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Effect of Naringin Supplementation on Performance and Physiological Responses of Heat Stressed Lambs

Ibrahim A. Alhidary and Mutassim M. Abdelrahman
Department of Animal Production, College of Food and Agriculture Sciences,
King Saud University, 11451 Riyadh, Saudi Arabia

Abstract: Eighteen Awassi lambs (4 month old; mean BW 23.8±1.3 kg) were used in a 60 days trial to investigate the effects of naringin supplementation on performance and physiological responses of heat stressed lambs. The experiment was undertaken during the period of summer months of Saudi Arabia. The lambs were individually housed in a shaded pen and randomly assigned to 1 of 3 treatments: 0 (control), 1 and 2 g of naringin which was weekly administered as an oral dose of naringin (Naringin 98%; Blackburn Distribution, UK). Feed intake was measured weekly and lambs were weighed on days 1, 15, 30, 45 and 60. Blood were collected on days 1, 30 and 60 for measurement of concentrations of metabolic profile. Oral administration with 1 g naringin resulted an increase in ADG (5.2%; $p<0.02$), a greater gain to feed ratio (17.1%; $p<0.05$) compared with those in other groups. Lambs receiving orally administered 1 g naringin had a greater ($p<0.03$) serum concentration of albumin and less ($p<0.01$) creatinine concentration than non-treated lambs. These results indicate that naringin supplementation has reduces the adverse effects of heat stress and important implications for the sheep industry.

Key words: Heat stress, awassi lambs, naringin, physiological response, sheep industry

INTRODUCTION

High environmental conditions have negatively influence in sheep industry in many parts of the World (e.g., Australia, United State and Middle East). During periods of hot conditions, physiological, immunological and molecular responses occur which allow sheep to cope with this stress or when Temperature-Humidity Index (THI) unit exceeds 78. However, such these responses may have adverse effects on the performance and health of sheep, causing a substantial loss of production for the sheep industry (Fuquay, 1981, Finch, 1984; Silanikove, 2000; Finocchiaro *et al*., 2005). Moreover, heat stress is associated with changes in antioxidant status by promoting oxidative stress and reducing the blood concentrations of antioxidant micronutrients (vitamins, minerals and flavonoids) in ruminants (Bernabucci *et al*., 2002; Saker *et al*., 2004; Burke *et al*., 2007).

Naringin as a flavanone glycoside, plays important roles in the growth and health of animals. It is involved in a wide range of biological functions (e.g., metabolic, immune and antioxidant roles in the body through its participation in several important enzymes and antioxidant defence system (Kim *et al*., 2004; Cushnie and Lamb, 2011; Oskoueian *et al*., 2013). Naringin is often added to diets to improve animal productivity and antioxidant status and also to enhance immune competence (Gladine *et al*., 2007; Bodas *et al*., 2012). Moreover, the addition of naringin to ruminants' diets may be useful particularly when animals are under elevated environmental temperatures to reduce or ameliorate the adverse effects of thermal stress. Thus, the objectives of the study were to evaluate the effects of naringin supplementation on productive performance and physiological responses of lambs under hot environmental conditions.

MATERIALS AND METHODS

Animals and experimental design: The study was undertaken at the Experimental Farm Animal Centre, Department of Animal Production, King Saud University, Riyadh, Saudi Arabia with the approval of King Saud University Animal Ethics Committee.

Eighteen 4 month old Awassi lambs (mean BW, 23.8±1.3 kg) were used in a 60 days trial. The experiment was carried out through Summer months (July and August) of Saudi Arabia. The lambs were individually housed in a shaded pen (1.5 m long×1.0 m wide). Each pen

Corresponding Author: Ibrahim A. Alhidary, Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, P.O. Box 2460, 11451 Riyadh, Saudi Arabia

was equipped with a feed trough and a 10 L plastic water bucket. Lambs were adapted to the pens and adjusted to the diet being fed, over 14 days period before the commencement of the study. On day 1 of the experimental period, the lambs were randomly assigned to 1 of 3 treatments: 0 (control), 1 and 2 g of naringin which was weekly administered as an oral dose of naringin (Naringin 98%; Blackburn Distribution, Lancashire, United Kingdom). Lambs assigned to the control group were orally administered with equal amount of PBS (1 mL; pH 7.4; Pharmaceutical Solutions Industry, Jeddah, Saudi Arabia). All lambs were offered a commercial total mixed ration (WAFI, ARASCO, Riyadh, Saudi Arabia) which contained 1.95 Mcalof ME_m and 13.0% CP/kg (DM basis) at maintenance level (2.5% of initial bodyweight; NRC, 1985) twice daily at 0700 h and 1500 h. Water was available *ad libitum*.

Measurements and sampling: Ambient Temperature (T_a) and Relative Humidity (RH) were measured at 30 min intervals throughout the experimental period using temperature/RH sensors and measurements were stored using a data logger (Wireless Vantage Pro2, Davis Instruments Corp, Hayward, CA, USA) until downloaded. Sensors were calibrated prior to the study and checked for accuracy at the end of the study. T_a and RH were recorded every 30 min and hourly means were calculated and an hourly Temperature-Humidity Index (THI) was calculated using the following equation (Thom, 1959): $THI = (0.8\times T_a)+[(RH/100)\times(T_a-14.3)]+46.4$.

The weights of offered feed and feed refusals were measured daily at 0700 h and feed intake was calculated on a DM basis. Lambs were weighed individually before morning feeding time on day 1, 15, 30, 45 and 60. Body weight gain per kg DMI was calculated for each lamb. Respiration Rate (RR) was determined by counting flank movements over a 20s period which was then converted to breaths per minute (bpm). Rectal Temperature (RT) was measured using a digital rectal thermometer. The RT and RR were measured at 0800, 1200 and 1600 h on days 15, 30 and 45.

Blood samples (10 mL) were collected from each lamb before morning feeding via jugular venipuncture on days 1, 30 and 60 using 10 mL Vacutainer tubes (BD, Franklin Lakes, NJ, USA) for serum collection. The samples were allowed to clot for 2 h at room temperature (25°C) before being centrifuged. Serum was obtained by centrifugation at 2.400×g for 15 min at 4°C and was then frozen at -20°C until analyzed. The serum was assayed for glucose, total protein, albumin, globulin, creatinine and urea using commercial kits (Randox Laboratories, Antrim, United Kingdom) and determined by using amicroplate reader (Multiskan EX, Thermo Fisher Scientific Inc., Waltham, MA, USA) according to the manufacturer's procedures.

Statistical analysis: All data were analyzed using repeated measures and the Proc Mixed Model (SAS Institute Inc., Cary, NC). Treatment (naringin), lambs within treatment and day of measurement were included in the model as main effects and treatment x day was included as the interaction term. The effects of time of day were added to the model for analysis of RT and RR. Lambs within treatment were used as a random variable (error term). Data are presented as the least square mean±SE and differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Climate conditions: Mean climatic conditions for the current study are presented in Fig. 1. During the experimental period (July and August), T_a ranged from 25.2°C (at night time) to 45.4°C (at daytime) with a mean of 38.3°C, RH ranged from 12.6-28.4% with a mean of 19.6% and THI ranged from 69.4-86.8 with a mean of 81.6. It is generally accepted that the upper critical temperature of the sheep lies between 25 and 32°C and heat stress occurs when sheep are subjected to temperatures in excess of 32°C or a THI value of 78 (Fuquay, 1981; Silanikove, 2000). In current study, the mean THI over the daytime period was (84.1) indicated that lambs were subjected to moderate heat stress (THI between 78 and 89; Fuquay, 1981). Although, the lambs in the current study were not subjected to severe or extreme climatic conditions, the conditions were harsh enough to invoke a heat load response.

Dry matter intake, bodyweight change and feed efficiency: In the current study, there was no observed difference ($p>0.05$) in DMI between lambs administered orally with naringin and control lambs during heat stress (Table 1) which is consistent studies indicating that there is no beneficial effect of naringin supplementation (1.5 or 3.0 g kg^{-1} DM) on feed intake in Merino sheep under transportation stress (Gladine *et al*., 2007; Bodas *et al*., 2011) or in Holstein heifers fed high-concentrate diets (Balcells *et al*., 2012; Oskoueian *et al*., 2013).

The effects of naringin supplementation on growth rate (bodyweight, ADG and G:F ratio) of Awassi lambs in the current are presented in Table 1. There was difference ($p<0.05$) in growth rate between treatment groups over the experimental period. Supplementation of lambs with 1 g of naringin in the current study resulted increases in ADG

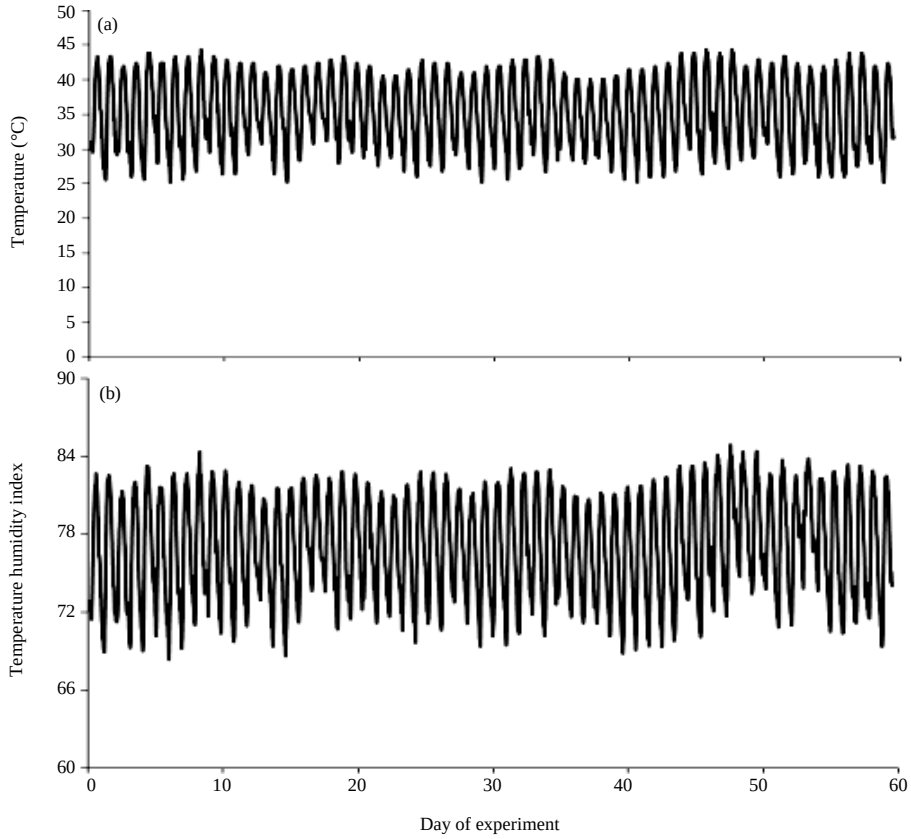

Fig. 1: a) Mean ambient temperature (above) and b) temperature-humidity index (below) during the experimental period. Measurements were made every 30 min for 60 days

Table 1: Effects of naringin supplementation on dry matter intake, bodyweight change and feed efficiencyin lambssubjected to heat stress[1]

	Oral dose of naringin (g/day)				
Items	0.0	1.0	2.0	SE	p-values
Dry matter intake (kg/day)	1.61	1.56	1.59	0.06	0.23
Initial bodyweight (kg)	23.90	24.10	23.60	1.23	0.87
Final bodyweight (kg)	36.90^{b}	38.80^{a}	37.20^{ab}	1.47	0.04
Average daily gain (g/day)	246.00^{b}	278.00^{a}	258.00^{ab}	38.00	0.02
Gain:Feed ratio	152.00^{b}	178.00^{a}	161.00^{ab}	23.00	0.05

$^{a, b}$Within a row, means without a common superscript differ ($p<0.05$); Values are for lambs (n = 18) subjected to heat stress for 60 days

(5.2%; $p<0.02$), final bodyweight (13.1%; $p<0.04$) and greater feed efficiency (17.1%; $p<0.05$) compared with lambs that did not receive naringin supplementation (Table 1). These observations are consistent with the results of previous studies with different species (poultry, rats and rabbits) indicating that growth rate is increased by dietary naringin (Jeon *et al*., 2001; Lien *et al*., 2008). The mechanism by which naringin improves growth rate are not clearly known but could involve improvement of antioxidant defence system (Peterson and Dwyer, 1998; Gladine *et al*., 2007; Bodas *et al*., 2011) orthe regulation of carbohydrate and lipid metabolism (Jung *et al*., 2003; Seo *et al*., 2003; Bodas *et al*., 2011) which would affect positively feed efficiency and growth rate.

Physiological responses: The protective effect of naringin supplementation on rectal temperature and respiration rate ofboth humans and animals to the knowledge has not been demonstrated previously. Moreover, the main RT and RR were similar across treatments in the current study (average, 39.34°C and 57.8 bpm, respectively) over the 3 days of measurement period and there supplementation with naringin did not affect ($p>0.05$) RT or RR (Table 2).

Serum biochemical variables: Serum concentrations of glucose, total protein, globulin and urea, albumin, total in all lambs across treatments in the current study during were unaffected ($p>0.05$) by naringin supplementation (Table 3) and were within the reference ranges reported by previous studies (Arello, 1998; Abdoun *et al*., 2012; Alhidary *et al*., 2012). However, an increase in the serum concentration of albumin was observed ($p<0.03$) in lambs administered orally with 1 g of naringin in the present

Table 2: Effects of naringin supplementation on thermoregulatory response oflambssubjected to heat stress[1]

	Oral dose of naringin (g/day)				
Items	0.0	1.0	2.0	SE	p-values
Rectal temperature (°C)					
0800 h	38.92	39.03	38.97	0.19	0.36
1200 h	39.86	39.97	40.02	0.38	0.51
1600 h	39.47	39.33	39.56	0.23	0.16
Respiration rate (bpm)					
0800 h	37.40	36.60	39.10	4.51	0.24
1200 h	64.70	68.30	67.30	7.07	0.78
1600 h	56.30	52.70	57.10	5.34	0.56

[a, b]Within a row, means without a common superscript differ ($p<0.05$); Values are for lambs (n = 18) subjected to heat stress for 60 days. RT and RR were collected 3 times on day 15, 30 and 45

Table 3: Effects of naringin supplementation on serum concentrations of biochemical variables of lambs subjected to heat stress[1]

	Oral dose of naringin (g/day)				
Analytes	0.0	1.0	2.0	SE	p-values
Glucose (mM)	4.26	4.34	4.41	0.32	0.13
Total protein (g L^{-1})	72.55	70.84	71.58	3.27	0.57
Albumin (g L^{-1})	34.35[b]	36.93[a]	33.32[b]	2.71	0.03
Globulin (g L^{-1})	38.21	37.58	39.71	4.37	0.32
Creatinine (μM)	103.80[a]	91.30[b]	96.20[ab]	9.19	0.01
Urea (mM)	4.91	5.05	4.75	0.94	0.64

[a, b]Within a row, means without a common superscript differ ($p<0.05$); Values are for lambs (n = 18) subjected to heat stress for 60 days. Blood samples were collected on day 1, 30 and 60

study compared with those in other groups (Table 3) and thus it seems likely that this increase in albumin concentration was due to the improvements in antioxidant defence system by the protective role of naringin (Kim *et al*., 2004; Cushnie and Lamb, 2011; Oskoueian *et al*., 2013). It has been found that the blood concentrations of antioxidant capacity including albumin are markedly decreased during a period of high environmental conditions (Miller *et al*., 1993; Gaughan *et al*., 2009; Bernabucci, 2012). Conversely, the oral administration of naringin (1 g/day) decreased ($p<0.01$) concentrations of serum creatinine in the current study (Table 3). This suggests that the reduction in serum concentration of creatinine could be attributed to an increase in the ability of kidneys to creatinine clearance or to an increase muscle activity in the respiratory system and thoracic cavity caused by an increased RR and increased oxygen consumption during hot environmental conditions (Kurahashi and Kuroshima, 1977; Brosnan and Brosnan, 2010).

CONCLUSION

The results from the current study indicate that repeated doses of naringin as oral administration to lambs under Summer month conditions of Saudi Arabia can be used to reduce or alleviate of the consequences of thermal stress including the improved body weight and physiological responses which have important implications for the sheep industry. As several of the indicators observed in this study have not been reported previously further studies are required to elucidate the effects of naringin on productivity and physiological responses in sheep subjected to hot environmental conditions. Different naringin administrations (oral, injection or dietary supplementation) and levels should be considered to determine the most efficient method and levels of naringin administration for sheep when they are exposed to heat stress.

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