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Physiological and productive response of lactating dairy cows to the alpine transhumance at the end of the summer grazing*

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ABSTRACT
The study aimed at verifying if the prolonged walking and fasting of the driving down transhumance from an alpine pasture to the farm of origin at the end of the summer grazing may impair health and productive response of lactating cows belonging to two specialised dairy breeds. The study considered a herd of 380 dairy cows and heifers of Italian Holstein and Italian Brown breeds that faced a driving down transhumance with a vertical drop of about 1050 m. The animals walked for about 40 km completing the journey in 23 h, including three resting periods. Ten Holstein and nine Brown lactating cows were selected and monitored before and after the driving down transhumance taking individual blood samples and recording milk yield. The prolonged physical exercise and the limited water and energy intake during the driving down transhumance impaired cows’ metabolic profile resembling a status of ketosis. A significant drop in milk yield was observed in all the monitored animals regardless of the breed. The production loss was more severe (49.4%) in cows with high milk yield and less days in milk. These animals required also a prolonged time to recover from the milk drop due to the driving down transhumance and, based on these findings, they should be excluded from this practise.

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Animal welfare; blood analyte; dairy cow; milk yield; transhumance

Introduction
In the traditional management system of dairy herds in the Alps, cows and replacement cattle are kept indoors from autumn to spring and moved to the mountain pastures during summer (Corazzin et al. 2010). In recent years, the practise of cattle transhumance walking back from the alpine pastures to their farms of origin at the end of the summer grazing has become the target of touristic attractiveness in many valleys. Transhumance festivals and celebrations that involve popular banquets and folk dancing are organised in early autumn in many villages to welcome the homecoming of cattle herds. Cows and young stocks are adorned with wreaths, colourful ribbons, bells and flowers to celebrate their return to the home farms. It could be assumed that the driving down transhumance events may be stressful for the animals, particularly if the journeys on gravel trails and/or asphalt roads are long (Coulon et al. 1998). Moreover, from an animal welfare perspective, the negative effects of the driving down transhumance might be amplified by the recent evolution of the dairy sector in the mountains. In many alpine regions, autochthonous cattle breeds, well adapted to the alpine environment, have been indeed massively replaced by specialised dairy breeds that have more difficulty to cope with pasture grazing (Battaglini et al. 2014; Knaus 2015; Stergiadis et al. 2015). According to D’Hour et al. (1994), the extreme walking exercise of the transhumance could be particularly detrimental for high producing dairy cattle when managed under extensive systems. Therefore, the rationale behind this study was the hypothesis that high producing dairy cows would suffer more for the fatigue imposed by the driving down transhumance than low producing ones. The study aimed at comparing the effect of an alpine driving down transhumance on the productive and metabolic response of Italian Holstein and Italian Brown lactating cows with different milk yield levels.

Materials and methods

Animals
The study considered a herd of 380 dairy cows and heifers of Italian Holstein and Italian Brown breeds that at the onset of the grazing season were transferred by truck from the farm of origin to an alpine shed located...
in the highland of Asiago (Italy). The herd grazed for about 3 months on a free choice pasture located at an average altitude of 1100 m a.s.l. All the animals were monitored during the driving down transhumance to their farms of origin and a sample of 19 lactating cows was randomly selected for the assessment of metabolic parameters and the recording of milk yields before and after the transhumance. The sample consisted of 10 Holstein (seven primiparous and three multiparous) and nine Brown cows (two primiparous and seven multiparous). The individual milk yield recorded at the day preceding the departure (Day 0) was used to assign these cows to two levels of production according to the median value of the group milk yield (24.0 kg): low if < 24 kg (four Holstein and five Brown) and high if ≥ 24 kg (six Holstein and four Brown).

Location and description of the transhumance

The driving down transhumance began on 6 October 2009 from the alpine shed located at 1062 m a.s.l in Bocchetta Galgi, Conco (latitude 45° 51’ 57.6” N; longitude 11° 36’ 28.8” E) and it ended at the farms of origin (Day 1). The individual milk yield recorded at the day preceding the departure (Day 0) was used to assign these cows to two levels of production according to the median value of the group milk yield (24.0 kg): low if < 24 kg (four Holstein and five Brown) and high if ≥ 24 kg (six Holstein and four Brown).

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Experimental measures

The health status of the entire herd was assessed before the driving down transhumance by the farmer and the farm veterinarian through a visual inspection of all cows and heifers. Animals that showed signs of impaired locomotion were excluded from the transhumance and they were transported by truck to the farm of origin. During the driving down transhumance, the herd was supervised by an animal science student who took note of any particular event and recorded all the animals that withdrew.

The individual milk yield of the selected cows was monitored on Day 0, after the arrival at the farm of origin (Day 1) and on Day 2, 3, 4, 10 and 38 after the driving down transhumance. Individual blood samples were collected from all selected cows during the milking period prior to departure (Time 0) and at the first milking after arrival at the farm of origin (Time 1). Samples were taken from the jugular vein into vacuum tubes containing 150 USP units of lithium heparin (Vacutainer; Becton Dickinson Co., Franklin Lakes, NJ). Blood analytes considered for the analysis were: total protein, albumin, globulin, urea, glucose, non-esterified fatty acids (NEFA), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), creatinine kinase (CK), cholesterol, direct bilirubin, total bilirubin, calcium, phosphorous, magnesium and microhaematocrit. Laboratory analyses were carried out as described by Cozzi et al. (2011).

Statistical analysis

Parity, days in milk (DIM), milk yield and blood analytes of the selected cows recorded before the driving down transhumance were analysed with Student’s t-test to evaluate differences between breeds at Time 0. The effect of the driving down transhumance on productive and physiological traits was studied by the effect of sampling day (time), pre- and post-transhumance. Milk yield data were processed using a mixed model that considered the fixed effects of breed (Holstein versus Brown), productive level (low versus high) and parity (primiparous versus multiparous), DIM as covariate, the repeated effect of sampling day (Day 0, 1, 2, 3, 4, 10 and 38) and the interactions between sampling day and fixed effects. The individual animal was inserted as repeated and random effect. Blood analytes concentrations were processed using a mixed model that considered the fixed effects of sampling time (Time 0 and 1), breed, productive level, and parity, DIM as covariate, the interactions sampling time × breed, sampling time × productive level, and sampling time × parity and animal as repeated random effect. The post-test multiple contrasts technic between estimated least square mean values was used with the Bonferroni adjustment. All the analysis were performed with SAS 9.4 statistical software.
Table 1. Age, parity, days in milk, milk yield and blood analytes concentrations at sampling time 0, before the outset of the driving down transhumance from the alpine pasture to the farm of origin according to breed (Lsmeans ± SEM).

|                    | Italian Holstein | Italian Brown | p value |
|--------------------|-----------------|---------------|---------|
| Cows, n            | 10              | 9             |         |
| Age, months        | 43.9 ± 9.5      | 63.9 ± 19.9   | *       |
| Parity, n          | 1.4 ± 0.1       | 2.4 ± 0.4     | *       |
| Days in milk       | 265 ± 47        | 264 ± 55      | ns      |
| Milk yield, kg/d   | 25.4 ± 2.6      | 21.6 ± 3.1    | ns      |
| Protein and energy markers |
| Total protein, g/l | 76.5 ± 2.7      | 81.5 ± 2.8    | ns      |
| Albumin, g/l       | 34.8 ± 0.8      | 33.1 ± 0.8    | ns      |
| Globulin, g/l      | 41.7 ± 2.8      | 48.4 ± 2.9    | ns      |
| Urea, mmol/l       | 7.20 ± 0.59     | 7.27 ± 0.61   | ns      |
| Glucose, mmol/l    | 3.33 ± 0.11     | 3.03 ± 0.11   | ns      |
| NEFA, mmol/l       | 0.21 ± 0.01     | 0.21 ± 0.01   | ns      |
| Enzymes and hepatic markers |
| AST, U/L           | 103.1 ± 8.6     | 104.3 ± 8.8   | ns      |
| GGT, U/L           | 17.2 ± 2.9      | 25.0 ± 3.0    | ns      |
| CK, U/L            | 188.5 ± 59.7    | 213.9 ± 70.5  | ns      |
| Cholesterol, mmol/l| 4.55 ± 0.53     | 4.47 ± 0.54   | ns      |
| Direct bilirubin, μmol/l | 5.68 ± 0.82 | 3.68 ± 0.82 | ns      |
| Total bilirubin, μmol/l | 11.9 ± 1.1 | 9.84 ± 1.13 | ns      |
| Minerals |
| Calcium, mmol/l    | 2.48 ± 0.04     | 2.53 ± 0.04   | ns      |
| Phosphorus, mmol/l | 2.17 ± 0.11     | 2.08 ± 0.12   | ns      |
| Magnesium, mmol/l  | 1.09 ± 0.05     | 1.20 ± 0.05   | ns      |
| Microhaematocrit, %| 30.5 ± 0.65     | 28.9 ± 0.63   | ns      |

*p<0.05.
ns, not significant.

Results

Descriptive information of the selected sample of cows at the day preceding the driving down transhumance from the alpine pasture to the farm of origin was reported in Table 1. Holstein cows were younger than Brown cows. The cows were in a late phase of lactation, on average over 260 d and there was a relevant variability of the DIM within the breed. There was no significant breed effect on the average milk yield or on the blood analytes concentrations before the beginning of the driving down transhumance.

The two groups of cows classified according to the milk yield level at Day 0 had similar numbers of parities (high 1.9 ± 0.35 versus low 1.9 ± 0.35; p > 0.05) and differed for the average DIM being 203 d for the high-yield group and 332 d for the low-yield group (p < 0.05).

All the animals that took part to the driving down transhumance were in a good health at its start with no evident signs of impaired locomotion. Four Holstein cows that were not part of selected sample of monitored animals withdrew from the transhumance during the first leg of the journey when the herd walked along the steep unpaved mule track, however. These four cows showed signs of severe fatigue or impaired locomotion and they were immediately loaded on the trailer that was following the herd. Direct observations (performed along the whole journey with no systematic methodology) of the herd showed that cows preferred to align along the roadside trying to avoid the asphalt when walking on roads in the second and third leg of the journey.

Milk yield data showed a significant effect of the interaction between sampling day and level of production (Figure 1A). Compared to the milk yield measured at Day 0, the high-yield group of cows showed a significant drop (49.4%) in production at the farm of origin (Day 1) and it required two more days to recover a yield similar to that measured before the driving down transhumance. Conversely, the loss of milk recorded in the low-yield group of cows at Day 1 (32.1%) and in the following sampling days was minor and it never differed from that of Day 0. The trend of milk yield across sampling days was not affected by breed (Figure 1B) and by parity (Figure 1C).

Most blood analytes were affected by the sampling time (Table 2) while they were similar (p > 0.05) between breeds, production levels and numbers of parities. Among protein and energy markers, total protein, albumin, globulin, urea and NEFA concentrations were significantly higher on the arrival at the farm of origin (Time 1) compared to those recorded before the departure (Time 0). On the contrary, glycaemia was lower at Time 1 than at Time 0. Regarding enzymes and hepatic markers, concentrations of AST, GGT, CK and cholesterol increased after the driving down transhumance, while no sampling time differences were observed for direct and total bilirubin concentrations. Considering blood minerals, calcium concentrations varied according to the sampling time × production level interaction (p < 0.05). A more relevant drop of calcium was observed after the driving down transhumance in the high-yield group (2.28 mmol/l at Time 1 versus 2.50 mmol/l at Time 0) than in the low-yield one (2.48 mmol/l at Time 1 versus 2.52 mmol/l at Time 0). After the driving down transhumance, magnesium concentration decreased (p < 0.05) and phosphorous concentration was unvaried. The packed cell volume (microhaematocrit) showed a significant increase (p < 0.001) after the transhumance (Table 2).

Discussion

Only healthy animals took part to the driving down transhumance and all completed it except for four Holstein cows who denied to keep on walking. The good health status of the cows selected for the
monitoring of productive and physiological traits at the outset of the journey was confirmed by the concentrations of blood analytes that fell within the confidence intervals of the reference values for mid-lactating Holstein cows (Cozzi et al. 2011). The rationale behind comparing the two breeds at Time 0 was to assess that there were no differences between them at the outset of the driving down transhumance. Including the breed effect in the further analysis aimed at pointing out possible differences in their suitability to the alpine transhumance since both breeds are specialised for milk production but Italian Brown, being a strain of an ancient alpine breed, could have coped better with the driving down transhumance.

The driving down transhumance resulted in a stressful practise since it affected milk yield and metabolic profile of the monitored cows, regardless of their breed. Most of the blood analytes showed, indeed, significant variations according to the sampling time pre- and post-transhumance and not due to the other fixed effects. The increase of total protein, albumin, globulin and urea concentrations, along with the raise in microhaematocrit, recorded after the transhumance could indicate a certain degree of dehydration of the cows (Russell & Roussel 2007). Despite the planned periods for feeding, drinking and resting, it is likely that cows were not able to fulfil their water requirement during the journey. This could be an explanation also for the impaired milk synthesis. Moreover, the prolonged walking also increased the cow’s energy requirement (National Research Council 2001) and feed intake during the transhumance was probably insufficient to meet this demand. Support to this hypothesis comes from the highest NEFA and the lowest glucose concentrations measured at Time 1 (D’Hour et al. 1994; Block et al. 2001). The partial fasting of the animals induced a blood change similar to that described for the ketosis (Shearer & Van Horn 1992; Andrighetto et al. 1996).

Figure 1. Sampling time interactions with (A) production level: high (—) versus low (—); (B) breed: Holstein (—) versus Brown (—) and (C) parity: primiparous (—) versus multiparous (—) on cows milk yield. Different letters indicate significant differences ($p < 0.05$) for the interaction sampling time × production level.
According to Puppione (1978), blood lipids transport in lactating dairy cows requires a large amount of lipoproteins, which might explain the increased cholesterol concentration at the farm of origin at Time 1 in the current study. It could be assumed that the driving down transhumance induced also a certain degree of liver distress since AST and GGT concentrations were higher at Time 1 while bilirubin concentrations remained similar to those measured at Time 0 (Shearer & Van Horn 1992; Cozzi et al. 2011). The increase of CK concentration might indicate some skeletal muscle impairment caused by the extreme physical exercise experienced by the cows (Hoffmann & Solter 2008). The supervision of the herd during the walking on asphalt roads showed cows aligning along the unpaved roadside as an attempt to improve their walking comfort, although data were not collected with a systematic methodological approach. This might prove that cattle prefer to walk and rest on soft soils rather than on hard ground, asphalt or concrete as observed by Telezhenko et al. (2007), likely due to a more elastic ground surface that allows a uniform load transmission on the claws as suggested by Hinterhofer et al. (2006). In addition, the driving down transhumance likely caused a slight metabolic alkalosis, resulting in the parallel decrease of calcium and magnesium, as suggested by Russell and Roussel (2007). The significant sampling time × production level interaction observed for plasma calcium indicates that this metabolic status was exacerbated in the high-yield cows that probably experienced a more severe negative energy balance during the long walking.

The good practises of the traditional dairy farming in the Alps would suggest the transfer of the herds to the alpine pastures in early June with all lactating cows having seasonal calving in order to start the summer grazing in a late stage of lactation (Cozzi et al. 2009). The recent evolution of the dairy sector in the mountains has upset these guidelines due to the increase of high producing deseasonalised herds in which many cows have high milk yield and low DIM at the end of the pasture season, however. The present study, despite its small sample size, clearly shows that cows facing the driving down transhumance in this productive stage suffer more for the negative energy balance imposed by this practise with detrimental effects either on milk yield (49.4% drop) or on the time required to recover the milk drop due to the transhumance. Support to these results comes from D’Hour et al. (1994) who reported a lowering effect on milk yield and a delayed recovery of milk production in high-producing cows exposed to extreme walking on a mountain pasture. Coulon et al. (1998) reported that the relevant drop in milk yield and the changes in blood analytes provide evidence that high producing dairy breeds are not suitable for the transhumance.

### Conclusions

The alpine driving down transhumance at the end of the pasture season proved to be a stressful experience for specialised dairy breeds. Based on the outcome of the study, despite its small sample size, it could be concluded that high producing cows with low number

### Table 2. Effect of sampling time (ST) and its interactions with the breed (B), production level (PL) and parity (P) on blood analytes of the 19 cows monitored.

| Parameter | 0   | 1   | PSE ST ST × B ST × PL ST × P |
|-----------|-----|-----|-----------------------------|
| **Protein and energy markers** |     |     |                             |
| Total protein, g/l | 77.5 | 82.1 | 1.8 *** | ns | ns | ns |
| Albumin, g/l | 34.1 | 35.9 | 0.6 ** | ns | ns | ns |
| Globulin, g/l | 43.4 | 46.3 | 1.9 ** | ns | ns | ns |
| Urea, mmol/l | 7.31 | 8.80 | 0.53 ** | ns | ns | ns |
| Glucose, mmol/l | 3.16 | 2.67 | 0.11 ** | ns | ns | ns |
| NEFA, mmol/l | 0.21 | 0.81 | 0.06 *** | ns | ns | ns |
| **Enzymes and hepatic markers** |     |     |                             |
| AST, U/L | 104.1 | 116.5 | 6.5 * | ns | ns | ns |
| GGT, U/L | 21.1 | 26.6 | 2.4 * | ns | ns | ns |
| CK, U/L | 335.2 | 373.0 | 70.5 * | ns | ns | ns |
| Cholesterol, mmol/l | 4.47 | 5.14 | 0.45 ** | ns | ns | ns |
| Direct bilirubin, μmol/l | 4.68 | 3.56 | 0.45 ns | ns | ns | ns |
| Total bilirubin, μmol/l | 10.9 | 11.0 | 0.7 ns | ns | ns | ns |
| **Minerals** |     |     |                             |
| Calcium, mmol/l | 2.51 | 2.38 | 0.04 * | ns | * ns | ns |
| Phosphorus, mmol/l | 2.18 | 2.18 | 0.09 ns | ns | ns | ns |
| Magnesium, mmol/l | 1.15 | 1.01 | 0.04 * | ns | ns | ns |
| Microhaematocrit, % | 29.5 | 33.7 | 0.62 *** | ns | ns | ns |

***p<0.001; **p<0.01; *p<0.05. ns, not significant; PSE, pooled standard error.
of DIM at the end of the pasture season should be excluded from the driving down transhumance since their high milk yield and energy expenditure for locomotion could not be counterbalanced by adequate water and feed intake. Less productive cows, managed according to a seasonal calving scheme, are likely to be more suitable to the driving down transhumance than the high producing ones.

 Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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