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The effect of water-restriction on various physiological variables in intensively reared Lacaune ewes

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ABSTRACT: The effects of water restriction on rectal temperature, respiratory rate and selected blood variables in intensively reared Lacaune ewes were evaluated. The tests were carried out over the course of 28 days in spring on 30 lactating sheep divided into three groups of 10 animals each, matched by lactation status and body weight and allocated into individual boxes. The animal groups were as follows: control group W100 with drinking water for the whole day (24 h/24 h), while the two experimental groups each received 80% (W80 group) and 60% (W60 group) of the water ration of the control group, respectively. The feed, in according with physiological and production needs of the animals, was administered in three daily meals (08:00, 14:00 and 20:00 h). Water intake was recorded three times per day (at 08:00, 14:00 and 20:00 h) and the daily feed intake at 08:00 h, while the rectal temperature, respiratory rate were measured and blood samplings were carried out on Day 0, Day 14 and Day 28. In water-restriction groups (W80 and W60) a lower ($P < 0.01$) feed intake of meadow hay, and a lower ingestion of alfalfa hay pellet in the W60 group compared to W100 group were observed. In addition, the water restriction regimen resulted in a significant decrease of respiratory rate ($P < 0.05$) and an increase in serum total protein, urea, creatinine, sodium, chlorine, reactive oxygen metabolites, cortisol, haemoglobin and mean corpuscular haemoglobin concentration ($P < 0.05$), red blood cell counts, and haematocrit ($P < 0.01$). These results show the important role of water, as limiting factor for animal breeding in low-water availability environments. Where possible, the management of low-dairy breeds should be well adapted to support the productive performance.

Keywords: *Ovis aries*; water stress; blood metabolites; adaptation

Drinking water is an important requirement for livestock and the lack of a sufficient source of water can be a critically limiting factor in animal physiology and productivity (Alamer 2010). In fact, low water intake leads to a lower intake of feed in various animal species, with considerable differences existing in the ability of water use among species in relation to the geographical areas of origin (Shoeman and Visser 1995). Animals living in deserts and arid regions have acquired various adaptation mechanisms and are able to maintain a high water economy by efficiently using their bodily reserves; therefore, the production performance of such animals are maintained within an acceptable range during periods of water scarcity (Silanikove 1994). Compared to other species, ruminants are more resistant (for instance, compared to the rat), to the effects of dehydration, because of a lower energy deficiency as a result of the reduced intake of dry matter. This is due both to an improvement of the digestibility of the feed during the period of water shortage (Silanikove 1985; Brosh et al. 1987) and a decreased metabolic rate during dehydration (Brosh et al. 1986). Moreover, in contrast to monogastric animals, the rumen in ruminants acts as a water reservoir (15% of animal body weight) that can be used during periods of water scarcity (Silanikove 2000). Compared to most other mammals, where water loss exceeding 15% body mass may be lethal (Al-Ramamneh et al. 2012), ruminants such as cattle, sheep, camels and Bedouin goats are able to tolerate losses exceeding 18, 20, 25 and 40% of their body masses, respectively (Al-Ramamneh et al. 2012). Water, as a necessary substance for the maintenance of body heat balance, is the primary solvent of intra- and

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extracellular bodily fluids; it represents 90% of the blood plasma and is essential to maintain a high blood flow in lactating animals (Carter and Grovum 1990; Holter and Urban 1992; Andrew et al. 1995). Water scarcity can also have a negative impact on the endocrine and metabolic balance of animal, reducing adaptive capacity. Choshniak et al. (1995) observed a reduction in metabolic activity by about 20% and an increase of respiratory rate of 40–60% in dehydrated Bedouin goats, compared to sheep with free access to water. Water restriction also affects other physiological variables; we previously reported an increase in some blood metabolites in Comisana sheep (Casamassima et al. 2008). During the summer dehydration almost always results in a reduction in plasma volume caused by a recall of water from tissues (Schaefer et al. 1990). Although several authors (Laden et al. 1987; Dahlborn et al. 1988; Olsson and Dahlborn 1989; Abdelatif and Ahmed 1994; Ghanem 2005) agree that a reduction of corpuscular volume concentration and haemoglobin serve as good indicators of dehydration in plasma, other authors do not report these findings; in fact, the haematocrit concentration was higher in Awassi and Merinos sheep in conditions of water stress (Laden et al. 1987; Ghanem 2005; Ghanem et al. 2008). Moreover, under the same conditions other authors have failed to detect any significant variations in this as well as other breeds (Aganga et al. 1989; Igbokwe 1993; Li et al. 2000). These conflicting results may be dependent on the ability of the sheep to maintain their plasma volume and distribute bodily water when already adapted to water scarcity (Ashour and Benlemlih 2000). In our previous study (Casamassima et al. 2008) carried out on water-restricted Comisana sheep, we did not observe any effect of experimental treatment on the quantity or quality milk production and feed intake, while our analysis revealed a progressive and significant increase of some blood metabolites like cholesterol, triglycerides, total proteins, albumin, sodium, creatinine, urea and potassium. The ovine breeds differ in their respective abilities to respond to water restriction, and experiments on different breeds have yielded conflicting results. Yankasa sheep survive five days without drinking but undergo severe physiological changes (Aganga et al. 1989; Igbokwe 1993); on the other hand, Awassi sheep can withstand more than a month without significant physiological changes while drinking every two days, while watering every five days results in important alterations (Jaber et al. 2004). Australian Merino sheep survive 10 days without water (MacFarlane 1964) and the two-horn desert sheep can survive for up to 15 days (Farid et al. 1979; Turner 1979); however, Egyptian Barki sheep do not survive for more than three days without water (Farid et al. 1979). On the basis of existing reports in the literature, this study was aimed at investigating selected physiological variables in intensively-reared Lacaune sheep in response to different levels of water restriction. This could lead to better assessment of the relationship between water availability, environmental temperature and the welfare of animals, and might be useful in preventing the deleterious effects of water stress, especially in warm-arid regions where water is often a limiting factor in livestock breeding.

MATERIAL AND METHODS

Animal and diet. Ewes were healthy and their condition was judged as good at the beginning of the experiment. All experimental procedures were performed in accordance with European Community Guidelines (86/609/CEE) regarding the protection of animals used for scientific purposes.

The trial lasted 28 days and was carried out during the spring season on 30 lactating Lacaune sheep, divided into three homogeneous groups of 10 animals each, by stage of lactation (63 ± 14 days), body weight (54 ± 5 kg) and body condition score (2.10 ± 0.8). Animals were allocated into individual boxes of the same size (1.5 m^2/animal).

In order to promote the animal's adaptation to the experimental conditions without any water restriction, a 7-day pre-experimental period followed the final formation of the groups. The feed, according to the physiological and production needs of animals, consisted of 1 kg of meadow hay, 400 g of alfalfa hay pellet and 700 g of concentrated feed, divided into three daily meals, at 08:00 h (200 g of concentrated feed, 200 g of alfalfa hay pellet, 500 g of meadow hay), at 14:00 h (200 g of concentrated feed and 200 g of alfalfa hay pellet) and at 20:00 h (300 g of concentrated feed and 500 g of meadow hay).

The chemical composition of feeds and diet, according to the Association of Official Analytical Chemists (AOAC 2006), is reported in Table 1.

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Table 1. Chemical composition of feeds and diet

Specification	Feeds			Diet
	concentrate	alfalfa hay pellet	meadow hay	
DM (%/FM)	87.45	88.52	86.47	87.19
Crude protein (%/DM)	19.20	18.11	10.12	14.61
Ether extract (%/DM)	58.06	38.69	37.99	44.81
Fat (%/DM)	3.25	3.10	2.65	2.94
Crude fiber (%/DM)	11.47	28.00	40.44	28.41
NDF (%/DM)	13.79	48.56	65.61	45.09
ADF (%/DM)	5.82	36.51	48.74	39.10
Lignin (%/DM)	3.82	9.49	14.97	10.21
Ash (%/DM)	8.20	12.10	8.80	9.23

ADF = acid detergent fibre, DM = dry matter, FM = fresh matter, NDF = neutral detergent fibre

The experimental protocol. In control group W100, sheep received water daily *ad libitum* at three different times of the day, at 08:00, 14:00 and 20:00 h in order to determine the quantity of water for experimental groups, water intake was recorded at every administration.

In experimental group W80, sheep received drinking water daily at a level of 80% of the intake recorded in the W100 group, at three set times; this was achieved by restricting water intake by 20% compared to the intake of the control group.

In experimental group W60, sheep received drinking water daily at a level of 60% of the intake recorded in the W100 group, at three set times; this was achieved by restricting water intake by 40% compared to the intake of the control group.

Feed and water containers were placed outside and in front of each box to eliminate any leaks.

Experimental measurements. During the trial, the temperature and the ambient relative humidity was continuously recorded through the use of a thermograph positioned at the same level of the animal. The collected data were used to calculate the temperature humidity index (THI) to characterise the climatic and environmental conditions which the animals were exposed to. For the calculation of the THI the following formula of from the National Council of Research (NCR 1971) was used:

$$THI\ (°C) = (1.8 \times Ta + 32) - (0.55 \times 0.55 - RH/100) \times [(1.8 \times Ta + 32) - 58]$$

where:

Ta = ambient temperature inside the farm (°C)

RH = relative humidity (%)

In addition, we carried out rectal temperature recording with a mercury thermometer at 06:00 h and determined respiratory rate by observing the movement of the body-side per minute, every 14 days (Day 0, Day 14 and Day 28). Individual water intake was recorded three times daily at 08:00, 14:00 and 20:00 h. Individual feed intake was recorded daily.

Blood samples were taken from fasting animals at 07:00 h, immediately after the milking of the sheep, at the start of the trial (Day 0), in the middle (Day 14) and at the end of the test (Day 28). The blood was taken using a vacutainer system (Venoject, Terumo Europe N.V., Louvain, Belgium) from the external jugular vein into two tubes, the first with gel separator for the isolation of serum and the second with EDTA for blood count. The blood was centrifuged for 15 min at 1800 *g* and from serum we determined the following parameters: glucose, total cholesterol and high density lipoprotein (HDL) cholesterol, triglycerides, non-esterified fatty acids (NEFA), total protein, albumin, urea, creatinine, calcium and chlorine using a semi-automated Analyser for clinical chemistry (ARCO Biotech Instruments, SpA, Italy); sodium and potassium were determined using a SEAC flame photometer (Radim model Company, Italy). The concentration of ROMs was read spectrophotometrically using the colorimetric method proposed by Diacron (Diacron International, Italy) at a wavelength of 505 nm, using a specific commercial kit (Cesarone et al. 1999). The results were expressed in U Carr (1 Carratelli Unit corresponds to 0.024 mmol/l H_2O_2). The blood concentration of cortisol was measured with a specific kit (Radim,

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Cortisolo EIA Well KS18EW, Italy) using a photometric reader for immunometry on microplates (DV 990B/V4, Italy) at a wavelength of 450 nm with a sensitivity of 0.8 ng/ml of plasma.

Whole blood was tested for haematological parameters, including red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT) and mean corpuscular haemoglobin concentration (MCHC) using the SEAC automatic blood cell counter (Radim Company, Italy).

Statistical analysis. After evaluation of normal frequency distributions, all variables were subjected to statistical analysis using the GLM procedure for repeated measures from the statistical package SPSS 19.0 (SPSS Inc., Chicago, IL). The fixed effect of water treatment (W100, W80, W60), the day of sampling and their interaction were included in the model. The relationships between blood parameters were assessed using Pearson correlation coefficients. The individual ewe formed the experimental unit. The data were presented as mean values of each group and the differences were considered significant at $P < 0.05$.

RESULTS

Feed and water intake

During the experiment, the mean daily water intake in the *ad libitum* W100 group was 4.93 l (Table 2). As expected, no residual water was detected in either W80 or W60 groups that consumed in the period 0–28 days, respectively, 3.94 l and 2.96 l. The lowest water intake found in the experimental groups was observed in both the first (0–14 days) and the second experimental period (14–28 days), and resulted, from 0 to 28 days, in a progressive and significant ($P < 0.01$) reduced intake of meadow hay (911.1 g W100 vs 764.8 g W80 vs 583.9 g W60) and alfalfa hay pellets, between the W100 (400.0 g) and W60 (329.5 g) groups. No effect on concentrate intake due to water restriction was found; all groups consumed it in its entirety over the course of the whole trial.

Rectal temperature and respiratory rate

The water restriction carried out on experimental groups, under rearing temperatures ranging between 16.6 °C and 25.8 °C and relative humidity of 71.3%, did not influenced the rectal temperature (Table 3).

Blood variables

The water restriction treatment did not influence blood glucose, total cholesterol, HDL cholesterol, NEFA, albumin and calcium; therefore, these values are not reported in the table.

Water restriction significantly influenced the serum concentration of total protein, urea, creatinine, sodium, chlorine, ROMs and cortisol (Table 4).

Total proteins showed a progressive increase from the first (Day 0) to the third sampling (Day 28), reaching statistical significance only at Day 28 ($P < 0.05$), between the W100 group and the experimental W60 group (72.7 vs 82.0 g/l). Also, a time-dependent effect resulted in a significant increase ($P < 0.05$) in both experimental groups, culminating in an increase of values from Day 0 to Day 28 of 10.1% and 13.3%, respectively, in the W60 and W80 groups; no changes were recorded in the W100 group over the same time period.

Urea content markedly increased ($P < 0.01$) in the W60 compared to the W100 group (16.2%) and compared to the W80 group (9.7%). Also, the duration of the experiment influenced ($P < 0.01$) the blood concentration of urea, with an increase of values of 13.3% and 21.4%, respectively, in the W60 and W80 groups, while no significant differences in the W100 group were observed.

Serum creatinine concentrations exhibited significantly increased ($P < 0.05$) values due to the water restriction effect, to an extent of 15% in both experimental groups compared to the W100 group. A time-dependent effect of the experimental treatment was observed, from Day 0 to Day 28, which resulted in an average increase of 14% for creatinine ($P < 0.05$) in the W60 and W80 groups, while values remained unchanged in the W100 group.

The serum concentrations of total proteins, creatinine and urea also revealed a significant interaction of the experimental treatment with the duration of the test; in particular, a positive correlation between water restriction and duration of the trial was observed. A positive correlation ($P < 0.001$) was also found when analysing the three considered blood parameters, probably due to both the increasing values in the experimental groups and the reduction in blood volume.

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Table 2. Individual water and feed intakes of control and water-restricted Lacaune ewes

Specification	Groups			SEM	Effect		
	control	experimental					
	W100	W80	W60		G	T	G × T
Water intake (l/day)					**	ns	**
Day 0–14	4.839[1]	3.865[2]	2.901[3]	0.158			
Day 14–28	5.018[1]	4.014[2]	3.012[3]	0.167			
Day 0–28	4.928[1]	3.940[2]	2.957[3]	0.162			
Feed intake (g/day)							
Meadow hay					**	**	*
Day 0–14	888.4[1]	755.4[2]	559.3[3a]	9.57			
Day 14–28	933.8[1]	774.3[2]	608.5[3b]	23.12			
Day 0–28	911.1[1]	764.8[2]	583.9[3]	27.63			
Alfalfa hay pellet					**	ns	*
Day 0–14	400.0[1]	395.9	328.2[2]	12.24			
Day 14–28	400.0[1]	392.4	330.7[2]	13.43			
Day 0–28	400.0[1]	394.2	329.5[2]	12.73			
Concentrate							
Day 0–28	700.0	700.0	700.0	–	ns	ns	ns

G = water restriction effect, G × T = interaction of water restriction × time, T = time effect

[1,2,3]Values within a row with different superscripts differ significantly

[a,b]Values within a column with different superscripts differ significantly

*$P < 0.05$, **$P < 0.01$

Table 3. Rectal temperatures and respiratory rates of control and water-restricted Lacaune ewes

Specification	Group			SEM	Effect		
	control	experimental					
	W100	W80	W60		G	T	G × T
Rectal temperature (°C)					ns	ns	ns
Day 0	38.55	38.63	38.41	0.052			
Day 14	38.78	39.01	39.06	0.063			
Day 28	38.85	38.88	38.94	0.058			
Respiratory rate (*n*/min)					*	**	*
Day 0	26.60	29.20[a]	25.20[a]	0.778			
Day 14	24.40[1]	21.90[2b]	18.60[3b]	0.947			
Day 28	26.20[1]	22.60[2b]	20.20[3b]	1.076			

G = water restriction effect, G × T = interaction of water restriction × time, ns = not significant, T = time effect

[1,2,3]Values within a row with different superscripts differ significantly

[a,b]Values within a column with different superscripts differ significantly

*$P < 0.05$, **$P < 0.01$

Water restriction resulted in a progressive increase in serum concentrations of sodium and chlorine in the W60 group, with values that statistically significant increases ($P < 0.05$) of 9.7% and 8.8% respectively, compared to the W100 group. Also, there was a significant time-dependent increase ($P < 0.05$) in sodium and chlorine values in the W60 group, while no statistical differences were found in the W100 and W80 groups.

Blood ROM levels were significantly influenced ($P < 0.01$) by water restriction, and analysis revealed a progressive increase of the values in both experi-

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Table 4. Blood parameters of control and water-restricted Lacaune ewes

Specification	Group			SEM	Effect		
	control	experimental					
	W100	W80	W60		G	T	G × T
Total protein (g/l)					*	*	*
Day 0	72.9	72.5[a]	72.4[a]	0.443			
Day 14	72.7	78.3	79.0	0.865			
Day 28	72.7[1]	79.8[b]	82.0[2b]	0.849			
Urea (mmol/l)			**	**	**		
Day 0	16.6	16.7[a]	17.0[a]	0.170			
Day 14	16.8[1]	17.4[1]	19.2[2b]	0.287			
Day 28	17.8[1]	18.9[1b]	20.7[2c]	0.331			
Creatinine (mmol/l)					*	*	*
Day 0	90.4	91.9[a]	93.7[a]	1.265			
Day 14	90.7	97.8	95.5	1.248			
Day 28	91.8[1]	104.8[2b]	105.7[2b]	1.924			
Sodium (mmol/l)			*	*	*		
Day 0	149.60	150.50	150.70[a]	0.786			
Day 14	150.40[1]	154.20	159.60[2b]	1.124			
Day 28	150.90[1]	154.50	165.50[2b]	0.661			
Chlorine (mmol/l)					*	*	*
Day 0	89.31	89.47	90.44[a]	0.363			
Day 14	87.94	88.90	90.07[a]	0.262			
Day 28	90.67[1]	94.16	98.61[2b]	0.433			
ROMs (IU/Carr)					**	**	**
Day 0	87.21	92.86[a]	89.06[a]	3.678			
Day 14	92.83[1]	102.45[2b]	113.43[3b]	4.953			
Day 28	89.52[1]	140.41[2c]	155.72[3c]	5.633			
Cortisol (mmol/l)					**	**	**
Day 0	47.94[a]	48.94[a]	48.74[a]	2.321			
Day 14	50.30[1]	63.31[3b]	56.36[2b]	3.475			
Day 28	52.18[1b]	73.08[2c]	93.13[3c]	3.298			

G = water restriction effect, G × T = interaction of water restriction × time, T = time effect

[1,2,3]Values within a row with different superscripts differ significantly

[a,b,c]Values within a column with different superscripts differ significantly

*$P < 0.05$, **$P < 0.01$

mental groups; by 56.8% (W80 group) and 73.9% (W60 group) compared to the control group. Also, the duration of the experiment significantly influenced ($P < 0.01$) this parameter, which increased its plasma concentration in both experimental groups.

Plasma concentrations of cortisol were also affected by water restriction ($P < 0.01$), leading to a progressive increase in values during the trial of 40.1% in the W80 group and 78.5% in the W60 group compared to the W100 group. The time-dependent effect of water restriction in both experimental groups was also evident in an increase ($P < 0.01$) of cortisol, from the first to the third sampling, of 49.3% in the W80 group and 91.1% in the W60 group; the W100 group exhibited an increase of 8.8%.

Examination of blood counts (Table 5) shows that the number of RBC exhibited progressive and significantly increased values ($P < 0.01$) in response to water restriction; values rose by 12.9% and 27.9%, respectively, in the W80 and W60 groups compared to the W100 groups; whereas a difference of 13.3% was observed between the two experimental groups. The duration of water restriction

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Table 5. Haematological parameters of control and water-restricted Lacaune ewes

Specification	Group			SEM	Effect		
	control	experimental					
	W100	W80	W60		G	T	G × T
RBC (10^3/mm^3)					**	**	*
Day 0	8.63	8.57[a]	8.60[a]	0.184			
Day 14	8.69[1]	9.23[1]	11.17[2b]	0.240			
Day 28	8.71[1]	9.83[2b]	11.14[3b]	0.212			
Hb (g/l)					*	*	*
Day 0	99.1	100.5	100.4[a]	1.717			
Day 14	100.6[1]	106.0	116.5[2b]	2.544			
Day 28	100.8[1]	106.5	119.3[2b]	2.299			
HCT (proportion of 1)					**	**	*
Day 0	0.23	0.23[a]	0.24[a]	0.005			
Day 14	0.24[1]	0.25	0.28[2]	0.006			
Day 28	0.24[1]	0.29[2b]	0.30[2b]	0.007			
MCHC (g/l)					*	*	ns
Day 0	378.4	379.3[a]	377.1[a]	2.298			
Day 14	385.7	394.8	388.4	2.648			
Day 28	387.8[1]	420.8[2b]	421.4[2b]	5.992			

G = water restriction effect, G × T = interaction of water restriction × time, Hb = haemoglobin, HCT = haematocrit, MCHC = mean corpuscular haemoglobin concentration, RBC = red blood cell, T = time effect

[1,2]Values within a row with different superscripts differ significantly

[a,b]Values within a column with different superscripts differ significantly

*$P < 0.05$, **$P < 0.01$

showed, in both experimental groups, a significant effect ($P < 0.01$) in the number of red blood cells; to an extent of 14.7% in the W80 group and 29.5% in the W60 group. No variation was observed in the control group.

The concentration of HGB was significantly higher ($P < 0.05$) at the end of the test in the W60 group compared to the W100 group (119.3 vs 100.8 g/l). The duration of the trial showed a statistical effect ($P < 0.05$) on the W60 group whose haemoglobin values rose by 18.8%.

Water restriction elicited a significant increase ($P < 0.01$) in HCT values, by 19.7% in the W80 group and by 24.1% in the W60 group, compared to the W100 group. The duration of the experimental period influenced ($P < 0.01$) haematocrit values; these increased by 24.5% in the W80 group and by 25.8% in the W60 group. The control group showed no changes.

MCHC values were affected ($P < 0.05$) by water restriction; the values rose at the third sampling by 8.5% in both the W60 and W80 experimental groups, compared to the W100 group. Over the course of the entire test a significant increase in values ($P < 0.05$) was observed; by 10.9% in the W80 group and by 11.7% in the W60 group. In contrast, the values in the control group were unchanged.

Blood count values, which were statistically affected by water restriction, were also shown to have a significant interaction ($P < 0.05$) between treatment groups in a time-dependent manner, highlighting the progressive effects of water restriction during the period of the trial.

DISCUSSION

In similar experiments on water restriction (Maloiy et al. 2008), carried out at an ambient temperature of 22 °C, a significant and progressively increasing reduction in feed intake from fat-tailed sheep (–48%), to Zebu (–50%) and Turkana goats (–58.3%) was reported; the same authors also noted a dietary depression in non-domestic species such

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as Grant's gazelles (–34%; *Nanger granti*) and oryx (–40%; *Oryx beisa*). In comparable experiments conducted by other authors (Aganga et al. 1989; Ikhatua et al. 1992; Abdelatif and Ahmed 1994) in Yankasa sheep and native goats, marked effects of water deficiency on feed intake under various regimens of water restriction were detected.

The decrease in feed intake under water restriction is also linked to the type of feed that animals receive; in fact, in goats fed legume hay a feed intake reduction of only 18.8% was observed, while this percentage rose to 21.2% when animals were fed meadow hay, which has lower protein content (Muna and Ammar 2001). In water restriction experiments carried out on sheep, goats and cattle, low feed intake has been described to be partially offset by reduced intestinal peristalsis, which leads to an increased time of exposure of feed to the intestinal microflora with beneficial effects on digestibility and feed utilisation (Musimba et al. 1987; Ajibola 2000; Hadjigeorgiou et al. 2000; Muna and Ammar 2001).

Results on rectal temperature and respiratory rate are in agreement with what Jaber et al. (2004) reported on Awassi sheep water restricted for two and four days, and with what other authors observed (Degen 1977; Hamadeh et al. 2006) in similar research on sheep under different regimens of water restriction.

Alamer (2010) also reported no change in rectal temperatures in water-restricted Aardi goat. However, Alamer and Al-Hozab (2004) described a significant increase in rectal temperature under conditions of water restriction during the spring and summer season in Awassi and Najdi rams; other authors (Ghanem et al. 2008) made the same findings in Awassi sheep, which are due to the adaptive response of animals to the breeding environment that serve to reduce water loss through mechanisms of thermoregulation for the defence from heat.

Water restriction in the W80 and W60 experimental groups, both at Day 14 and Day 28 samplings, elicited a significant reduction ($P < 0.05$) in the number of respiratory acts per minute, reaching at the end of the test (Day 28) values of –13.7% in the W80 group and –22.9% in the W60 group compared with the W100 group (Table 3). A time-dependent effect ($P < 0.05$) was observed in the period from Day 0 to Day 14, while from Day 14 to Day 28 the respiratory rate remained stable. The reduction of respiratory acts under water restriction is one of many defence mechanisms which the animal employs to prevent the loss of water and dehydration through pulmonary evaporation. Alamer and Al-Hozab (2004) also observed a significant reduction in respiratory rate in Awassi and Najdi sheep, subjected to water deprivation for three days. Al-Ramamneh et al. (2012), in experiments carried out on water-restricted Boer goats and black-head sheep in an arid environment, reported a significant decrease in the respiratory rate only for sheep; Dmi'el (1986) observed a 50% increase in respiratory rate after water deprivation for 72 h during the summer, in experiments conducted on Bedouin desert goats. This increase in rate is primarily aimed at maintaining normal body temperature especially that of the brain (Robertshaw and Dmi'el 1983). Rahardja et al. (2011), in their experiments evaluating water restriction by 50% in fat-tailed sheep and Kacang goats in a hot and dry area, observed a significant increase of respiratory rate to prevent an increase in body temperature only in sheep.

Water restriction significantly changed the serum concentration of total protein, urea, creatinine, sodium, chlorine, ROMs and cortisol. A marked increase in the concentration of serum proteins was observed by Mengistu (2007) in Ethiopian-Somali goats water-restricted for four days, and by Jaber et al. (2004) in Awassi ewes subjected to two different regimens of water restriction. Hossaini-Hilali et al. (1994) reported the same findings from similar experiments performed on goats in Morocco. According to Khan et al. (1978), the increased serum concentrations of proteins helps maintain blood colloidal osmotic pressure in water-stressed animals, as the loss of water results in an over-concentration in a smaller volume of blood (Schalm et al. 1975).

Urea is produced by the liver and is eliminated by the kidneys and in this way the liver eliminates ammonium ions produced by amino acid metabolism, the rumen and by bacterial flora in the intestine. The increased serum concentrations of urea that we found in our experiments, is to be attributed to the water-restricted condition that produced a state of dehydration with haematic concentration of metabolites and led to the inability of the kidney to perform its function. Our results are in agreement with what Jaber et al. (2004) reported in Awassi ewes, which showed progressively increasing values of urea from the control group through

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the two and four day-watering groups. Water deficiency leads to increased water reabsorption at the nephron level; consequently, increased urea reabsorption is expected as it is a highly permeable molecule. In other experiments conducted on Merino (MacFarlane et al. 1961) and Yankasa sheep (Aganga et al. 1989; Igbokwe 1993) similar results have been obtained. Laden et al. (1987) also reported a gradual increase in urea concentrations in response to two and five days of water restriction in Awassi sheep. Osbaldiston (1971) reported that hypovolaemia due to water insufficiency is expected to cause a decrease in renal blood flow, thus leading to a decreased filtration rate; in fact, 95% of creatinine, the final metabolite of creatine, is of muscular origin and it is excreted by the kidneys in proportion to the muscle mass and to its rate of proteolysis. When kidney water stress leading to slower glomerular filtration and reabsorption of urea excretion affects kidney excretion function (Keenan and Allardyce 1986; Igbokwe 1993; Silanikove 2000; Marini et al. 2004; Kataria and Kataria 2007), the blood creatinine levels increase, as noted in similar experiments on sheep (Alamer 2005; Hamadeh et al. 2006). Laden et al. (1987) reported an increased creatinine value of 88% in five days water-restricted Awassi sheep, while Abd El-latif et al. (1997) reported an increase of 13% in blood levels of creatinine after three days of water restriction in Barki sheep. However, Jacob et al. (2006) did not observe any changes in creatinine after water restriction for two days in Poll Dorset × Merino lambs.

The increase of serum chlorine and sodium concentrations is due to the reduction in plasma volume and increased serum levels of aldosterone and vasopressin. This promotes increased sodium retention and water adsorption at the kidney level, leading to a haemo-dilution in an attempt to restore the physiological values of sodium and chlorine. In similar previous studies on sheep, goats and cattle (Burgos et al. 2001; Mengistu et al. 2007; Casamassima et al. 2008; Ghanem et al. 2008) similar variations in sodium and chlorine in response to increased restrictions in water were reported. Other authors (Rawda 2003; Hanna 2006; Ghanem et al. 2008) reported a strong influence of water intake on sodium ion concentrations in sheep plasma; the reduction of plasma volume in response to water restriction causes an increase in blood plasma osmolality, and leads to the concentration of electrolytes in plasma (Qinisa et al. 2011), in particular, an increase in sodium and chlorine ions as has been reported by different authors for sheep and goats (Alamer 2005; Jacob et al. 2006; Karnib 2009).

High levels of ROMs in the blood are an indication of oxidative stress, which stems from an imbalance between the production of reactive oxygen metabolites and the capacity of the endogenous antioxidant system to neutralise them (Sies 1991). In fact, when free radicals accumulate in the blood, this leads to damage to cellular membranes, altered metabolism, cellular aging, as well as potentially mutagenic damage to DNA. Zhou et al. (2012) reported an increase of 71% in the activity of mitochondrial enzymes, which produce reactive oxygen species in the kidney medulla, and a decrease of 41% in the activity of NADPH oxidase in experiments on rats subjected to water restriction.

Cortisol plays an important role in maintaining the water balance and plasma electrolytes, although its mechanism of action is not yet clear (Parker et al. 2003). Kataria and Kataria (2007), in accordance with the results of our research, reported a significant increase in the concentration of cortisol in Marwari sheep, fed *ad libitum* and subjected to water deprivation for eight days; while Parker et al. (2003) found that such experimental conditions do not activate the HPA (hypothalamus-pituitary-adrenal) axis in Merino sheep undergoing water deprivation for three days, and suggested that an increase in plasma cortisol represents a response to a longer period of water restriction. Parrot et al. (1996) similarly observed a lack of cortisol release in sheep after feed and water restriction for 48 h.

The increase in the values of some blood count variables (RBC, HGB, HCT and MCHC), found in the present study, can be attributed to water restriction, which produced a reduction in plasma volume and in their haematic concentration.

Al-Toum and Al-Johany (2000) reported a progressive increase in haemoglobin values from trials carried out in winter or during the summer in gazelles subjected to water stress. The same results were reported by other authors (Li et al. 2000; Ghanem 2005; Hamadeh et al. 2006) in experiments conducted on sheep after water restriction.

The increased haematocrit values are consistent with what was observed also by other authors in Awassi sheep (Laden et al. 1987; Ghanem et al. 2008), Merino sheep (MacFarlane et al. 1961) and goats (Hossainini-Hilali et al. 1994), subjected to different regimens of water deprivation.

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This has also been reported by Hamadeh et al. (2006) in Awassi sheep. In particular, the progressive increase in RBC, in response to water restriction, leads to secondary polycythaemia due to a gradual reduction in circulating blood volume and not due to an increase in red blood cells. When this condition persists over time, it leads to a slowing of blood flow in animals that could in turn cause several pathological events. Other authors, however, in similar water deprivation experiments conducted on Yankasa (Aganga et al. 1989; Igbokwe 1993), Awassi (Jaber et al. 2004) and Australian Merino sheep (Li et al. 2000) failed to find any significant effects on blood count parameters.

In conclusion, this research shows that water restriction by 20% (W80) and 40% (W60) results in a significant and progressive reduction of meadow hay and alfalfa hay pellet feed intake, a reduction of respiratory rate per minute and an increase in selected blood variables. These results show the important role of water, as a limiting factor for animal breeding in low-water availability environments. Where possible, the management of low-dairy breeds should be well-designed to support production performance.

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