Influence of housing conditions and calving distance on blood metabolites in water buffalo cows

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ABSTRACT

The aim of this study was to evaluate whether housing conditions allowing the animals to lie in the mud and perform more physical exercise can negatively affect reserve mobilization and milk production. In addition, the effect of calving distance on blood metabolites was assessed. The experiment was conducted on twenty-eight lactating buffalo cows, equally allocated to two treatments. Fourteen cows were group-housed in a loose open-sided barn with a concrete floor and equipped with self-locking stanchions, where they received 10 m²/head of space allowance, as in intensive systems (Group IS). Fourteen others were group-housed in a similar barn but they also had the benefit of an outdoor yard with 500 m²/head as space allowance, including spontaneous vegetation and potholes for bathing and wallowing, as in traditional systems (Group TS). Animals were included in the experimental groups 5 days after calving. Daily milk yield, and milk fat, protein and somatic cell content were determined 4 weeks after grouping (about 35 days after calving) and then at monthly intervals (5 recordings). Blood samples were collected from the jugular vein in vacuum tubes 10 days after grouping (i.e. 15 days after calving) and then at 10 day intervals (17 collections). After centrifugation, plasma and serum aliquots were frozen stored until metabolite determinations (glucose, cholesterol, triglycerides, NEFA, urea, creatinine, albumins, total proteins, calcium, phosphorus, bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase and γ-glutamyl transferase). Treatment did not affect daily milk yield and milk fat, protein and somatic cell content were determined (glucose, cholesterol, triglycerides, NEFA, urea, creatinine, albumins, total proteins, calcium, phosphorus, bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase and γ-glutamyl transferase). Treatment did not affect daily milk yield and milk fat, protein and somatic cell content. Blood metabolite levels were not affected by treatment and interaction treatment x time. Conversely, as expected, calving distance had an influence on most of these variables (P<0.05÷0.001). In particular, in the first two months after parturition, glucose concentration decreased, whereas NEFA and cholesterol increased as a consequence of the high energetic requirements of animals in the first stage of lactation. The systems that allow the expression of some basic natural behaviour did not have any detrimental effect on buffalo metabolism and production, thus they should be encouraged.

Key words: Buffalo cow, Housing system, Metabolic profile, Milk production

RIASSUNTO

INFLUENZA DEL SISTEMA DI TENUTA E DELLA DISTANZA DAL PARTO SU ALCUNE VARIABILI EMA- TOCHIMICHE NELLA BUFALA

Scopo della ricerca è stato quello di verificare se l'adozione di un sistema di allevamento in grado di soddisfare alcuni dei
Introduction

For centuries and up until a few decades ago, in Italy, dairy water buffalo farming has been conducted with extensive and traditional rearing systems in low-lying swampy areas. Recent intensification of buffalo farming determined the introduction of modern rearing techniques already in use for dairy cattle, such as artificial rearing, machine milking, reduction of pasture utilization, introduction of a second daily milking, etc.

Although water buffaloes are known to possess a high degree of adaptability, the current rearing techniques aimed at maximizing animal performance impose marked environmental stressors on them. Therefore, the reaction of buffaloes to environmental stimuli should be assessed in order to identify technically and economically suitable farming conditions more compatible with animal welfare.

In intensive farming, animals are exposed to an "artificial" environment and, if subjects are not properly managed, numerous environmental factors may become noxious. In particular, space restriction may result in a dramatic reduction of animal welfare due to the inhibition of movement, inability to choose the right social and physical microenvironment (Napolitano et al., 2000), reduction of resting time (Haley et al., 2000) and increase of lesions and injuries (Sandoe et al., 1997). Overcrowding may also cause chronic stress with altered behavioral, endocrine and immune responses, lower growth rates and a higher incidence of disease (Maton and Daelemans, 1989; Fisher et al., 1997). If buffaloes are free to express their natural behavior they often perform wallowing and bathing in the mud (de Francis, 1992). A previous study where different welfare indicators were used demonstrated that buffaloes benefit from potholes and high space allowance (Tripaldi et al., 2004). A number of authors studied the effect of calving distance on metabolic profile (reviewed by Campanile et al., 1998). According to Bertoni et al. (1994), the buffalo metabolic response to lactation shows a different pattern compared to other ruminants, as also demonstrated by the low incidence of metabolic disorders.
It has been stated that the study of blood metabolites can help in understanding the peculiar features of water buffaloes in many different physiological, environmental and management conditions in order to adjust farming and feeding systems to buffalo needs (Bertoni et al., 1994; Montemurro et al., 1997; Campanile et al., 1998).

The aim of the present study was to evaluate whether housing conditions allowing the animals to lie in the mud and perform more physical exercise, can negatively affect reserve mobilization and milk production. In addition, the effect of calving distance on blood metabolites was assessed.

Material and methods

The experiment was conducted from February to July on twenty-eight lactating buffalo cows. Animals aged about 68 months at the start of the study, with a parity of 2.5 and a mean live weight of 667.5 kg, were equally allocated to two treatments. Fourteen cows were group-housed in a loose open-sided barn with a concrete floor and equipped with self-locking stanchions, where they received 10 m$^2$/head of space allowance, as in standard intensive systems (Group IS). The straw bedded resting area and the feeding area were covered by a roof, whereas the exercise area (35% of the total surface of the barn), located between them, was uncovered. Straw was routinely added when necessary. Fourteen others were group-housed in a similar barn, but they also had the benefit of an outdoor yard with 500 m$^2$/head as space allowance, including spontaneous vegetation and potholes for bathing and wallowing, as in traditional systems (Group TS). After parturition experimental animals were separated from calves and allocated to either group where other lactating non-experimental subjects were already present. The two experimental groups were constituted over two-week period. When all the animals had been included, there were 40 buffaloes balanced for parity, milk production and body weight in each group: fourteen experimental and twenty-six non-experimental animals.

Every day at 0800 h subjects were offered unifeed meal for ad libitum consumption. The ingredients constituting the unifeed meal were the following (dry matter basis): maize silage (31.3%), meadow hay (21.5%), lucerne hay (15.9%), barley meal (11.0%), maize meal (11.0%), concentrate (8.3%), and mineral mix (0.92%). Chemical composition of the diet (dry matter basis) was: 0.88 milk FU, 13% crude protein and 19.1 crude fiber. Feed intake was not apparently different between groups. For each group an ample drinking trough was available.

The buffalo cows were scored for body condition (BCS) at the beginning (5 days after calving) and at the end (175 days after calving) of the experimental period (Scale 1-5 units; Edmonson et al., 1989).

Animals were milked twice daily at 05.30 and 17.30 in a herring-bone parlor using pipeline milking machines (Tecnozoo, Zelo Buon Persico, Italy). Daily milk yield, and milk fat, protein and somatic cell content were determined 4 weeks after grouping (about 35 days after calving) and then at monthly intervals (5 recordings). Milk yield was recorded by means of graduated measuring cylinders attached to individual milking units. Subsequently, for each milking, individual samples were withdrawn from cylinders thoroughly mixed and placed in 40-ml plastic containers. Afternoon milk was refrigerated and proportionally mixed to morning milk to obtain only one sample. The latter was stored at 4°C until analysis. Samples were analyzed for fat and protein content (IDF, 1990) using an infrared spectrophotometer (Milko Scan 605; Foss Electric, Hillerød, Denmark) and somatic cell count (IDF, 1995) using a Somacount 300 Bentley Instruments (Chaska, USA).

Blood samples were collected from the jugular vein in vacuum tubes 10 days after grouping (approximately 5 days after calving) and then at 10 day intervals (17 recordings). After centrifugation plasma aliquots were frozen stored until metabolite determinations. Glucose, cholesterol, NEFA (using the kit Wako Chemicals GMBH, Neuss, Germany), triglycerides, urea, creatinine, albumins, total proteins, calcium, phosphorus, bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and γ glutamyl transferase (γGT) were determined on
plasma using a Monarch 1000 model 661 (Chemistry System, International laboratory, Lexington, MA, USA).

Data were analyzed with the Statistical Analysis System package (SAS, 1990). Blood metabolites, BCS and milk data were analyzed with analyses of variance for repeated measures with treatment (IS and TS) as a non repeated factor and time and time x treatment as repeated factors. Square root (NEFA and phosphorous data) and logarithmic (somatic cell count) transformations were used to normalize skewness. Initial and final BCS data were separately analyzed using an ANOVA with one factor (treatment). Where appropriate, the t-test was used to identify differences between least square means.

Results and discussion

Initial (3.17 and 2.86 BCS units, SEM = 0.12, for IS and TS, respectively) and final (3.87 and 3.55; SEM = 0.11, for IS and TS, respectively) BCS did not differ significantly between groups. The higher values of final BCS can be attributed to the recovery of body weight after the peak of lactation.

Treatment did not affect daily milk yield, or milk fat, protein or somatic cell count content. As expected, an effect of time on these variables was detected (Table 1).

Table 2 shows that blood metabolite levels were not affected by treatment and interaction treatment x time. Only plasma LDH tended to be higher in TS than IS (P<0.10). Conversely, as expected, calving distance was able to influence most of these variables (P<0.05÷0.001; Table 3).

Results are mainly discussed in relation to dairy cattle since only few studies are available for water buffaloes.

Housing system did not markedly affect the metabolic status of buffalo cows. Therefore, although the higher levels of physical exercise allowed for group TS determined a slightly higher level of LDH, they did not have any detrimental effect on reserve mobilization. Milk yield and quality were also unaffected by housing conditions. Braghieri et al. (2001) observed a reduced milk production in grazing dairy ewes compared to confined subjects. However, in that study experimental groups were subjected to different feeding regimens, whereas in our study the ration offered to TS and IS was the same.

Although group TS could benefit from pot-holes for wallowing and bathing no significant increase in somatic cell count was observed, thus

Table 1. Milk yield, protein, fat and somatic cell count during the lactation (least square means ± SE) of buffalo cows kept in intensive and traditional rearing systems.

| Rearing system (RS) | Month after parturition (Time) | Significance | SE |
|---------------------|---------------------------------|--------------|----|
|                     | 1 | 2 | 3 | 4 | 5 | RS | Time |
| Milk yield kg/d     |   |   |   |   |   | ns | 0.358 |
| Intensive           | 10.14 | 10.15 | 8.36 | 7.36 | 6.13 |   |   |
| Traditional         | 9.66 | 9.60 | 7.71 | 7.20 | 7.35 |   |   |
| Protein content %   |   |   |   |   |   | ns | 0.082 |
| Intensive           | 4.51 | 4.28 | 4.52 | 4.73 | 4.40 |   |   |
| Traditional         | 4.58 | 4.44 | 4.52 | 4.63 | 4.32 |   |   |
| Fat content %       |   |   |   |   |   | ns | 0.221 |
| Intensive           | 7.88 | 7.73 | 7.89 | 8.28 | 8.10 |   |   |
| Traditional         | 7.29 | 7.13 | 8.29 | 8.26 | 8.22 |   |   |
| Somatic cell count n/ml |   |   |   |   |   | ns | 47,741 |
| Intensive           | 174,900 | 206,750 | 290,333 |   |   |   |   |
| Traditional         | 281,444 | 253,200 | 278,375 |   |   |   |   |

SE: standard error
←: not recorded
ns: not significant
indicating that such housing conditions have no negative effect on this aspect of milk hygiene. The provision of housing conditions closer to the peculiar environmental needs of buffaloes may result in increased reproductive performances (Di Palo et al., 2001; Zicarelli et al., 2001) and higher levels of animal welfare in terms of behavioral activities and endocrine response (Tripaldi et al., 2004).

Blood metabolites were, instead, obviously influenced by calving distance. In particular, in the first two months after parturition glucose concentration decreased (P<0.001), whereas NEFA (P<0.001) and cholesterol (P<0.001) increased as a consequence of the high energetic requirements of animals in the first stage of lactation. It has to be added that the first milk recording started approximately 35 days after calving, when the lactation was likely to be already in the persistency phase, thus the increased metabolic demand for milk production could not be detected. The subsequent downward trend for NEFA and cholesterol and the corresponding steadiness of glucose indicated that buffalo nutritive requirements were satisfied in the following lactation phase. Such results confirm those obtained by Zicarelli et al. (1986) and Campanile et al. (1995). Similar trends can be observed in dairy cows, although in these animals the postpartum energetic deficit is much more evident. The lower lipid mobilization of buffalo cows at the beginning of lactation, indicating a reduced negative energetic balance, can be attributed to the lower milk yield compared to dairy cattle, as also stated by other authors (Bertoni et al., 1993 and 1994; Campanile et al., 1998). Bertoni et al. (1994) stated that dairy cows and buffaloes have a similar metabolic behavior, but the processes arranging for lipidic reserves mobilization, their hepatic metabolism and mammary picking up of milk precursors may be different and further studies are needed also in relation to feeding. These differences should be taken into account when buffalo rationing is formulated, although the general rules applied for dairy cows can be used. Therefore, we suggest verifying the adequacy of buffalo rations by evaluating the animals’

### Table 2. Metabolic variables (least square means ± SE) of buffalo cows kept in intensive (IS) and traditional (TS) systems.

| Treatment | IS    | TS    | SE  |
|-----------|-------|-------|-----|
| Glucose   | 3.48  | 3.44  | 0.05|
| Cholesterol | 2.81  | 2.74  | 0.12|
| Triglycerides | 0.10  | 0.10  | 0.01|
| NEFA      | 0.32  | 0.26  | 0.03|
| Urea      | 7.96  | 7.88  | 0.19|
| Creatinine | 140.20 | 135.07 | 3.13|
| Albumins  | 41.80 | 40.92 | 0.37|
| Total proteins | 86.85 | 86.50 | 1.05|
| Calcium   | 2.49  | 2.50  | 0.02|
| Phosphorus | 1.77  | 1.68  | 0.06|
| Bilirubin | 4.34  | 3.80  | 0.50|
| AST       | 146.84| 164.68| 6.74|
| ALT       | 55.35 | 58.49 | 2.40|
| ALP       | 370.11| 443.12| 54.91|
| LDH       | 1500.41| 1603.17| 50.15 |
| γGT       | 26.95 | 27.43 | 1.62|

^: P < 0.10.
Table 3. Variation of the blood metabolites (least square means ± SE) in relation to calving distance.

| Metabolite     | Units   | 15  | 25  | 35  | 45  | 55  | 65  | 75  | 85  | 95  | 105 | 115 | 125 | 135 | 145 | 155 | 165 | 175 |
|----------------|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glucose        | mmol/l  | 3.79| 3.71| 3.66| 3.58| 3.49| 3.43| 3.33| 3.36| 3.33| 3.33| 3.14| 3.07| 3.06| 3.29| 3.43| 3.23| 0.08***|
| Cholesterol    |         | 2.05| 2.50| 2.76| 2.85| 3.03| 3.01| 3.01| 2.91| 2.91| 2.98| 2.86| 2.80| 2.64| 2.60| 2.72| 2.70| 0.05***|
| Triglycerides  |         | 0.09| 0.11| 0.10| 0.10| 0.11| 0.11| 0.10| 0.10| 0.09| 0.09| 0.10| 0.09| 0.10| 0.10| 0.10| 0.11| 0.01|
| NEFA          |         | 0.27| 0.30| 0.43| 0.34| 0.38| 0.29| 0.43| 0.39| 0.23| 0.26| 0.18| 0.33| 0.25| 0.28| 0.14| 0.16| 0.32| 0.04***|
| Urea          |         | 6.03| 7.18| 7.37| 7.86| 8.66| 8.21| 8.26| 8.31| 8.39| 9.31| 7.95| 8.48| 7.61| 8.09| 7.45| 7.34| 8.17| 0.26***|
| Creatinine    | µmol/l  | 129.13| 120.60| 124.71| 128.18| 128.50| 132.62| 132.92| 136.07| 137.02| 135.25| 135.16| 141.97| 144.09| 147.45| 150.81| 150.01| 160.62| 1.85***|
| Albumin       | g/l     | 37.78| 39.96| 39.68| 39.14| 41.07| 40.77| 41.21| 40.46| 41.29| 41.47| 42.47| 42.61| 41.96| 42.90| 41.71| 43.72| 44.95| 0.47***|
| Total proteins|         | 86.29| 87.64| 88.82| 88.11| 93.36| 90.34| 88.25| 87.32| 86.32| 86.71| 86.69| 85.81| 85.02| 84.88| 82.15| 82.31| 83.41| 1.10***|
| Calcium       | mmol/l  | 2.53| 2.55| 2.55| 2.46| 2.50| 2.45| 2.46| 2.50| 2.45| 2.41| 2.50| 2.46| 2.46| 2.49| 2.50| 2.53| 2.59| 0.02**|
| Phosphorus    |         | 1.64| 1.79| 1.49| 1.70| 1.71| 1.71| 1.65| 1.75| 1.63| 1.67| 1.87| 1.62| 1.79| 1.74| 1.80| 2.02| 1.64| 0.51*|
| Bilirubin     | µmol/l  | 9.28| 6.53| 7.57| 6.90| 6.29| 5.86| 4.09| 4.34| 2.63| 3.16| 2.33| 2.95| 2.23| 2.74| 1.57| 2.63| 2.70| 0.84***|
| AST           | U/l     | 162| 155| 147| 150| 156| 155| 158| 153| 159| 157| 151| 165| 160| 154| 152| 151| 164| 4.03|
| ALT           |         | 46| 52| 51| 57| 58| 60| 64| 58| 60| 59| 58| 60| 59| 59| 55| 55| 57| 1.85***|
| ALP           |         | 344| 383| 385| 394| 410| 427| 410| 411| 426| 432| 425| 411| 406| 415| 444| 402| 389| 12.1***|
| LDH           |         | 1,615| 1,618| 1,581| 1,605| 1,670| 1,666| 1,622| 1,526| 1,611| 1,557| 1,469| 1,560| 1,333| 1,527| 1,469| 1,335| 1,617| 40.2***|
| γGT           |         | 26| 23| 25| 25| 26| 27| 27| 27| 28| 28| 29| 29| 30| 29| 28| 28| 27| 1.09*|

* P < 0.05; ** P < 0.01; *** P < 0.001.
responses in terms of milk quality, milk yield, feed intake, general health status, etc..

As to protein metabolism, urea levels were higher (P<0.001) in the first three months of lactation due to both increased food intake and higher tissue mobilization, as also observed by Bertoni et al. (1994). In dairy cows Cappa et al. (1989) observed that tissue mobilization was correlated with decreased levels of creatinine which indicated a reduction of muscular mass. Total protein slightly increased from parturition to the fifth bleeding, which occurred at about 55 days post-partum, and, subsequently, slightly and continuously decreased (P<0.001), whereas creatinine and albumin increased throughout the experimental period (P<0.001). Similar results for the albumin were found by Aganga et al. (2002) in sheep and goats. Lower levels of this parameter may be indicative of higher protein requirements, which can occur at beginning of lactation.

Due to hormonal control, calcium and phosphorus level changes are generally contained in a rather limited range. Nevertheless, in our study calcium concentration in the blood was lower (P<0.01) when milk production peaked, possibly because a higher amount of the element passed to the milk. In the present study, buffalo cows showed calcium plasma levels similar to those of dairy cows, whereas phosphorous concentration was higher (P<0.05), as also observed by Bertoni et al. (1994).

With regard to enzyme activity, significant effect (P<0.05÷0.001) of calving distance can be attributed to the high variability of these parameters. However, buffaloes showed a higher activity for AST and γGT and a lower level of LDH compared with dairy cattle as reported by Bertoni et al. (1994) and ASPA (1999).

Bilirubin can be considered an indicator of liver function and the downward trend observed in relation to calving distance may be attributed to a higher demand for hepatic metabolism in the first lactation phase compared to the end of lactation. This trend is similar to that observed for the NEFA, thus indicating a general increment of the metabolic demand. A positive correlation between these two parameters was found by Bertoni (1990).

Conclusions

In this study, as expected, calving distance was an important source of variation, demonstrating many points in common with dairy cows. Particular emphasis should be given to the fact that in buffalo cows, even in the first lactation phase, no marked metabolic changes typical of dairy cows can be observed. This peculiarity can represent a possible explanation for the low predisposition of buffaloes to metabolic disorders.

In the present research we found no negative effects of traditional housing system on the BCS, metabolic state, milk production, composition and hygiene of buffalo cows. Therefore, farming conditions which allow the expression of basic natural behaviors should be encouraged whenever possible, as positively affecting buffaloes.

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