The effect of rearing system on behavioural and immune responses of buffalo heifers

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ABSTRACT: From November 2005 to October 2006 thirty-two buffalo heifers from the same commercial farm were used to evaluate the effect of rearing system on a range of behavioural and immune variables. Sixteen heifers were group-housed in indoor slatted floor pen (3.0 m^2/animal) with an outdoor paddock (3.0 m^2/animal) at ~5m above sea level (group IR). Sixteen others were kept at ~600m above sea level. They grazed a fenced Mediterranean maquis of ~40ha (group ER). At the end of the experimental period the animals were subjected to a novel object test. Each animal was exposed to a novel environment (a 6x6-m paddock), where in the middle a traffic cone was present. Avoidance distance at manger was evaluated by an assessor who walked slowly (1 step per second) toward each animal with one hand slightly forward until signs of withdrawal. Phytohaemagglutinin (PHA, 1mg/animal) was used to perform a skin test based on specific delayed type hypersensitivity. During the novel object test, IR animals touched more and devoted more time to the traffic cone than ER animals (P =0.003 and P=0.008, respectively). Avoidance distance at manger was lower in ER animals than IR (P=0.004). Skin thickness after PHA injection was higher in ER heifers than in IR (P=0.003). The results indicated that an extensive rearing system based on pasture seems to be a valid method to promote welfare and sustainability of buffalo heifer.

Key words: Buffalo heifers, Behaviour, Immunity, Extensive rearing system, Welfare.

INTRODUCTION - Dairy water buffalo (*Bubalus bubalis*) farming is a traditional Italian enterprise which has been conducted for centuries with extensive rearing systems. Thus, buffaloes still present several morphological features acquired through natural selection which reinforce their ability to thrive well in open environments . For instance, the melanin-pigmented skin of buffaloes is useful for defence against ultraviolet rays. In addition, at least in cattle, animals prefer to graze rather than feed on mixed rations and to spend some time outdoor every day, even in bad weather conditions (Krohn *et al.*, 1992). However, more recently buffalo farming has been subjected to a marked intensification of rearing techniques which has determined a marked reduction of animal welfare (Tripaldi *et al.*, 2004; Zicarelli *et al.*, 2001). Recent European union policy oriented to de-intensify animal production and promote a sustainable development of otherwise marginal Mediterranean areas, have led to renewed interest in extensive rearing systems for species which are well adapted to the environment and, in particular, for categories such as heifers, which can be extensively reared with no negative effects on production. Thus, the present study aims to evaluate the influence of rearing system (extensive *vs.* intensive) on buffalo heifer welfare as assessed by a range of behavioural and immune variables.

MATERIAL AND METHODS - From November 2005 to October 2006 sixteen Italian Mediterranean buffalo heifers (*Bubalus bubalis*), were group-housed in indoor slatted floor pen (3.0 m^2/animal) with an outdoor paddock (3.0 m^2/animal) at Eboli, Salerno province, SW Italy (15°03'E, 40°37'N; ~5m above sea level). They received a complete mixed diet for *ad libitum* consumption (group IR). Sixteen others were kept at Gioi Cilento, Salerno province, SW Italy (15°13'E, 40°17'N; ~600m above sea level). They grazed on a fenced Mediterranean maquis of ~40ha (group ER) and, from December 2005 to March 2006, were supplemented with dehydrated beet-pulps and meadow hay administered in the barn. The experimental animals, aged about 8-9 months at the start of experiment, came from the same commercial farm and were managed at origin under the same farming condition (group-housed in indoor slatted floor pen with an outdoor paddock). Both groups were temporary gathered just prior blood sampling for assays not reported in this study, at 10-day intervals. At the end of the experimental period the animals were subjected to a novel object test. Each animal was exposed to a novel environment (a 6x6-m paddock), where, in the middle, a traffic cone (novel object) was present, and isolated from tactile and visual contact with other animals for 5 min. However, they were able to receive auditory and olfactory stimuli from them. The number of vocalisation, number of times touching the traffic cone, duration of exploration of the traffic cone (animal with head pointed towards the novel objector or sniffing it), latency time to the first touch of the traffic cone, duration of locomotion (walking slowly, looking in front or around) and duration of sustained walking (walking energetically, looking in front or around) were recorded. Avoidance distance at manger was evaluated by an assessor who walked slowly (1 step per second) toward each animal with one hand slightly forward until signs of withdrawal. Phytohaemagglutinin (PHA, 1mg/animal dissolved in 1 ml of sterile saline solution) was used to perform a skin test based on specific delayed type hypersensitivity. PHA was injected intradermally into the middle of two circles stamped on shaved skin in the upper side of each shoulder. The skinfold thickness was determined before PHA injection, and 24 h after with a caliper. For each animal, an average increase was computed using the two measurements gathered from each shoulder. Data were analysed with SPSS statistical package version 3.1 (Norusis, 1989). The heifer was used as experimental unit. Data were analysed with Mann-Whitney U test.

RESULTS AND CONCLUSIONS - Results are displayed in Table 1. During the novel object test, IR animals touched more and devoted more time to the traffic cone than ER animals ($P = 0.003$ and $P = 0.008$, respectively). This result indicated that intensively reared animals, due to the lack of stimuli in their home environment, were more motivated in exploring a novel object, whereas Group ER showed a lower interest for novel stimuli as they were accustomed to a more complex environment. Avoidance distance at manger was lower in ER animals than IR (P=0.004). Measuring animals' reactions to humans enables to gain information about how they perceive human beings (Waiblinger *et al.*, 2006). Such reactions reflect a mixture of different emotions. Fear is likely to be of primary importance, depending on the type of animal and husbandry system, but inferences can also be drawn about its social attachment to humans, the nature of its past experience with people, and the quality of stockmanship. In this study, animals were from the same farm and the sole difference was the free access to pasture (Group ER). It can be hypothesized that for ER animals people were a part of an environment which was positively perceived by the buffalo heifers, thus allowing the establishment of a better human-animal relationship.

Skin thickness after PHA injection was higher in ER heifers than in IR (P=0.003).The skin test can be used to evaluate the effect of different housing systems on animal health and well-being (Napolitano *et al.*, 2004). Accordingly, in a previous study on buffalo calves' a higher spatial density determined reduced hypersensitivity to PHA, thus indicating that space restriction may have detrimental effects cellular immune reactivity (Grasso *et al.*, 1999). We concluded that ER animals were less reactive to novel stimuli and developed a better relationship with humans and a higher immune responsiveness. Therefore, an extensive rearing system based on pasture seems to be a valid method to promote welfare and sustainability of buffalo heifer.

Table 1 Mean (± se) of behavioural and immune variables recorded on buffalo heifers.

	ER	IR	P
Vocalisation, n	15.88 ± 3.84	24.79 ± 5.14	0.162
Touching traffic cone, n	1.56 ± 0.26	4.50 ± 0.86	0.003
Exploring traffic cone, s	13.44 ± 4.75	61.86 ± 14.53	0.008
Latency time to touch the traffic cone, s	60.06 ± 18.99	56.86 ± 21.07	0.950
Locomotion time, s	79.50 ± 11.43	72.57 ± 12.93	0.739
Sustained walking, s	0.06 ± 0.06	0.86 ± 0.44	0.040
Flight distance, m	0.54 ± 0.13	1.36 ± 0.21	0.004
Skin thickness, mm	3.74 ± 0.28	2.38 ± 0.34	0.003

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REFERENCES – **Grasso**, F., Napolitano, F., De Rosa, G., Quarantelli, T., Serpe, L., A. (1999). Effect of pen size on behavioral, endocrine and immune responses of water buffalo (*Bubalus bubalis*) calves. J. Anim. Sci., 77: 2039-2046. **Krohn,** C.C., Munksgaard, L., Jonasen, B., (1992). Behaviour of dairy cows kept in extensive (loose housing/pasture) or intensive (tie stall) environments. I. Experimental procedure, facilities, time budgets-diurnal and seasonal conditions. Appl. Anim. Behav. Sci. 34:37-47. **Napolitano**, F., De Rosa, G., Grasso, F., Pacelli, C., Bordi, A., 2005. Influence of space allowance on the welfare of weaned buffalo

(*Bubalus bubalis*) calves. Livestock Production Science. 86:117-124. **Norusis,** M. J. (1989). SPSS User's Guide: SPSS-PC+ Basal Manual and Advanced Statistics (Version 3.1). SPSS Inc., Chicago, IL. **Tripaldi**, C., De Rosa, G., Grasso, F., Terzano, M.G., Napolitano, F. (2004). Housing system and welfare of buffalo (*Bubalus bubalis*) cows. Anim. Sci., 78: 477-483. **Waiblinger,** S. Boivin, X., Pedersen,V., Tosi, M.V., Janczak, A. M., Visser, E. K., Jones, R. B.,. (2006). Assessing the human-animal relationship in farmed species: A critical review. Appl. Anim. Behav. Sci. 101:185-242. **Zicarelli**, L., Midea, D., Piccolo, G., Di Palo, R., Neglia, G., Campanile, G., 2001. Influenza della presenza di una vasca con acqua e fertilità nella bufala. Proc. 1st Nat. Congr. on buffalo management, Eboli, Italy, 327-331.