0 kg of milk per day) (193.9 g CP and 2830 kcal ME kg DM^{-1}) as shown in Table 1. Determination of CP was implemented according to the Association of Official Analytical Chemists (AOAC 1990) and that of fiber fractions (NDF and ADF) according to Van Soest et al. (1991). The metabolizable energy value of the diet was calculated based on chemical analyses using computer software from the National Research Council (NRC 2001).

Table 1- Composition of feed mixture and roughages fed by experimental buffaloes

Ingredient Composition	*Concentrate Feed Mixture*	*Roughages for buffaloes*	
		Alfalfa hay	*Corn silage*
Barley, g kg^{-1}	330		
Wheat, g kg^{-1}	340		
Sunflower meal, g kg^{-1}	310		
Marble powder, g kg^{-1}	10		
Salt, g kg^{-1}	7.5		
Vitamin+minerals[1], g kg^{-1}	2.5		
Total	1000		
Nutrient composition			
DM[2], g kg-1	887.0	894.4	310.3
OM, g kg^{-1}	850.7	803.8	261.1
CP, g kg^{-1}	193.9	146.5	66.2
EE, g kg^{-1}	19.4	15.2	23.2
CELL, g kg^{-1}	114.2	330.4	190.0
CA, g kg^{-1}	36.3	90.6	49.2
NFE, g kg^{-1}	523.2	311.7	18.2
Starch, g kg^{-1}	332.0	20.0	218.1
NDF, g kg^{-1}	220.0	409.9	428.6
ADF, g kg^{-1}	174.0	370.4	307.2
ADL, g kg^{-1}	43.0	90.6	63.2
ME (kcal/kg DM)[3]	2830	1780	696

[1]Trace minerals and vitamins (per kg): 50.000 mg Niacin; 150 mg Co; 800 mg Iyot; 150 mg Se; 50.000 mg Mn; 50.000 mg Fe; Zn 50.000 mg; Cu 10.000 mg; 15.000.000 IU Vitamin A; 3.000.000 IU Vitamin D3;20.000 mg Vitamin E; [2]DM: Dry Matter; OM: Organic Matter; CP: Crude Protein; EE: Ether Extract; CELL: Cellulose; CA: Crude Ash; NFE: Nitrogen Free Extract; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre;[3] ADL: Acid Detergent Lignin; [3]ME: Metabolizable Energy.

2.3. Shelters

Gas measurements were carried out with 3 gas measuring devices in 6 months and a total of 24 days of data were recorded. Temperature, relative humidity, and air velocity were measured with Testo 435 (Testo, Germany), and CH_4, NH_3, and CO_2 gases were measured with MultiRAE Lite multi-gas meter (Wireless Portable Multi-Gas Monitor- RAE Systems by Honeywell, USA) to show indoor conditions and air quality indicators. In this period, to be able to determine the gas condensation in the shelters, 24-hour data records were collected with instruments hung on measurement points in the shelter (Figure 2).

Figure 2- The general condition of measuring devices in the shelter

2.4. Statistical analysis

Variance analysis was used in determining the differences between the averages of in-shelter gas measurements, and the F test, test in determining the significance level of the differences observed between the averages (Turan 1995). Models were developed for the changes in gas concentration and indoor temperature and humidity in the buffalo shelter and multivariate regression analysis was applied. The linear regression method, which is the SPSS (2006) automatic regression determination system was used.

3. Results and Discussion

The daily average and maximum indoor CH_4, NH_3, and CO_2 gas concentrations are shown in Table 2.

As indicated in Table 2, methane concentrations increased from May to August as a result of temperature enhancement in the buffalo shelters. In March and April, no measurement was recorded because CH_4 concentrations were below the measurement limit value of the device. When the gas production results of methane are examined, the highest value was obtained in August with 7.12±0.15%. The other values were 4.88±0.13% for July, 3.72±0.09% for June, and 1.32±0.30% for May. Differences between all means were found to be statistically significant ($P<0.05$). As observed in Table 2, the average ammonia production value in the shelter of buffaloes during the experimental period varied between 11.12±0.42 and 2.40±0.18 (ppm). The highest average ammonia value in the shelter was obtained in August, followed by July, June, May, April, and March. Differences were found to be statistically significant ($P<0.05$). As reported in Table 2, the average CO_2 levels inside the shelter of buffaloes during the experimental period varied between 620±14.14 and 1196±40.61 ppm; CO_2 values were the highest in August and the lowest in March. It was observed that the differences among months in terms of CO_2 concentrations were statistically significant ($P<0.05$). The temperature inside the shelter has increased continuously throughout the experimental period from 15.60±0.08 to 28.15±0.28 (°C), with the highest temperature detected in August and the lowest in March. It was observed that the differences observed among months were statistically significant ($P<0.05$). As presented in Table 2, the humidity values in buffalo shelters varied between 49.08±3.023 and 75.57±0.74 (%). Humidity was the highest in March and the lowest in August ($P<0.05$). Finally, air velocity recorded in the buffalo barn varied between 0.157±0.08 and 0.025±0.03.

Table 2- Gas concentrations measured in the buffalo shelter during the summer period (mean±SE)

Buffalo shelter	*March* $\bar{X} \pm S_{\bar{X}}$	*April* $\bar{X} \pm S_{\bar{X}}$	*May* $\bar{X} \pm S_{\bar{X}}$	*June* $\bar{X} \pm S_{\bar{X}}$	*July* $\bar{X} \pm S_{\bar{X}}$	*August* $\bar{X} \pm S_{\bar{X}}$
CH_4 (%)	0.00	0.00	1.32±0.30^{d}	3.72±0.09^{c}	4.88±0.13^{b}	7.12±0.15^{a}
NH_3 (ppm)	2.40±0.18^{e}	5.84±0.41^{d}	6.32±0.20^{c}	7.28±0.60^{c}	9.04±0.40^{b}	11.12±0.42^{a}
$C0_2$ (ppm)	620±14.14^{c}	632±18.90^{c}	644±13.01^{c}	676±10.45^{c}	920±42.81^{b}	1196±40.61^{a}
T (°C)	15.60±0.08^{d}	20.92±0.49^{c}	24.44±0.35^{b}	25.84±0.28^{b}	27.63±0.18^{a}	28.15±0.28^{a}
V (m/s)	0.157±0.08^{a}	0.133±0.01^{b}	0.137±0,09^{b}	0.047±0.04^{c}	0.060±0.05^{c}	0.025±0.03^{d}
RH (%)	75.92 ±0.74^{a}	67.74 ±2.13^{b}	67.87 ±2.15 ^{b}	64.80 ±2.15^{bc}	59.96±2.78 ^{c}	49.07±3.02 ^{d}
TDMC (kg d^{-1})	11.00±0.12^{d}	11.60±0.10^{c}	12.50±0,09^{b}	13.20±0.06^{a}	12.30±0.14^{b}	11.50±0,15^{c}
The milk yield (kg d^{-1})	4.90±0.07^{c}	5.50±0.97^{b}	6.67±0.027^{a}	6.30±0.06^{a}	6.00±0.07^{b}	5.45±0.09^{bc}

CH_4; average Methane, NH_3; average ammonia, $C0_2$; average Carbon dioxide, T; average temperature, V; air velocity, RH; average humidity, TDMC; average total dry matter consumption, a-b, c-d: ($P<0.05$) Different letters in the same line are significantly different.

It was determined that the gas concentration data obtained in the present study remained at normal values when compared with the literature. As indicated, the highest values for methane concentration were found during the summer period. Bjorneberg et al. (2009) confirm these findings since the CH_4 concentration in the compartments increased in June and September compared to January and March in their experiment. Controversial findings have emerged in studies conducted with ammonia during the summer period, and it has been reported that these discrepancies could be possibly attributed to the environmental conditions inside the shelter. Indeed, the researchers reported ammonia values within the shelter from 5.3 ppm (Jungbluth et al. 2001), 1.4-7 ppm (Zhao et al. 2007 & Zhang et al. 2007) to 8.2 ppm (Snell et al. 2003).

The CO_2 and NH_3 concentrations in the buffalo shelter recorded during the experimental period varied between 620-1200 and 2.40-11.10 ppm, respectively. According to the previous literature, values for CO_2 and NH_3 were within the normal limits (3300-5000 and 20-50 ppm, respectively) (Brose et al. 1998; Jungbluth et al. 2001) and can be considered as not dangerous for animal and human health.

The wide range of gas concentrations could be possibly attributed to the different manure removal systems applied in the shelters, the duration of the manure in the shelter, the different ventilation systems of the shelters, the different rationed proteins given to the animals, and the structural differences of the shelters (Snell et al. 2003; Ndegwa et al. 2008; Zhang et al. 2008; Merino et al. 2008; Angel et al. 2008).

As can be observed in Table 2 and Figure 3, the total dry matter intake (TDMI) of buffaloes increased continuously until mid-summer. It was determined that TDMI varied between 11.00±0.12 and 13.20±0.06 kg during the experimental period. Water buffaloes had the highest feed intaken (13.20 kg) in June due to their lactation stage. However, the feed intake of buffaloes decreased as an effect of the increase in air temperature. It was observed that the differences observed among months in terms of feed intake were statistically significant ($P<0.05$). Although the milk yield of buffalo was low in March (4.90±0.07), increased in April (5.50±0.97). The milk yield of buffalo showed a linear increase and reached the highest level of 6.67±0.027 in May. Afterward, the milk yield of buffaloes was decreased. The temperature increase in July and August further accelerated the decline in milk yield. It was observed that the difference observed among months in terms of milk yield was also statistically significant ($P<0.05$). It can be concluded that this decrease is the result of the increase in ambient temperature, the increase in temperature caused by the breakdown of nutrients in the body, triggering heat stress in the buffaloes and the resulting reduction in feed intake, leading to insufficient ingestion of nutrients for milk synthesis (Degirmencioglu 2020). In addition to this, sudden temperature changes are thought to cause the aforementioned implications, as well as a decrease in the endocrine system function (endocrine glands) (Gantner et al. 2011). The findings obtained from the present research regarding the dry matter intake and milk yield as a result of the in-house gas densities in buffaloes are consistent with the findings of previous researchers (West et al. 2003; Fournel et al. 2017).

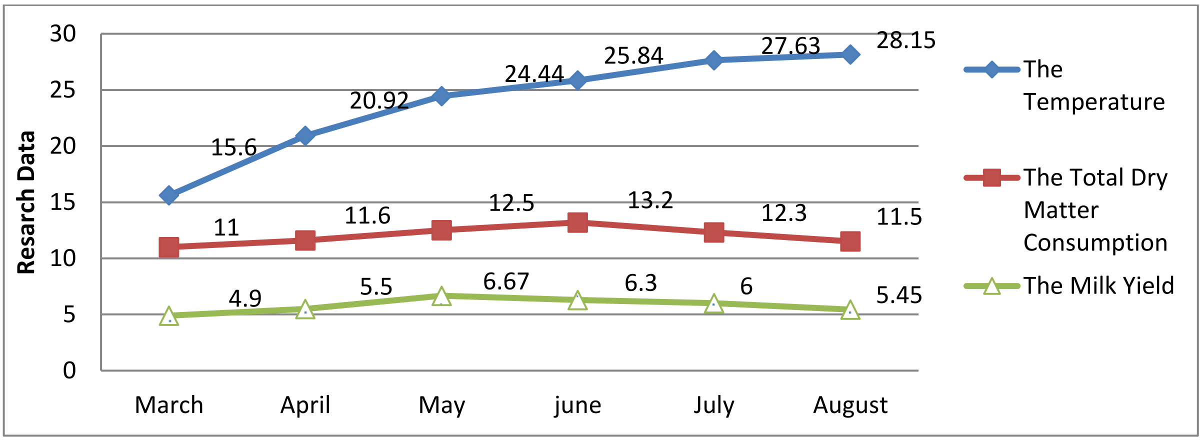

Figure 3- The Effects of Temperature Changes in Shelter on Dry Matter Consumption and Milk Yield

Monitoring the environmental conditions and gas concentration changes in the shelter is important in terms of controlling the health and productivity of the animals. The gas exchange model in the buffalo shelter is shown in Table 3. As indicated, while there is a negative interaction between humidity and indoor temperature in a buffalo shelter, there is a positive interaction between CO_2, CH_4, and NH_3 concentrations and indoor temperature.

Table 3- Gas exchange model in the buffalo shelter

Parameter	*Polluting*	*Regression equation*	*R*	*R^2*	*P*
	RH	*92.470-1.188T**	*-0.40*	*0.16*	*0.00*
	NH_3	1.388+0.399T**-0.49RH*-7.483V	0.76	0.57	0.00
	CH_4	-2.940+0.351T**-0.021RH-12.659 V**	0.86	0.73	0.00
	CO_2	77.744+29.602 T**	0.55	0.29	0.00
The milk yield (kg d^{-1})	T (°C)	4.257 +0.065 T**	0.44	0.19	0.00
	RH	6.011-0.03RH	0.06	0.04	0.44
	T RH	3.60+0.074T**+0.07RH	0.46	0.21	0.00
<25 (°C)		2.074+0.658 TDMC **	0.77	0.59	0.00
25> (°C)		1.724+0.347 TDMC **	-0.54	0.29	0.00
TDMC(kg d^{-1})	T (°C)	9.901+0.089T**	0.44	0.19	0.00

T: Temperature; RH: humidity (%); NH_3; ammonia, CH_4; methane, V: air velocity (m/s) TDMC; total dry matter consumption *P<0.05, **P<0.01

This interaction was statistically significant ($P<0.01$). The fact rise in the temperature increases the NH_3 release supports the findings of Zhang et al. (2005). On the other hand, Kılıc (2011) stated that there is a negative interaction between other gas concentrations other than CO_2 and indoor temperature in dairy cattle barns. In our study, it was determined that there is a negative interaction between CH_4 and NH_3 concentrations and humidity and air velocity in buffalo shelters, with the inverse relationship between NH_3 concentration and humidity being statistically significant ($P<0.05$). The results obtained by Zhang et al. (2008) support that CH_4 and NH_3 emissions can be reduced by properly designed ventilation systems in animal shelters. The regression equation between humidity and temperature was determined as Y= 92.470-1.188T, regression coefficient (R^2) 0.16 and correlation coefficient (r) -0.40 (P<0.001). There is an inverse and moderate relationship between them. The coefficients calculated in this study are below the values obtained by Turkmen (2018) (R^2= 95.36 and r= -0.98). The multiple regression equation between ammonia and temperature, humidity and air velocity in the studied buffalo shelter was Y = 1.388 + 0.399T ** - 0.49RH * -7.483V, R^2= 0.57 and r= 0.57 (P<0.001).

Regression equation between methane and temperature, humidity, and air velocity was Y= -2.940 + 0.351T ** - 0.021RH-. 12.659 V **, R^2= 0.73 and r= 0.86 (P<0.001).

Regression equation between milk yield and temperature in buffaloes was Y= 4.257 +0.065 T **, R^2= 0.19 and r= 0.44 (P<0.001). The coefficients calculated in this relationship are lower than the values calculated by Turkmen (2018) (R^2= 66.2% and r= 0.74). On the other hand, although the temperature rise increased the milk yield, a decrease of 370 mL was detected in milk yield after May. The regression equation between milk yield and relative humidity was Y= 6.011-0.03RH and showed a low degree of interaction.

Even at the highest humidity of 75.92% in March, the continuation of milk and feed intake of buffaloes was due to its harmony with nature and ability to endure hardships.

The multiple regression equation between milk yield and temperature, humidity and air velocity in the studied buffalo shelter is Y= 3.60 + 0.074T ** + 0.07RH, R^2= 0.21 and r= 0.46 (P<0.001). As variables, it had a positive effect with 0.074 coefficient in temperature and 0.07 coefficient in humidity.

As regression equality between milk yield and total dry matter intake at temperatures below 25 °C in buffaloes is Y= 2.074 + 0.658 TDMI, R^2= 0.59 and r= 0.77. They showed a moderate interaction (P<0.001). Regression equation between milk yield in buffaloes and total dry matter consumption at temperatures above 25 °C is Y= 1.724-0.347 TDMI **, R^2= 0.29 and r= -0.54, so they showed a moderate inverse interaction (P<0.001). In the studied buffalo shelter, it was determined that regression equation between feed intake and temperature was Y= 9.901 + 0.089T **, R^2= 0.19 and r= 0.44 (P<0.001). Basically, for an increase of +1 °C in air temperature, a 0.50 kg decrease in dry matter intake and a decrease of 264 ml in milk yield occurred. West et al. (2003) partially support the previous findings stating that the DM intake of cows decreased by 0.85 kg in case of an increase of 1 °C in air temperature. It can be concluded that the water buffaloes are less exposed to heat stress because they enter the pond to cool off and the gas density decreases with the manure cleaning and natural ventilation provided in the shelter, compared to dairy cows. Fournel et al. (2017) support the hypothesis that the decrease in feed intake and milk yield can be confined by reducing the temperature and humidity index of dairy cattle in open animal shelters where airflow is provided by the panel and basket-type fans.

4. Conclusions

Generally, it has been determined that in-barn temperature values reduce the feed intake and milk yield of buffaloes. For this purpose, positive contributions can be made to feed intake and milk yield in buffalo farms by installing fans especially throughout the feeder. It has been observed that in the in-house gas exchanges, the airflow is partially effective in reducing the level of methane, humidity, and ammonia. In hot weather, due to fact that long walks increase thermal stress for buffaloes, freeride areas and shower systems should be included outside the shelter.

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