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Changes in physiological and blood parameters in water restricted Awassi ewes supplemented with different levels of Vitamin C

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Summary

Vitamin C administration is not common in ruminant production. The current study was designed to assess the effect of oral vitamin C supplementation on alleviating water restriction in Awassi ewes. Experiment 1 included four groups, four animals in each, with one group as control receiving daily water, the remaining three groups were watered once every four days; the third and fourth groups were supplemented with a daily oral dose of 3g and 5g vitamin C, respectively. Experiment 2 was similarly designed except that the third group was given 3g Vitamin C daily, while the last group was administered one 10g vitamin C oral dose at the beginning and at the middle of the experiment. The vitamin C treatments seemed to slightly reduce weight loss induced by water restriction. Serum protein, albumin, globulin urea, creatinine, Na^+ and Cl^- concentrations were increased in all water restricted animals. Inconclusive results were observed for the effect of vitamin C on urea, creatinine, and electrolytes concentrations, under water restriction. On the other hand, albumin concentration seemed to be higher in vitamin supplemented animals, while protein and globulin concentrations were not significantly different in all water restricted groups. Further research is warranted to elucidate the different aspects of the role of vitamin C in water stress alleviation.

Introduction

The Awassi sheep, indigenous to the Eastern Mediterranean region, often face harsh conditions of water scarcity and low feed quality. However, its adaptation and ability to sustain production ensured the survival of this vital livestock sector (6), in this region and in other dry areas around the world, such as Australia, where the Awassi has been exported (9).

Water restriction results in a wide range of physiological changes. Generally, a decrease in feed intake and consequently weight loss are observed (3, 5, 8). Previous research has consistently reported increases in at least one of the following parameters: blood proteins, albumin, hemoglobin and packed cell volume in response to water deprivation (5, 8, 11). However, the Awassi seems to have the capacity for denoting high water retention at the level of the kidneys to prevent excessive losses (11).

The use of vitamin C for stress alleviation was tested in different animals, with only scarce reports on its use in ruminants (6). Previous work on water restricted Awassi has

shown that oral vitamin C supplementation could help in reducing weight loss and alleviating the water stress physiological effects (3).
This study was designed to evaluate the effects of different doses of vitamin C supplementation in water stressed Awassi ewes.

Materials and methods

Two experiments were conducted on the campus of the American University of Beirut. The animals were housed in a well ventilated closed barn. The animals were fed a 60:40 mixture of wheat straw and concentrate to cover their maintenance requirements (12).

In experiment 1, Sixteen dry Awassi ewes, 1 - 2 years of age, with an average weight of 32 kg, were randomly divided into four groups, each one subjected to one of the following treatments: “Control” had free access to water without Vitamin C supplementation; Groups “WR”, “V3” and “V5” received *ad libitum* water once every 4 days (repeated four times); groups “V3” and “V5” were orally supplemented with 3g and 5g vitamin C (fine powder extra pure, MERCK. Germany) respectively, dissolved in 50 mL water/animal. Group “WR” was administrated daily 50 mL water/animal without vitamin C.

In experiment 2, the animals were 2-3 years of age, with an average weight of 59.3 Kg. The experimental design and treatments were as in experiment 1 except that instead of group “V5”, the fourth group “V10” received 10 g Vitamin C/50 mL water/animal on the first day of the experiment, and had a similar dose 8 days later.
All animals were subjected to a preparation period, prior to the start of the experiment, during which the vitamin supplemented groups received daily 10 g vitamin C/50 mL water/animal.
The weather conditions in terms of maximum (30 - 31ºC and 18 - 21ºC,) and minimum (26 - 27ºC and 14 - 17ºC) temperature and maximum (85% - 86% and 83% - 93%) and minimum (72% - 73% and 65% - 77 %) relative humidity were recorded in experiment 1 and 2 respectively; similarly water and feed intake were daily monitored. Body weight measurement and blood sampling were performed on 5 occasions: at the onset of the water restriction period, and prior to watering for the water restricted groups (every 4 days); with an additional blood sampling 24 hours after watering (only in experiment 1).

Serum analysis for total serum protein, albumin, globulin, cholesterol, urea and creatinine concentrations was performed using a Roche/Hitachi 912 analyzer (Roche Diagnostics Gmbh Laboratory systems, D-68298 Mannheim), while blood pH and electrolytes were assessed using an automated blood-gas analyzer (model 860, Chiron Diagnostics Limited, Essex, UK).

Data were analyzed using the one–way ANOVA for 2-factor (4x4) completely randomized design (MSTAT 1991; Michigan State University, MI, USA; and SPSS 15.0, SPSS Inc., USA). The two factors were the treatment groups (4 groups in each experiment) and sampling time (4 samplings). Results of the first sample at the beginning of each experiment were used as a covariate. Means were then separated using Duncan’s Multiple Range test.

Results and Discussion

The results in the current study agree with previous findings regarding weight loss (Table I) under water restriction (3, 5, 8). This loss may be attributed to a reduction in total body water as well as loss in body solids since the animals reduced their feed intake and resorted to their body reserves. However, the rates in both trials are comparable to previously reported results ranging from -1.2% up to -33% in water restricted Awassi (8, 11), under similar ambient temperatures. Vitamin C groups showed slightly lower weight loss as compared to the water restricted-unsupplemented animals, supporting similar findings previously reported in our laboratory (3), and other reports positively correlating vitamin C intake with body weight in pregnant sheep and new-borns (4). An explanation for this observation may be found in the slightly higher feed intake seen under Vitamin C. Improved feed intake and feed conversion have been reported in young stressed animals supplemented with vitamin C (1, 13).

Table I. Body weight and feed intake in control, water restricted and vitamin C supplemented Awassi ewes.

	Experiment 1[(a)]				Experiment 2[(b)]			
	Control	WR	V3	V5	Control	WR	V3	V10
Initial Body weight (Kg)	33.6	29.2	34.0	30.5	52.2	60.0	61.0	64.2
Final Body weight (Kg)	30.0	21.6	26.4	23.0	62.7	53.2	54.6	54.4
Weight change (%)	-8.2$^{(a)}$ ±12.72	-26.2$^{(a)}$ ±7.54	-22.5$^{(a)}$ ±3.70	-23.0$^{(a)}$ ±1.73	+6.2$^{(a)}$ ±3.16	-10.4$^{(b)}$ ±1.60	-7.7$^{(b)}$ ±0.74	-7.8^{b} ±3.15
Feed intake (Kg/animal.day)	0.7$^{(a)}$ ±0.0.5	0.3$^{(b)}$ ±0.07	0.3$^{(b)}$ ±0.07		1.5$^{(a)}$ ±0.20	1.1$^{(b)}$ ±0.45	1.2$^{(b)}$ ±0.49	

[a] Control: animals were watered daily; WR: animals were watered every four day; V3: animals were watered every 4 days and received 3g of vitamin C per day; V5: animals were watered every 4 days and received 5g of vitamin C per day.

[b] Control: animals were watered daily; WR: animals were watered every four day; V3: animals were watered every 4 days and received 3g of vitamin C per day; V5: animals were watered every 4 days and received 10g of vitamin C at the beginning and at the middle of the experiment.

Values within a row with different superscripts are significantly different ($P<0.05$), for each experiment.

The effect of water restriction on blood chemistry has been reported in many studies; most of the previous work agrees with the current results (Table II) showing an increase in the concentration of the various assessed blood parameters (3, 5, 8). This general trend may be due to the decrease in total blood volume under dehydration; such is probably the case of total protein and albumin (experiment 1) which are commonly used as dehydration indicators (2). Albumin concentrations seemed to be higher in the vitamin C treated animals, while no significant differences were observed in protein concentrations between all water restricted groups. Ghanem *et al.* (3) reported opposite trends whereby protein and albumin concentrations were lower in vitamin C treated water restricted ewes. On the other hand, Sahin *et al.* (13) reported an increase in protein and albumin concentrations when both dietary vitamin C and E were increased in Japanese quails.

Table II. Serum chemistry[a] of control, water restricted and vitamin C supplemented Awassi ewes.

	Experiment 1[b]				Experiment 2[c]			
	Control	WR	V3	V5	Control	WR	V3	V10
Protein (g/L)	$70.1^{(b)}$ ±0.85	$82.0^{(a)}$ ±2.39	$83.8^{(a)}$ ±1.03	$79.9^{(a)}$ ±1.48				
Globulin (g/L)	$39.8^{(b)}$ ±0.64	$50.3^{(a)}$ ±1.65	$46.7^{(a)}$ ±0.64	$49.3^{(a)}$ ±0.86				
Albumin (g/L)	$28.8^{(c)}$ ±0.39	$32.3^{(b)}$ ±1.03	$34.7^{(a)}$ ±0.43	$34.2^{(a)}$ ±0.74				
Creatinine (µmol/L)	$62.9^{(c)}$ ±5.84	$97.6^{(b)}$ ±5.31	$103.2^{(b)}$ ±4.25	$118.2^{(a)}$ ±4.64	$61.3^{(c)}$ ±1.43	$72.6^{(a)}$ ±0.90	$75.5^{(a)}$ ±1.96	$66.4^{(b)}$ ±1.56
Urea (mmol/L)	$5.8^{(c)}$ ±0.71	$11.2^{(b)}$ ±0.64	$10.8^{(b)}$ ±0.53	$13.4^{(a)}$ ±0.58	$6.7^{(c)}$ ±0.15	$8.1^{(b)}$ ±0.20	$9.1^{(a)}$ ±0.31	$8.9^{(a)}$ ±0.46

[a] The presented values are the means of each parameter, assessed on the fourth day of water restriction (right before watering of the restricted group) on 4 consecutive water restriction cycles; the mean of each parameter recorded right before the start of the first restriction cycle, was used as covariate.
[b] Control: animals were watered daily; WR: animals were watered every four day; V3: animals were watered every 4 days and received 3g of vitamin C per day; V5: animals were watered every 4 days and received 5g of vitamin C per day.
[c] Control: animals were watered daily; WR: animals were watered every four day; V3: animals were watered every 4 days and received 3g of vitamin C per day; V5: animals were watered every 4 days and received 10g of vitamin C at the beginning and at the middle of the experiment.
Values within a row with different superscripts are significantly different ($P<0.05$), for each experiment.

Water restriction may also activate mechanisms leading to an actual increase in the quantity of certain blood parameters. For instance, under dehydration, the increase in urea reabsorption at the level of the kidneys contributes to the higher blood urea concentrations (7). Renal reabsorption is mainly activated to prevent water losses; dehydrated Awassi are reported to produce small amounts of highly concentrated urine (16). In addition, renal urea reabsorption may help in decreasing urea losses in urine and improve its recycling to the gut thus improving nitrogen balance, especially since the animals reduced their dietary intake (14). The increase in creatinine may be a result of the decrease in feed intake (10), in addition to the decrease in blood volume. The vitamin C treatments yielded inconclusive results. Literature on this issue is scarce; however, Sahin *et al.* (13) reported a decrease in serum urea when both vitamin E and C are increased in heat stressed Japanese quails.

The increased Na^+ and Cl^- concentration observed under water restriction (Table III) were expected. This is probably due to the combined effect of decreased blood volume and an increase in renal retention as reported in previous literature (3, 5, 8). Similar to our results, literature suggests that the response of K^+ to water restriction is inconsistent (3, 5, 8). Vitamin C treated groups showed significant differences from their unsupplemented counterparts only in experiment 1. Ghanem *et al.* (3) had reported that daily vitamin C supplementation alleviated the water restriction effects on blood Na^+, Cl^- and Ca^{++}, while the opposite was observed in group V3 (experiment 1) of the current study. Furthermore, Vitamin C seemed to have a slightly blood acidifying effect as reflected through the pH, while Avci *et al.* (1) observed no significant changes in blood pH due to ascorbic acid supplementation to Japanese quails.

Table III. Blood electrolytes concentrations [a] and pH of control, water restricted and vitamin C supplemented Awassi ewes.

	Experiment 1 [b]				Experiment 2 [c]			
	Control	WR	V3	V5	Control	WR	V3	V10
pH	7.488 [a]	7.477 [a]	7.459 [ab]	7.440 [b]	7.419 [a]	7.441 [a]	7.410[a]	7.409[a]
	±0.020	±0.013	±0.009	±0.016	±0.030	±0.016	±0.014	0.008
Na^+(mmol/L)	145.3 [d]	157.9 [c]	168.3 [a]	163.0 [b]	148.2 [b]	155.0 [a]	154.6[a]	155.4[a]
	±1.27	±2.97	±1.14	±3.04	±0.96	±1.00	±0.93	±1.35
Cl^-(mmol/L)	112.7 [c]	121.4[b]	129.8[a]	124.1[b]	117.3[b]	123.5[a]	122.4[a]	123.2[a]
	±2.59	±3.93	±2.99	±3.73	±1.42	±1.53	±1.11	±0.89
K^+(mmol/L)	4.3[c]	4.3[bc]	4.5[ab]	4.7[a]	5.1[a]	4.6[b]	4.3[b]	4.6[b]
	±0.74	±0.08	±0.22	±0.12	±0.18	±0.16	±0.08	±0.08

[a] The presented values are the means of each parameter, assessed on the fourth day of water restriction (right before watering of the restricted group) on 4 consecutive water restriction cycles; the mean of each parameter recorded right before the start of the first restriction cycle, was used as covariate.
[b] Control: animals were watered daily; WR: animals were watered every four day; V3: animals were watered every 4 days and received 3g of vitamin C per day; V5: animals were watered every 4 days and received 5g of vitamin C per day.
[c] Control: animals were watered daily; WR: animals were watered every four day; V3: animals were watered every 4 days and received 3g of vitamin C per day; V5: animals were watered every 4 days and received 10g of vitamin C at the beginning and at the middle of the experiment.
Values within a row with different superscripts are significantly different ($P<0.05$), for each experiment.

Upon rehydration (experiment 1), the differences in electrolytes and pH due to the different treatments seem to disappear (Table IV), except for Cl^- that dropped below the control values, in the water restricted groups. In addition, the sustained high creatinine and urea concentrations, particularly under vitamin C supplementation, suggest that the animals may have not restored their fully hydrated status. In fact, Laden at al. (11) have observed that the Awassi are slow to get fully hydrated after a period of water deprivation. They suggested that it might take more than 24 hours for normal renal function and full hydration to be restored. The role of vitamin C in urea and creatinine dynamics during dehydration and rehydration in sheep warrants further investigation.

The comparison of the different vitamin C treatments revealed that daily oral supplementation with 3 g per animal seems to be an adequate treatment for inducing the physiological changes observed. In fact, groups V3 in both experiments recorded the lowest weight loss compared to the other restricted groups. Previous research showed that a daily dose of 2.5 g per ewe was able to elicit physiological responses similar to our current findings (8). With respect to daily versus single dosing of vitamin C, experiment 2 results indicate that daily supplementation elicits greater response. Similarly, Hidiroglou *et al.* (6) have previously observed that multiple dosing of vitamin C yielded higher plasma concentrations of the vitamin as compared to single dosing.

Table IV. Blood electrolytes concentrations$^{(a)}$ and pH of control, water restricted and vitamin C supplemented Awassi ewes, after rehydration

	Mean after rehydration (Experiment 1$^{(b)}$)			
	Control	WR	V3	V5
pH	7.481$^{(a)}$ ±0.017	7.478$^{(a)}$ ±0.015	7.466$^{(a)}$ ±0.010	7.456$^{(a)}$ ±0.010
Creatinine (µmol/L)	68.0$^{(b)}$ ±5.63	68.4$^{(b)}$ ±4.59	73.6$^{(ab)}$ ±3.68	82.6$^{(a)}$ ±4.26
Urea (mmol/L)	5.7$^{(ab)}$ ±0.76	4.2$^{(b)}$ ±0.62	5.1$^{(ab)}$ ±0.52	6.6$^{(a)}$ ±0.59
Na^+(mmol/L)	141.3$^{(a)}$ ±1.36	139.7$^{(a)}$ ±1.46	142.1$^{(a)}$ ±1.16	138.8$^{a(a)}$ ±1.44
Cl^-(mmol/L)	111.6$^{(a)}$ ±1.07	106.7$^{(b)}$ ±0.68	108.5$^{(b)}$ ±0.99	105.7$^{(b)}$ ±0.71
K^+(mmol/L)	4.6$^{(a)}$ ±0.24	4.3$^{(a)}$ ±0.06	4.7$^{(a)}$ ±0.18	4.6$^{(a)}$ ±0.11

$^{(a)}$ The presented values are the means of each parameter, assessed on the day following watering (of the restricted groups) on 4 consecutive water restriction cycles; the mean of each parameter recorded right before the start of the first restriction cycle, was used as covariate.
$^{(b)}$ Control: animals were watered daily; WR: animals were watered every four day; V3: animals were watered every 4 days and received 3g of vitamin C per day; V5: animals were watered every 4 days and received 5g of vitamin C per day.
Values within a row with different superscripts are significantly different (P<0.05), for each experiment.
Values within a row with different superscripts are significantly different (P<0.05).

Conclusion

A daily oral dose of 3 g vitamin C was shown to slightly decrease weight loss in water restricted Awassi sheep. The mechanisms of action of vitamin C on different physiological parameters such as serum albumin, urea, creatinine and electrolytes are still not elucidated. The promising results suggesting a positive role of vitamin C in water stressed sheep warrant further research in the light of increased global warming and the need to exploit different livestock breeds' adaptive capacities.

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