

## Physiological and biochemical responses in German Fawn kids subjected to cooling treatments under Mediterranean climate conditions

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**Abstract:** This study was conducted to determine the effects of different physical modifications (sprinkler and fan) related to heat stress during summer on relevant blood biochemical measures and physiological responses, such as rectal temperature (RT), respiration rate (RR), heart rate (HR), testis temperature (TT), and head temperature (HT), in kids. In total, 32 male German Fawn × Hair kids at 6 months of age were divided into 4 groups of 8. Treatments were as follows: group A: (no sprinkler, no fan) (control); group B: sprinkler only (at 1000-1100 and 1400-1500), no fan; group C: fan (1.5 m/h between 1000-1800), no sprinkler; group D: sprinkler (at 1000-1100 and 1400-1500) and fan (1.5 m/h between 1000-1800).

Sprinkler + fan treatment decreased RR, RT, TT, and HT ( $P < 0.05$ ), whereas sprinkler + fan, sprinkler only, and fan only increased HR. Sprinkler + fan treatment significantly decreased T3 and T4 secretion ( $P < 0.05$ ). Time × fan × sprinkler interaction significantly affected HCT ( $P < 0.05$ ), whereas F × S interaction affected CHO ( $P < 0.05$ ). According to blood chemistry and physiological responses, fan treatment was more effective than sprinkler and may be recommended as a means to alleviate heat stress in goats under Mediterranean climate conditions; however, further research is needed to optimize sprinkler cooling.

**Key words:** Blood measures, heat stress, physiological response, cooling methods, kids

### Serinletilen Alman Alaca oğlaklarının Akdeniz iklim koşullarındaki fizyolojik ve biyokimyasal tepkileri

**Özet:** Bu çalışma, oğlaklarda sıcaklık stresi oluşumunu engellemek amacıyla duş ve fan gibi farklı fiziksel uygulamaların fizyolojik tepkileri (solunum sayısı, rektal sıcaklık, nabız sayısı, testis sıcaklığı ve kafa sıcaklığı) ve kandaki biyokimyasal değişikliklerin saptanması amacı ile yürütülmüştür. Altı aylık toplam 32 baş erkek oğlak 8'er baş olacak şekilde 4 eşit gruba ayrılmıştır. Deneme grupları şu şekilde oluşturulmuştur; Grup A (kontrol grubu, duş ve fan uygulaması yok), Grup B (1000-1100 ve 1400-1500 arasında duş uygulanmış), Grup C (1000-1800 arasında 1,5 m/s fan uygulanmış), Grup D (1000-1100 ve 1400-1500 arasında duşa ek olarak 1000-1800 arasında 1,5 m/s fan uygulanmış).

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Duş-fan uygulamasının birlikte kullanıldığı veya duş ya da fan uygulamalarının tek başına kullanıldığı oğlaklarda nabız sayısında artışa neden olmasına rağmen, duş ve fan uygulanan hayvanlarda rektal sıcaklık, solunum sayısı, testis sıcaklığı ve kafa sıcaklığı daha düşük bulunmuştur ( $P < 0,05$ ). Fan ve duş uygulamaları T3 ve T4 salgısını önemli düzeyde azaltmıştır ( $P < 0,05$ ). HCT değerleri üzerine zaman  $\times$  fan  $\times$  duş interaksiyon etkisinin olduğu tespit edilmiş ( $P < 0,05$ ) fan  $\times$  duş uygulamasının CHO değeri üzerine etkisi önemli bulunmuştur ( $P < 0,05$ ). Elde edilen bulgular ışığında Akdeniz iklim koşulları açısından fan ile serinletmenin oğlaklardaki sıcaklık stresinin azaltılmasında son derece etkili olduğu ve önerilebileceği belirlenmiştir. Ancak, duş ile serinletmenin optimizasyonu için daha detaylı araştırılması gerekmektedir.

**Anahtar sözcükler:** Kan ölçümleri, sıcaklık stresi, fizyolojik tepkiler, serinletme metodları, oğlak

## Introduction

The Mediterranean region of Turkey is characterized by high temperature and humidity from June to September. One of the main problems with raising kids during this time period is growth retardation because of the negative impacts of heat stress. Thus, alleviation of the detrimental effects of heat stress is important for maintaining productivity and animal welfare in hot environments (1-4).

Thyroid hormones—thyroxin T4 or triiodothyronine T3—play an important role in animal adaptation to environmental changes. T4 and T3 stimulate oxygen consumption and heat production by cells, which increase the basal metabolic rate, enhance glucose utilization, modify lipid metabolism, and stimulate cardiac and neural functions (5). Thyroid glands primarily secrete T4, which is monodeiodinated to T3 prior to interacting with the target cells. Low ambient temperatures increase thyroid activity, whereas high temperatures depress it (6).

Hemoglobin concentration decreases during heat stress due to depression of hematopoiesis and expansion of body fluids (7). Lu (1) reported that

blood pH,  $\text{HCO}_3$ , and CR increased, while  $\text{pCO}_2$ , BE (base excess), plasma Ca, CPK, and AST decreased when sheep were subjected to heat stress.

The aim of the present study was to compare the effects of sprinkler and fan (ventilation) treatments on biochemical indicators in blood in order to determine the best cooling method for kids.

## Materials and methods

The study was carried out on crossbred goats at Çukurova University, Faculty of Agriculture, Dairy Goat Research Farm. The farm is located in the Mediterranean region of Turkey, in which subtropical weather conditions prevail, with an average temperature of 35 °C, 65% relative humidity, and 1.1 km/h wind speed during the study period. It is 40 m asl (36°59'N, 35°18'E) and annual precipitation is 450 mm. The average temperature and relative humidity of the pens were recorded daily with a thermometer and barometer. Climatic data during the fattening trial for each treatment pen are given in Table 1.

Table 1. Average temperature and relative humidity in each experimental pen during the fattening trial.

Climatic data for pens	Treatment groups			
	Group A	Group B	Group C	Group D
Temperature (°C)	35.6	32.1	33.8	32.4
Relative humidity (%)	68	67	73	58

The animals used in the study were crossbred German Fawn and Hair kids (75% German Fawn and 25% Hair goat) born in 2005.

Kids were weaned at 3 months of age. They had free access to kid grower concentrate and medium quality alfalfa hay from 2 weeks of age during the pre-weaning period. After weaning, a 1-month transition period was used for adaptation to pens and fattening diets. Then they were fed with total mixed rations containing 90% concentrate (2550 Kcal ME/kg, 18% CP) and 10% alfalfa hay cut to a length of 1.5-2 cm. All kids had ad libitum access to food and water.

The experiment included 4 groups of 8 male kids. The treatments were as follows: group A: no sprinkler, no fan (control); group B: sprinkler only (at 1000-1100 and 1400-1500), no fan; group C: fan, no sprinkler (1.5 m/h from 1000-1800); group D: sprinkler (at 1000-1100 and 1400-1500) and fan (1.5 m/h). Eight kids were allocated into four 1.80 × 2.10-m pens (2 kids per pen). The Kentucky system was employed for cooling the goats. Parallel sprinkler pipelines were mounted 2 m above the ground. In total, 24 sprinklers supplied 2.5 L of water/min; 0.12 cm<sup>3</sup> of water was applied per cycle. The pipes were 2.85 cm in diameter. Two axial fans (air movement 1.5 m/h) were mounted (45 cm in diameter) in the middle of the paddocks (8).

Blood samples were taken twice a day (at 1120 and 1520) twice a week throughout July and August. Blood was collected via jugular venipuncture. Samples collected into EDTA tubes were analyzed for whole blood count (triiodothyronine (T<sub>3</sub>), thyroxin (T<sub>4</sub>), blood urea nitrogen (BUN), cholesterol (CHO), glucose, serum glutamic oxaloacetic transaminase (SGOT), hemoglobin (HGB), hematocrit (HCT), pH, bicarbonate (CHCO<sub>3</sub>), (LH-750, Coulter Counter Beckman), and blood gas (9); samples collected into heparin tubes were analyzed immediately (NOVA PHOX PLUS -USA).

Physiological measurements (rectal temperature, respiration and pulse rate, and head and testis skin temperature) were recorded 4 times a day—between 0600 and 0700, 1200 and 1300, 1800 and 1900, and

2400 and 0100. Rectal temperature was measured with a digital thermometer, and respiration and pulse were counted using a stethoscope. Skin temperature was measured with an infrared thermometer (Testo BP-960) positioned 10 cm from the head and testis skin.

Data were analyzed for the effects of the cooling methods (fan or sprinkler) using repeated measurements mode and the means were ranked by Duncan's multiple comparison test (10). Data analysis was conducted using SPSS for Windows v.11.5 (11). The statistical model was as follows:

$$\hat{Y}_{ijkl} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \pi_{l(ij)} + \gamma_k + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + \gamma\pi_{kl(ij)} + e_{ijkl}$$

where

$\hat{Y}_{ijkl}$  is the observation value (rectal temperature, respiration rate, pulse rate, testis temperature, head temperature, etc.);

$\mu$  is the overall mean;

$\alpha_i$  is the sprinkler cooling effect (S);

$\beta_j$  is the fan cooling effect (F);

$\pi_{l(ij)}$  is the random effect of  $l$  subject within  $i$  and  $j$  levels of S and F treatments;

$\gamma_k$  is the time effect (T);

$\alpha\beta_{ij}$  is the sprinkler × fan cooling interaction effect (S × F);

$\alpha\gamma_{ik}$  is the sprinkler cooling × time interaction effect (S × T);

$\beta\gamma_{jk}$  is the fan cooling × time interaction effect (F × T);

$\alpha\beta\gamma_{ijk}$  is the sprinkler × fan cooling × time interaction effect (S × F × T);

$\gamma\pi_{kl(ij)}$  is the random effect of  $l$  subject at  $k$  period within  $i$  and  $j$  levels of S and F treatments;

$e_{ijkl}$  is the random error (12).

**Results**

Thermo-physiological responses are presented in Table 2. Fan treatment decreased all the measures,

except HR ( $P < 0.01$ ). Fan + sprinkler treatment decreased TT and HT ( $P < 0.01$ ), whereas sprinkler treatment decreased RR ( $P < 0.01$ ).

Table 2. Thermo-physiological responses of the experimental kids.

	Observation time	Groups				Means for time	SEM
		A	C	B	D		
RT ( °C)	0600 - 0700	38.99	38.72	38.52	37.49	38.43 <sup>c</sup>	0.040
	1200 - 1300	39.59	39.26	39.08	38.03	38.99 <sup>a</sup>	0.044
	1800 - 1900	39.60	39.22	39.30	38.10	39.05 <sup>a</sup>	0.048
	2400 - 0100	39.22	38.88	38.82	37.17	38.66 <sup>b</sup>	0.040
	Means for S × F	39.35 <sup>a</sup>	39.02 <sup>b</sup>	38.93 <sup>b</sup>	37.83 <sup>c</sup>	38.78	0.072
Effects	S	F	S × F	Time	S × T	F × T	S × F × T
Significance levels	0.677	0.001	0.022	0.000	0.145	0.362	0.931
TT ( °C)	0600 - 0700	33.32	32.43	32.79	32.26	32.70 <sup>c</sup>	0.106
	1200 - 1300	35.05	35.19	34.65	35.05	34.98 <sup>a</sup>	0.077
	1800 - 1900	34.58	33.61	34.02	33.69	33.98 <sup>b</sup>	0.088
	2400 - 0100	33.50	32.61	33.02	32.20	32.83 <sup>c</sup>	0.083
	Means for S × F	34.11 <sup>a</sup>	33.46 <sup>b</sup>	33.62 <sup>b</sup>	33.30 <sup>b</sup>	33.62	0.107
Effects	S	F	S × F	Time	S × T	F × T	S × F × T
Significance levels	0.002	0.000	0.017	0.000	0.821	0.000	0.675
HT (°C)	0600 - 0700	35.58	32.82	33.45	33.56	33.35 <sup>c</sup>	0.173
	1200 - 1300	36.40	34.31	34.02	34.11	36.21 <sup>a</sup>	0.137
	1800 - 1900	36.48	33.49	34.90	34.58	34.61 <sup>b</sup>	0.124
	2400 - 0100	33.36	31.71	33.01	32.36	32.61 <sup>d</sup>	0.097
	Means for S × F	36.21 <sup>a</sup>	33.83 <sup>c</sup>	34.60 <sup>b</sup>	34.40 <sup>b</sup>	34.45	0.141
Effects	S	F	S × F	Time	S × T	F × T	S × F × T
Significance levels	0.458	0.001	0.000	0.000	0.144	0.003	0.271
RR (number/min)	0600 - 0700	63.84	48.90	50.01	46.23	52.24 <sup>c</sup>	1.773
	1200 - 1300	95.01	86.07	77.10	90.60	84.13 <sup>a</sup>	2.347
	1800 - 1900	85.71	68.34	74.79	67.62	74.11 <sup>b</sup>	2.188
	2400 - 0100	65.34	52.50	56.40	48.00	55.56 <sup>c</sup>	1.789
	Means for S × F	77.49	63.96	64.59	63.12	66.51	1.336
Effects	S	F	S × F	Time	S × T	F × T	S × F × T
Significance levels	0.004	0.007	0.311	0.000	0.021	0.170	0.393
PR (beat/min)	0600 - 0700	84.18	85.32	86.55	82.50	84.64 <sup>c</sup>	0.915
	1200 - 1300	84.96	90.78	92.61	89.82	90.29 <sup>ab</sup>	1.054
	1800 - 1900	90.00	91.77	92.16	90.06	91.00 <sup>a</sup>	0.974
	2400 - 0100	88.11	87.45	90.18	86.40	88.03 <sup>b</sup>	0.966
	Means for S × F	86.82	88.83	90.39	87.21	88.50	0.558
Effects	S	F	S × F	Time	S × T	F × T	S × F × T
Significance levels	0.571	0.725	0.185	0.000	0.715	0.473	0.867

A: No sprinkler, no fan (control group); B: sprinkler only; C: fan only; D: sprinkler and fan; RT: rectal temperature; RR: respiration rate; PR: pulse rate; TT: testis temperature; HT: head temperature; SEM: standard error of means; <sup>a,b,c,d</sup> (means with different superscript letters are significantly different [ $P < 0.05$ ]).

The differences in RT between treatments ( $P < 0.05$ ) and the time of observation were significant ( $P < 0.01$ ). Generally, cooled kids had lower RT than the control kids.

Daily fluctuations in respiration and pulse rates in all groups were statistically significant ( $P < 0.05$ ). Respiration and pulse rates in the control group were higher than those in cooled groups, whereas the pulse rates in the 3 cooled groups were similar.

Skin temperature in the cooled kids was lower than that in the control group. The effect of fan + sprinkler treatment on blood chemistry is shown in Table 3. T3 levels in the morning and afternoon with fan only, sprinkler + fan, and sprinkler only treatments were lower than in the controls.

Plasma T4 levels in the treatment groups (groups B-D) were lower than those in the control group. Cooling methods did not affect blood glucose or CHO blood levels.

Table 3. Changes in the least square means of blood chemistry parameters in kids.

	Observation time	Groups				Means for time	SEM
		A	C	B	D		
T3	Morning	235.41 <sup>a</sup>	222.63 <sup>b</sup>	209.43 <sup>b</sup>	228.38 <sup>ab</sup>	233.96	11.926
	Afternoon	255.64 <sup>a</sup>	227.95 <sup>b</sup>	241.21 <sup>ab</sup>	248.39 <sup>a</sup>	245.80	14.436
T4	Morning	6.68 <sup>a</sup>	6.18 <sup>a</sup>	5.40 <sup>b</sup>	5.43 <sup>b</sup>	5.91	0.214
	Afternoon	6.53 <sup>a</sup>	6.32 <sup>a</sup>	5.89 <sup>b</sup>	5.49 <sup>b</sup>	6.06	0.283
BUN	Morning	25.58 <sup>a</sup>	21.67 <sup>b</sup>	23.58 <sup>ab</sup>	22.92 <sup>b</sup>	23.44 <sup>b</sup>	1.016
	Afternoon	29.33 <sup>a</sup>	26.17 <sup>b</sup>	27.92 <sup>b</sup>	27.25 <sup>b</sup>	27.67 <sup>a</sup>	0.683
CHO	Morning	40.67 <sup>b</sup>	48.42 <sup>a</sup>	38.67 <sup>b</sup>	37.92 <sup>b</sup>	41.42 <sup>b</sup>	1.560
	Afternoon	43.50 <sup>b</sup>	53.58 <sup>a</sup>	48.92 <sup>ab</sup>	45.17 <sup>b</sup>	47.79 <sup>a</sup>	1.837
Glucose	Morning	65.50 <sup>a</sup>	66.67 <sup>a</sup>	63.83 <sup>b</sup>	64.67 <sup>ab</sup>	65.17 <sup>a</sup>	2.208
	Afternoon	55.83 <sup>b</sup>	62.00 <sup>a</sup>	58.17 <sup>b</sup>	57.5 <sup>b</sup>	58.38 <sup>b</sup>	2.172
SGOT	Morning	106.33 <sup>a</sup>	84.50 <sup>c</sup>	99.08 <sup>b</sup>	107.00 <sup>b</sup>	101.92	4.658
	Afternoon	117.08 <sup>a</sup>	89.33 <sup>b</sup>	102.92 <sup>a</sup>	104.33 <sup>a</sup>	99.23	5.150
HGB	Morning	9.37	8.73	9.33	8.58	9.13	0.248
	Afternoon	9.35	8.39	9.74	9.48	9.41	0.241
HCT	Morning	4.06 <sup>d</sup>	4.86 <sup>bc</sup>	5.56 <sup>ab</sup>	4.68 <sup>cd</sup>	4.79 <sup>b</sup>	0.299
	Afternoon	4.81 <sup>c</sup>	4.98 <sup>bc</sup>	5.53 <sup>ab</sup>	6.19 <sup>a</sup>	5.38 <sup>a</sup>	0.271
pH	Morning	7.35	7.37	7.36	7.38	7.36	0.004
	Afternoon	7.30	7.28	7.30	7.27	7.29	0.009
CHCO <sub>3</sub>	Morning	28.78 <sup>b</sup>	21.23 <sup>b</sup>	24.40 <sup>ab</sup>	25.34 <sup>a</sup>	24.60	0.783
	Afternoon	28.27 <sup>b</sup>	23.50 <sup>b</sup>	24.53 <sup>ab</sup>	26.58 <sup>a</sup>	24.47	0.892

A: No sprinkler, no fan (control group); B: sprinkler only; C: fan only; D: sprinkler + fan; SEM: standard error of means; <sup>a,b,c,d</sup> (means with different superscript letters are significantly different [ $P < 0.05$ ]).

Afternoon levels of blood glucose, SGOT, and pH were lower than the morning levels, whereas for CHO, HGB, HCT,  $T_3$ ,  $T_4$ , and BUN it was vice versa (Table 3). BUN, CHO, glucose, HCT, and pH levels were significantly different between observation times ( $P < 0.01$ ). The effects of treatments ( $P < 0.05$ ) and observation time ( $P < 0.001$ ) were significant. BUN levels in groups B, C, and D, especially in the morning ( $P < 0.01$ ) and afternoon ( $P < 0.05$ ), were lower than in group A. Differences between BE levels in the morning were significant ( $P < 0.05$ ), whereas no significant differences were observed in the afternoon measurements ( $P > 0.05$ ). Differences between  $\text{CHCO}_3$  levels were significant ( $P < 0.05$ ).

## Discussion

Rectal, head, and testicular skin temperatures, and respiration and pulse rates fluctuated during the daytime and returned to normal levels at night, in accordance with ambient temperature. Rectal temperature, considered a good index of deep body temperature, reflects thermal balance and might be used to evaluate the impact of heat stress (4,13,14). Rectal temperatures in the cooled kids rose during the day and returned to normal at midnight, indicating that the cooling measures did not completely ameliorate the heat load. Nevertheless, rectal temperatures in the control kids were higher than in the cooled kids in the morning, indicating that their heat load was higher. Overall, our data are consistent with previously reported observations in goats and kids of the same breed (15,16). The fact that rectal temperatures in the control kids did not return to normal levels until the early morning, unlike in the treated kids, further demonstrates that these measures increased heat loss and thus minimized the effect of heat stress during the day.

The lower midnight pulse and respiration rates in cooled kids suggest that they accumulated less heat during the day. The higher head temperatures in non-cooled kids suggest that they used this area to radiate excess heat, as reported by Knižková et al. (17).

Glucose and CHO concentrations decreased markedly, along with high ambient temperature,

which is consistent with previous reports (8,18,19). CHO levels in kids that were fan cooled only (Group C) were higher than in the kids cooled with other systems ( $P < 0.05$ ), suggesting it was better at preventing a drop in the concentration of this metabolite. Generally, CHO and glucose levels in the control kids were higher than in the kids cooled by sprinkler and sprinkler + fan, but CHO and glucose levels decreased as the ambient temperature rose.

The ability of the cooled goats to better maintain their blood glucose level was related to higher thyroxin hormone levels, suggesting that energy metabolism was not reduced as much as in the control goats, or that blood plasma volume expansion was lower (7,20,21).

Heat stress may cause an increase in SGOT (serum glutamic oxaloacetic transaminase) activity because of increased stimulation of gluconeogenesis by corticoids (20). Thus, the lowest SGOT values, both in the morning and afternoon, in kids cooled by fan suggest that they responded better to heat stress.

Heat stress often causes respiratory alkalosis as a result of hyperventilation and compensation, which results in urinary  $\text{HCO}_3$  loss in an attempt to balance the ratio of carbonic acid to bicarbonate in the blood (13).  $\text{CHCO}_3$  levels rose when animals were in heat stress. In the present study  $\text{CHCO}_3$  levels were the lowest in group C, further supporting the conclusion that this treatment was the most effective in ameliorating heat stress.

In conclusion, our findings show that the effects of high temperature can be alleviated by using cooling methods. In this study fan cooling was the most effective, which contradicts many similar studies on dairy cows that show that sprinkler cooling is the most effective cooling method (2,3,8,14-16,18,22). Thus, further research and potential improvements in sprinkling technology (e.g., preventing extra humidity with stronger fans) are needed to clarify this point in kids under sub-tropical climate conditions.

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