USING MULTIPLE BEHAVIORAL CRITERIA TO ASSESS BUFFALOES ON-FARM WELFARE

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ABSTRACT: *The present study was carried out at the research unit for animal behaviour, faculty of agriculture, Minufiya University, Egypt. This farm unit could be representing the main production systems in Egypt which used traditionally extensive production systems. The main aim of this research was to using multiple behavioral criteria to assess animal's on-farm welfare. Fifty three Egyptian buffaloes, distributed according to production status and its age (18 dairy buffalo cows, 14 heifers and 21 growing calves) were kept in closed housing system in individual tie-stall barn with hard surface. The experiment lasted one year from august 2014 to July 2015 representing different seasons; during this period buffaloes were video recorded for 72 h. /month with a total observation time of 864 hours for each animal using complete digital behavioural observation unit. Animal rectal temperature, pulse rate, respiration rate and serum cortisol level were estimated monthly after observation period. Results revealed that the suggested behaviour models for Egyptian buffaloes for lying, eating and ruminating bouts were 75.34, 30.90 and 27.04 min. /time resp., Representing 47.04, 28.31 and 30.95% of the day and frequent 8.99, 13.19 and 16.48 times per day with the same previous order. Egyptian buffaloes have 37.71 ^{o}C as rectal temperature, 26.42 (breath/min) for respiration rate, 63.36 (beat/min) for pulse rate and 4.45 [ng/ml] for serum cortisol. All previous physiological parameters were within normal range. There were some factors affected studied criteria such as season and animals status.*

Key words: *Animal welfare, Egyptian buffalo, animal behaviour, physiological parameters, serum cortisol*

INTRODUCTION

Mechanization and the implementation of new technologies (Intensive production) have resulted in major increases in efficiency of production. Consequently cattle are susceptible to a variety of stress factors; rough handling can cause them to become apprehensive of people and to be stressed in their presence, and poor housing conditions can lead to illnesses which may be directly linked to stress. The concept of quality has now broadened greatly to take account of the effects of production systems on human health, animal welfare and the environment.

The welfare of an animal has been defined by Fraser and Broom (1990) as its state at it seeks to cope with its environment. Welfare principally concerns both the physical and psychological wellbeing of an animal, which is largely determined by the standard of stockman ship, the system of husbandry and the suitability of the animal for the environment (FAWC, 2009). At present, some monitoring systems have been developed in Europe for assessing welfare at farm level. These include the animal welfare index TGI35L in Austria (Bartussek, 2001) and the related TGI200 in Germany (Sundrum, 2001). In addition, the development of a monitoring system for assessing buffalo welfare at farm level is needed either to provide a certification system for comparing different husbandry systems or as an advisory/management tool for the farmer. Unfortunately, few studies have been conducted on this issue.

Behavioral changes are the most manifest symptoms of poor welfare Candiani *et al.* (2008). The study and observation of behavior and the animals themselves has become an important tool in identifying situations where their welfare is compromised (Bracke m *et al.* 2001). Therefore, the main aim of this research was to using multiple behavioral criteria to assess animals on-farm welfare.

MATERIALS AND METHODS

The present study was carried out at the research unit for animal behaviour, belonging to the Faculty of Agriculture, Minufiya University, Egypt. This farm unit could be representing the main production systems in Egypt which used traditionally extensive production systems. The milk production level of the dairy buffaloes in this Unit reach an average of 2000 ± 300 kg / lactation period which represents the average of milk yield in Egyptian buffaloes (El kaschab, 1998).

Buffalo Herd and Management

The herd of studied buffaloes consists of a total numbers of 53 animals distributed according to production status and its age as follows: 18 dairy buffalo cows (after weaning calves), 14 heifers (15-20 month old) and 21 growing calves (from weaning up to 10 month of age). Animals were kept in closed housing system in individual tie-stall barn measured 1.2 × 1.7 m. with hard surface. The lighting was adequate to monitoring the animals at the day while industrial dim lighting was used at night. Feeding regime was practiced as follows: during the period from December to May, animals were feed an Egyptian clover (*Trifolium alexandrinum*), concentrate mixture and rice straw, while they fed clover hay, concentrate mixture and rice straw during the rest of the year. Concentrate mixture was offered according to their maintenance and productive requirements according to NRC (1985). Roughage was presented ad-lib twice daily. Water was available ad lib from automatic drinkers. Concentrate mixture was delivered during milking times for dairy animals while other animals feed on concentrate mixture twice daily at 6 am and 6 pm. Machine milking was performed twice daily (at 5 am and 5 pm) using portable, milking machine.

The experiment was conducted during one year from august 2014 to July 2015 representing different seasons (Table 1). During this period buffaloes were video recorded for 72 h. /month with a total observation time of 864 hours for each animal using complete digital behavioural observation unit, which consists of 4 digital observation camera, digital storage unit and control unit. Continuous observation methods were used to record the activity patterns of each animal during observation periods.

Studied Criteria
Daily activities
A. Frequency and total period of lying behaviour: Lie was defined as all legs relaxed with underside in contact with the floor (Weimer, 2012).
B. Frequency and total period of eating behaviour: defined as feeding in mouth, chewing, or head down in the manger close to the feed (Alzahal *et al.*, 2006).
C. Frequency and total period of ruminating behaviour: defined as the time when the animals were not eating and were re-chewing the bolus (Mezzalira *et al.,* 2012).

Physiological parameters:

Ambient temperature and relative humidity were recorded twice daily in animal farm, at observed days, with digital portable thermo-hygrometer. Temperature humidity index (THI) was computed according to the following National Research Council formula [NRC 1971]

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

where:

T_{db} – dry bulb temperature (°C);

RH – relative humidity (%).

Animal rectal temperature was recorded using thermometer. Pulse rate and respiration rate were taken for 1 min. with stopwatch by counting tail artery beats and flank movements. To estimate serum cortisol hormone level, Blood samples were collected from the jugular vein into test tubes and they were kept at room temperature for 30 to 60 min and then centrifuged at 3500 rpm for 10 minutes. After centrifugation, serum was stored in a deep freezer at -20°C till analysis. Cortisol level was estimated using ELISA kit at commercial lab. Rectal temperature, Pulse rate, respiration rate and serum cortisol hormone level were estimated monthly after observation period.

Statistical analysis:

Statistical analyses were conducted to study the effect of season and animal status on studied criteria. Data were analyzed using the general linear model of SPSS (1999).according to the following model:

$$Y_{ijk} = \mu + S_i + T_j + ST_{ij} + e_{ijk}$$

Where:

Y_{ijk} Criteria studied for buffaloes in the ijk subclass;

μ Overall mean;

S_i The fixed effect due to the i_{th} season, i = 1, 2, 3,4, where:
1= spring

2= summer
3= autumn
4= winter

T_j The effect due to the j_{th} animal status, j = 1, 2, 3, where:
1=dairy buffalo,
2= buffalo heifers,
3= buffalo calves;

e_{ijk} Random error.

Means between different groups were tested by Duncan test (Duncan, 1955).

RESULTS AND DISCUSSION

Stable climate condition in different seasons (Ambient temperature and relative humidity) were recorded twice daily in animal farm, at each observed milking as shown by Table (1).

Lying behavior

Means ($\bar{X}$) ± standard errors (SE) of lying behaviour was listed in Table (2). It's clearly appears that studied animals lying 8.99 ±0.39 times as an average per day and spent 677.37 ± 15. 07 minutes in lying position throughout the day representing 47.04 % of each day. This duration almost within normal range as accepted by previous studies (De Rosa *et al.,* 2009, Elaref , 2006 and Omar,1988) who noted that buffalo cows spent 789.12 min, 559.71 min. and 702 min. in lying position respectively. The suggested lying bout model for buffaloes in the present study was 75.34 min. /bout.

Table (1): Stable climate condition in different seasons.

Seasons	Months	Ambient temperature	Relative humidity	THI
Spring	March, April and May	23.70˚c	53.62%	70.35
summer	June, Julie and august	29.83˚c	65.60%	80.19
autumn	September, October and November	24.59˚c	65.39%	72.62
winter	December, January and February	17.80˚c	59.00%	62.51

Table (2): Means ($\bar{X}$) ± standard errors (SE) of lying behaviour.

Studied factors		Observation N	Lying Frequency $\bar{X}$ ±SE (time)	Total Lying period/day $\bar{X}$ ±SE (min.)	Total lying period in (%)
General mean (µ)		1614	8.99 ±0.39	677.37 ± 15. 07	47.04
Season	Spring	414	9.73[ab]±0.53	729.44 [b]± 21.20	50.65
	Summer	387	11.33[b]±0.56	736.61 [b]± 16.30	51.15
	Autumn	390	7.93[a]±0.33	709.44 [b]± 26.29	49.26
	Winter	423	7.00[a]±1.25	534.00 [a]± 49.52	37.08
Sig.			0.01	0.05	
Animals status	Dairy buffaloes	354	8.52[a]±1.08	672.77±21.08	46.72
	Heifers	504	9.32[b]±1.22	681.22± 29.57	47.31
	Calves	756	9.16[b]±0.97	678.10± 18.34	47.09
Sig.			0.05	NS	

a ,b, c means within each column with different superscript differ significantly

NS non-significant, P < 0.01 highly significant and P < 0.05 significant

Factors affecting lying behaviour.

There was some factors affected lying behavior such as season and animals status. Lying frequency was significantly higher (P<0.01) in summer (11.33 ± 0.56) than other seasons. Also total Lying period scored the highest value in summer (736.61 min.) and the lowest in winter (534.00 min.) followed by autumn and spring (709.44 and 729.44 resp.) this depression in winter may be due to bad isolation of concrete hard surface in experiment location. This in agreement with Winckler *et al.* (2003) who noted that in cattle, an inadequate environment may reduce the ease with which animals change position from standing to lying by increasing the risk of falls and collision against cubicle partitions.

Lying frequency significantly affected by the animal's status (P<0.05). Which was the shortest achieved by dairy buffaloes (8.52±1.08 times/day) followed by calves (9.16±0.97 times/day) then heifers (9.32±1.22 times/day). Heifers spent more lying period (681.22± 29.57 min.) than

calves and lactating buffaloes (678.10± 18.34 min. and 672.77±21.08 min. resp.). However differences among three groups were not significant.

Eating behaviour.

Means ($\bar{X}$) ± standard errors (SE) of eating behaviour are listed in Table (3). It's obvious that studied animals spent 407.63 ± 13. 07 min. daily (24 hours) eating, representing 28.31 % of each day, which frequent 13.19±0.59 times in average per day. Eating behaviour affected by many factors which could be illustrated the wide range of eating as founded in previous studied. De Rosa *et al.,* (2009) and Elaref (2006) noted that lactating buffalo cows spent 216 min. and 202.26 min. in eating respectively. While Omar (1988), Thind and gill (1986) and Sharma *et al.,* (1978) mentioned that buffalo spent 381 min, 349 min. and 273 min. in eating position respectively. The suggested eating bout model was 30.90 min. per eating time.

Table (3): Means ($\overline{X}$) ± standard errors (SE) of eating behaviour.

Studied factors		Observation [N]	eating Frequency $\overline{X}$ ±SE (time)	Total eating period/day $\overline{X}$ ±SE (min.)	Eating period in (%)
General mean (μ)		1614	13.19±0.59	407.63 ± 13. 07	28.31
Season	Spring	414	15.36[b] ± 0.43	419.00[b] ± 9.18	29.09
	Summer	387	16.94[b] ± 0.83	404.00[b] ± 19.65	28.05
	Autumn	390	6.15[a] ± 0.77	288.52[a] ± 21.56	20.04
	Winter	423	14.33[b] ± 0.33	519.00[c]± 8.18	36.04
Sig.			0.01	0.01	
Animals status	Dairy buffaloes	354	12.63 ±1.38	422.70[b] ±12.18	29.35
	Heifers	504	13.14±1.22	392.06[a]± 20.47	27.22
	Calves	756	13.82±1.09	408.12[a]± 11.84	28.34
Sig.			NS	0.05	

a ,b, c means within each column with different superscript differ significantly
NS   non-significant, P < 0.01 highly significant and P < 0.05 significant

Factors affecting eating behaviour.

Eating frequency and eating period significantly affected by season. Lowest frequency and duration scored in autumn (6.15 times and 288.52 min. resp.). However the highest duration was in winter (519.00 min.). This trend may be refers to ambient temperature decrease, The best recognized effect of heat stress is an adaptive depression of metabolic rate associated with reduced appetite (Silanikove, 2000), and high feed stuffs quality availability in winter, but only eating period significantly affected by animal status. Lactating animals were the most group scored eating period (422.70 ± 12.18 min.).

Ruminating activities.

Table 4 shows least square means ± standard errors ($\overline{X}$ ±SE) of ruminating behaviour for Egyptian buffaloes. From this table it could be mentioned Egyptian buffaloes spent 445.72 ± 22. 07 min. representing 30.95 % of the day in ruminating and frequency was 16.48 ±1.39 times. Previous studies by De-Rosa *et al.* (2009), Elaref 2006, Omar 1988, Thind and Gill (1986) mentioned that ruminating duration of buffaloes were 388.8, 419.69, 539 and 490 min. per day respectively. Some studies show that the rumination pattern and changes in rumination may be used to evaluate the responses of animals to acute stressors (Schirmann *et al.,* 2011). The suggested ruminating bout model was 27.04 min. per ruminating time.

Table (4): Means ($\overline{X}$) ± standard errors (SE) of ruminating behaviour.

Studied factors		Observation [N]	ruminating Frequency $\overline{X}$ ±SE (time)	Total ruminating period/day $\overline{X}$ ±SE (min.)	Ruminating period in (%)
General mean (μ)		1614	16.48 ±1.39	445.72 ± 22. 07	30.95
Season	Spring	414	16.24 ± 2.04	445.44[b] ± 17.14	30.93
	Summer	387	18.44 ± 0.93	489.32[b]± 28.42	33.98
	Autumn	390	14.26 ± 1.14	345.44[a] ± 17.14	23.99
	Winter	423	17.00 ± 2.00	502.67[b]± 22.55	34.91
Sig.			NS	0.01	
Animals status	Dairy buffaloes	354	15.84 ±0.88	453.24 ±23.11	31.47
	Heifers	504	16.42±1.02	435.35± 31.52	30.23
	Calves	756	17.19±0.97	448.56± 18.14	31.15
Sig.			NS	NS	

a ,b, c means within each column with different superscript differ significantly
NS non-significant, P < 0.01 highly significant and P < 0.05 significant

Factors affecting ruminating behaviour.

Ruminating behaviour did not affected by season or animals status unless ruminating period significantly affected by season, buffaloes scored the lowest frequency and duration in autumn (14.26 times and 345.44 min. respect.) and the highest duration was in winter (502.67 min.).Spring and summer had intermediate values (445.44 and 489.32 min. resp.). This finding was in agreement with that reported by (Collier *et al.* 1982) they noted that heat stress in high-producing lactating dairy cows results in a dramatic reduction in rumination.

Modeling welfare using physiological parameters

Some physiological parameters that could be indicating animal welfare are

illustrated in Table (5). It clearly appears that Egyptian buffaloes have 37.71 ± 0.03 °C as Rectal temperature, 26.42 ± 0.22 (breath/min) for respiration rate, 63.36 ± 0.24 (beat/min) for pulse rate and 4.45 ±0.65 [ng/ml] for serum cortisol. all previous physiological parameters were within normal range. Cohen *et al.* (1997) noted that Serum cortisol concentrations have been used as physiological marker of stress in domestic animals.

Factors affecting studied physiological parameters

There was no significant difference in rectal temperature among seasons. On the other hand respiration and pulse rate significantly elevated (P<0.05) during summer season (28.06 breath/min. and 65.60 beat/min. resp.) against the others. Serum cortisol scored the highest value

during summer (9.13 ng/ml.) and the lowest for spring (3.90 ng/ml.). Autumn and winter have intermediate value 4.35 and 4.43 ng/ml. resp.). The previous results agree with Elvinger *et al.* (1992) they reported that heat stress increased rectal temperature, respiration rates and plasma cortisol concentration, and decreased milk yield of lactating cows. The significant increase in cortisol level in summer heat exposure of buffalo calves may be attributed to the hyper secretion of the adrenal cortex (Nessim, 2010).

Calves Rectal Temperature was the highest (38.03± 0.12 oc) followed by heifers (37.76± 0.23 oc) and dairy buffaloes was the lowest (37.34 ± 0.11 oc) but differences among the groups were not significant.

Respiration rate significantly affected by the animals status (P < 0.05) which was (24.10 ± 0.35, 24.11± 0.73 and 31.06± 0.82 breath/min) for dairy, heifers and calves, respectively. Animals' status did not have any significant effect on pulse rate.

The highest value of serum cortisol achieved by calves (6.07± 0.35 *ng/ml*) followed by heifers (4.06±0.42 *ng/ml*) and finally dairy animals were the lowest (3.23±0.75 *ng/ml*). The differences among groups were highly significant (P < 0.01). on the other hand Habeeb *et al.,* (1996) supposed that with increasing milk yield, the increased concentrations of plasma cortisol are associated with the demand of udder for cortisol for milk synthesis.

Table (5): Means ($\overline{X}$) ± standard errors (SE) of Rectal Temperature, Respiration rate, Pulse rate and serum cortisol.

Studied factors		N	Rectal Temperature (^{o}C)	Respiration rate (breath/min)	Pulse rate (beat/min)	*Serum cortisol [ng/ml]*
General mean (µ)		538	37.71 ± 0.03	26.42 ± 0.22	63.36 ± 0.24	4.45 ± 0.65
Season	Spring	138	37.79± 0.02	26.09± 0.42	61.83± 0.34	3.9± 0.84
	Summer	129	37.83± 0.02	28.06± 0.35	65.60± 0.44	9.13± 1.34
	Autumn	130	37.69± 0.04	25.86± 0.34	63.93± 0.36	4.35± 0.92
	Winter	141	37.55± 0.03	25.66± 0.24	62.08± 1.19	4.43± 0.55
Sig.			NS	0.05	0.05	0.01
Animals status	Dairy buffaloes	118	37.34 ± 0.11	24.10[b] ± 0.35	63.57 ± 0.54	3.23[c] ± 0.75
	Heifers	168	37.76± 0.23	24.11[b]± 0.73	63.29± 1.03	4.06[b]± 0.42
	Calves	252	38.03± 0.12	31.06[a]± 0.82	63.20± 0.66	6.07[a]± 0.35
Sig.			NS	0.05	NS	0.01

a ,b, c means within each column with different superscript differ significantly

NS non-significant, P < 0.01 highly significant and P < 0.05 significant

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استخدام معايير سلوكيه متعدده لتقيم راحة الجاموس داخل المزرعه

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الملخص العربى

أجريت هذه الدراسة في وحدة ابحاث سلوك الحيوان بكلية الزراعة جامعة المنوفية– مصر . هذه المزرعه تمثل نظام الانتاج الاساسي بمصر حيث الانتاج التقليدي الغير مكثف. وقد اجريت التجربة بغرض استخدام معايير سلوكيه متعدده لتقيم راحة الحيوان داخل المزرعه. استخدم في الدراسه 53 جاموسه مصريه موزعه حسب العمر والحاله الانتاجيه (18 حلاب و 14 عجله و 21 عجول ناميه) سكنت داخل حظائر مغلقه بصوره فرديه في مرابط ذات ارضيه صلبه. استمرت التجربه نحو العام من (اغسطس 2014 حتي يوليو 2015م) حيث مثلت جميع فصول السنه. خلال هذه الفتره تمت مراقبة الحيوانات المدروسه 72 ساعه شهريا لكل حيوان بإجمالى عدد ساعات مراقبه 864 ساعه باستخدام وحدة المراقبة السلوكيه الرقميه. في نهاية كل فترة مراقبه شهريه تم تسجيل درجة حرارة ومعدل نبض ومعدل تنفس وتركيز هرمون الكورتيزول في الدم لكل حيوان.

وقد اشارت النتائج ان النموذج المقترح لسلوك الرقاد والاكل والاجترار هو 75,34 و 30,90 و 27,04 دقيقه/مره على التوالي. حيث يمثل سلوك الرقاد والاكل والاجترار 47,04% و 28,31% و 30,95% من اليوم على التوالي بتكرارات يوميه 8,99 و 13,19 و16,48 مره في اليوم . كانت درجة حرارة الجسم ومعدل التنفس ومعدل النبض وتركيز الكورتيزول بالدم 37,71 درجه مئويه و 26,42 نفس/دقيقه و 63,36 نبضه/دقيقه و 4,45 نانوجرام بالملي على التوالي. جميع القياسات الفسيولوجيه السابقه كانت داخل الحدود الطبيعيه. اثر كل من الموسم وحالة الحيوان على بعض الصفات المدروسه.