

# EFFECTS OF NICARBAZIN AND HOT TEMPERATURE ON EVAPORATIVE WATER LOSS, ACID-BASE BALANCE, BODY TEMPERATURE AND CARBON DIOXIDE EXHALATION IN ADULT ROOSTERS<sup>1</sup>

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## Summary

Two experiments were conducted to study the effects of ambient temperature and nicarbazin on SCWL adult roosters. In Experiment 1, the effects of nicarbazin supplementation (125 ppm) on the water metabolism, blood acid-base balance, and rectal temperature of 16 birds in normal (21°C) and hot (35-36°C) temperatures were investigated. In Experiment 2, the evaporative water loss and CO<sub>2</sub> exhalation from 8 birds were measured individually with an open-circuit gravimetric respiration apparatus in normal (21°C) and hot (33.5-34°C) temperatures. The amounts of water intake and evaporative water loss increased in birds under heat stress (HS). Nicarbazin exacerbated these effects in hot temperature. Also, nicarbazin decreased the blood pCO<sub>2</sub> and increased pH of HS birds. The rectal temperature of birds increased in hot temperature, and nicarbazin worsened this effect. The evaporative water loss, measured directly with the respiration apparatus (Experiment 2), was increased in hot temperature. HS decreased the amount of CO<sub>2</sub> exhalation. Nicarbazin did not exert any effect on either of these measurements, probably due to the limited duration (2 h) of the trial. The decrease in CO<sub>2</sub> exhalation by HS birds could be explained by reduced metabolic rate, which helps homeothermy of birds in hot temperature.

**(Key Words):** Heat Stress, Nicarbazin, Water Balance, Acid-Base Balance, Carbon Dioxide Exhalation, Adult Rooster)

## Introduction

Nicarbazin was introduced for use nearly four decades ago (Cuckler et al., 1956), and is still used extensively because of its effectiveness against various field isolates of coccidia (Mathis and McDougald, 1982). The disadvantage of nicarbazin is its growth-depressing effect, even when used at a level of 125 ppm, the recommended level, or less (Bartov, 1989a,b).

The use of nicarbazin during periods of high environmental temperature is especially restricted due to the increased mortality (McDougald and McQuisiton, 1980). Farny (1965) and Beers et al. (1989) reported that nicarbazin-fed broilers

developed hyperthermia more rapidly than control birds during heat stress (HS).

Two experiments were conducted to study the effects of nicarbazin and hot temperature on the water balance, blood acid-base balance and rectal temperature (Experiment 1), and evaporative water loss and CO<sub>2</sub> exhalation (Experiment 2) in adult roosters.

## Materials and Methods

### Experiment 1

#### Animals, diets and management

Sixteen SCWL adult roosters (BW 2.20 kg) were housed in individual metabolic cages and fed one of the experimental diets as shown in table 1. They were exposed to normal (17-22°C) temperature for 4 d for adjustment, followed by 2-d collection period. Then the room temperature was raised to hot (35-36°C) temperature. There was a 3-d adjustment period, followed by 2-d collection period.

Each bird was offered 80 g of an experimental

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diet (as-fed basis) every morning. Water was provided *ad libitum* with a nipple-attached 1 L plastic bottle to each cage.

The total collection method was used to collect excreta for every 24 h. In order to obtain the moisture content, fresh excreta were collected for every 30 min from 09:00 to 17:00 and stored in an air-tight plastic container. Excreta samples were dried in an oven and the DM contents measured. Total water excretion through cloaca was calculated from the total DM excretion and moisture content of fresh excreta.

TABLE 1. COMPOSITION OF DIETS USED IN EXPERIMENT 1 AND 2

Ingredients	Diets	
	Control	Nicarbazin
	..... (%) .....	
Yellow corn	82	82
Soybean oil meal	15	15
Limestone	1.2	1.2
Calcium phosphate	1.0	1.0
Salt	0.3	0.3
Vit.-min. premix <sup>1</sup>	0.5	0.5
	100.0	100.0
Nicarbazin (mg/kg)	0	125

<sup>1</sup> Vit. min. premix contained the followings per kg: vit. A, 1,750,000 IU; vit. D<sub>3</sub>, 350,000 IU; vit. E, 2,000 IU; vit. K<sub>3</sub>, 880 mg; vit. B<sub>2</sub>, 1,000 mg; vit. B<sub>12</sub>, 3 mg; Ca pantothenate, 1.3 g; nicotinamide, 1.5 g; choline-Cl, 92.18 g; BHT, 0.2 g; ZnSO<sub>4</sub>, 26.4 g; CuSO<sub>4</sub> · 5H<sub>2</sub>O, 3.92 g; FeSO<sub>4</sub>, 24.9 g; MnSO<sub>4</sub>, 30 g; MgSO<sub>4</sub>, 17.3 g; CoCl<sub>2</sub>, 0.41 g; KI, 0.105 g.

The amount of metabolic water produced from each bird was calculated by the method of Newburgh et al. (1930). The evaporative water loss was assumed to be the difference between the total water input (water drunk + water in feed + metabolic water) and total water excretion through cloaca.

#### Blood collection and rectal temperature

At the end of each collection period, blood samples from brachial vein were collected from each bird with 1 mL heparinized syringes. Blood samples were kept on ice until injected into a blood gas analyzer (Corning CAT 477638). After the blood sampling, the rectal temperatures were measured by inserting a thermometer into the

rectum and kept it there for a few min until the temperatures were stabilized.

#### Statistical analysis

Data were subjected to 2 × 2 factorial ANOVA (Snedecor and Cochran, 1967). The main factors tested were ambient temperature and presence or absence of nicarbazin. A one-way ANOVA for 4 treatment means (2 × 2) was done for each variables and, when significant, comparisons were done by the method of Duncan (1955). The limit of probability accepted as being significant was  $p < 0.05$ .

#### Experiment 2

An open-circuit gravimetric respiratory apparatus (Kurihara et al., 1974) was prepared to measure the CO<sub>2</sub> exhalation and evaporative water loss from roosters. Eight SCWL roosters (BW 2.27 kg) were housed in individual metabolic cages and fed one of the diets shown in table 1. Each bird was offered 80 g of the diet at 08:00. In most cases, the birds gavaged themselves with their daily ration in less than an hour.

After 4 d of the preliminary period under a normal temperature (19°C), birds were transferred, one by one, to the respiratory chamber, in which temperature was 21°C. Each run of the respiration trial continued for 2 h. A liquid paraffin tray was placed beneath the cage inside the chamber to minimize the water evaporation from the excreta.

The room and chamber temperatures were 30-32°C and 33.5-34°C, respectively, during the HS episode. Birds were transferred, one by one, to the chamber and kept there for 2 h for the respiration trial. Statistical analyses for data obtained from Experiment 2 were done in the same way as in Experiment 1.

## Results and Discussion

#### Experiment 1

##### Water balance

Total water input was increased by HS (table 2). Nicarbazin increased total water input only under hot temperature. Water output through excreta was not affected by HS but by nicarbazin under normal temperature. HS increased

## HEAT STRESS AND NICARBAZIN ON ADULT ROOSTERS

TABLE 2. EFFECTS OF NICARBAZIN AND AMBIENT TEMPERATURE ON THE WATER BALANCE OF ADULT ROOSTERS (MEAN  $\pm$  SE)

Items	Ambient temperature	Nicarbazin (mg/kg)		$\bar{X}$
		0	125	
Total water input (g/kg BW per day)	Normal	60.7 <sup>a</sup> $\pm$ 4.66	61.3 <sup>a</sup> $\pm$ 1.45	61.0 $\pm$ 2.36
	Hot	93.3 <sup>b</sup> $\pm$ 8.23	122.0 <sup>c</sup> $\pm$ 12.91	108.2* $\pm$ 8.21
	$\bar{X}$	77.5 $\pm$ 6.30	91.6 $\pm$ 10.04	NS
Excreta water output (g/kg BW per day)	Normal	21.1 <sup>a</sup> $\pm$ 1.16	27.1 <sup>b</sup> $\pm$ 1.15	24.1 $\pm$ 1.11
	Hot	22.8 <sup>ab</sup> $\pm$ 2.13	24.0 <sup>ab</sup> $\pm$ 1.51	23.4 $\pm$ 1.27
	$\bar{X}$	22.0 $\pm$ 1.19	25.5* $\pm$ 1.00	NS
Evaporative water loss (g/kg BW per day)	Normal	39.7 <sup>a</sup> $\pm$ 4.92	34.2 <sup>a</sup> $\pm$ 1.09	36.9 $\pm$ 2.53
	Hot	71.6 <sup>b</sup> $\pm$ 6.68	98.0 <sup>c</sup> $\pm$ 12.93	84.8* $\pm$ 7.81
	$\bar{X}$	55.6 $\pm$ 5.75	66.1* $\pm$ 10.35	INT**

<sup>a,b,c</sup> Means with no common superscript among 4 treatments (2  $\times$  2) differ significantly ( $p < .05$ ).

\* Means with asterisks are significantly different from their counterpart ( $p < .05$ ).

\*\* A significant interaction ( $p < .05$ ) was found at 5% level between two main effects.

evaporative water loss, and nicarbazin exacerbated this effect under hot temperature. A significant interaction was found between hot temperature and nicarbazin.

Van Kampen (1981) reported that layers increased their evaporative water loss as the ambient temperature increased. Similar observation was made by Belay and Teeter (1993) with broilers. In order to maintain normal body temperature, homeotherms increase evaporative water loss in hot ambient temperature. In birds, however, evaporative water loss through skin is limited due to the lack of sweat gland. According to Van Kampen (1974), chickens lose similar amounts of heat through body surface and respiration in normal temperature. In hot temperature, however, they increase respiratory heat loss 6 times more than that of skin loss. Dawson (1958) observed that the rate of evaporative water loss in the cardinal increased more than 5 folds between 33 and 41°C, indicating that panting is an effective mechanism for heat dissipation in hot temperature. Nicarbazin increased evaporative water loss in HS roosters, suggesting that it somehow increases respiratory rate, either directly or indirectly.

#### Blood acid-base balance

HS birds increased blood pH and decreased blood pCO<sub>2</sub> only when they were fed nicarba-

zin (table 3), suggesting that hot temperature and nicarbazin act synergistically in incurring these effects.

Many workers (Linsley and Burger, 1964; Calder and Schmidt-Nielsen, 1968; Rottje and Harrison, 1985) observed that blood pCO<sub>2</sub> of birds in hot temperature decreased, and explained it with hyperthermal panting. However, Kleiber (1961) described that homeotherms decrease metabolic rate as the ambient temperature increases. Van Kampen (1981) and Belay and Teeter (1993) also reported that broiler chicks decreased heat production in hot temperature compared to normal ambient temperature. Therefore, it is debatable which one the real cause of blood pCO<sub>2</sub> reduction in HS birds is, either the hyperthermal panting or the decrease in metabolic rate. A direct comparison of CO<sub>2</sub> exhalation between normal and HS birds are necessary in this respect.

The observation of Beers et al. (1989) that nicarbazin decreased blood pCO<sub>2</sub> in HS broilers is similar to the result in table 3. Broilers fed nicarbazin displayed hyperthermal panting at lower temperatures than control birds, indicating that nicarbazin may exacerbate the stress of hot temperature.

#### Rectal temperature

The HS birds increased rectal temperature. Nicarbazin increased rectal temperature of birds

in hot temperature. A significant interaction was found between nicarbazin and ambient temperature. Farny (1965) and Beers et al. (1989) also reported similar results in this respect.

It appears that the increase in evaporative water loss in hot temperature (table 4) is not

sufficient to prevent the rise in body temperature. This is more so when birds are fed nicarbazin. Farny (1965) reported that nicarbazin increased metabolic rate of broilers even in normal temperature.

TABLE 3. EFFECTS OF NICARBAZIN AND AMBIENT TEMPERATURE ON THE BLOOD GAS VALUES OF ADULT ROOSTERS (MEAN  $\pm$  SE)

Items	Ambient temperature	Nicarbazin (mg/kg)		$\bar{X}$
		0	125	
pH	Normal	7.34 <sup>a</sup> $\pm$ 0.02	7.37 <sup>ab</sup> $\pm$ 0.01	7.36 $\pm$ 0.01
	Hot	7.35 <sup>a</sup> $\pm$ 0.01	7.42 <sup>b</sup> $\pm$ 0.02	7.38 $\pm$ 0.01
	$\bar{X}$	7.35 $\pm$ 0.01	7.39* $\pm$ 0.01	NS
pCO <sub>2</sub> (mmHg)	Normal	49.0 <sup>b</sup> $\pm$ 1.84	45.9 <sup>b</sup> $\pm$ 1.50	47.4* $\pm$ 1.22
	Hot	46.9 <sup>b</sup> $\pm$ 1.04	40.9 <sup>a</sup> $\pm$ 1.83	43.9 $\pm$ 1.28
	$\bar{X}$	47.9* $\pm$ 1.06	43.4 $\pm$ 1.31	NS
HCO <sub>3</sub> <sup>-</sup> (mM/L)	Normal	26.5 $\pm$ 0.53	26.6 $\pm$ 0.73	26.6 $\pm$ 0.44
	Hot	25.6 $\pm$ 0.42	25.9 $\pm$ 0.58	25.8 $\pm$ 0.35
	$\bar{X}$	26.1 $\pm$ 0.35	26.3 $\pm$ 0.46	NS
Base excess (mM/L)	Normal	0.63 $\pm$ 0.75	1.38 $\pm$ 0.84	1.00 $\pm$ 0.56
	Hot	0.25 $\pm$ 0.59	1.88 $\pm$ 0.64	0.81 $\pm$ 0.50
	$\bar{X}$	0.19 $\pm$ 0.48	1.63 $\pm$ 0.52	NS

<sup>a,b,c</sup> Means with no common superscript among 4 treatments (2  $\times$  2) differ significantly ( $p < .05$ )

\* Means with asterisks are significantly different from their counterpart ( $p < .05$ ).

TABLE 4. EFFECTS OF NICARBAZIN AND AMBIENT TEMPERATURE ON THE RECTAL TEMPERATURE OF ADULT ROOSTERS (MEAN  $\pm$  SE)

Item	Ambient temperature	Nicarbazin (mg/kg)		$\bar{X}$
		0	125	
Rectal temperature (°C)	Normal	40.6 <sup>a</sup> $\pm$ 0.21	40.6 <sup>a</sup> $\pm$ 0.11	40.6 $\pm$ 0.11
	Hot	42.3 <sup>b</sup> $\pm$ 0.16	42.9 <sup>c</sup> $\pm$ 0.13	42.6* $\pm$ 0.13
	$\bar{X}$	41.4 $\pm$ 0.25	41.7* $\pm$ 0.31	INT**

<sup>a,b,c</sup> Means with no common superscript among 4 treatments (2  $\times$  2) differ significantly ( $p < .05$ ).

\* Means with asterisks are significantly different from their counterpart ( $p < .05$ ).

\*\* A significant interaction was found at 5% level between two main effects.

## Experiment 2

When roosters were housed in the respiration chamber and offered HS for 2 h, nicarbazin-fed birds started to pant earlier than control birds, i.e., 30 min vs. 1 h. The birds did not consume any feed or water when kept in the chamber for

2 h respiration trial.

The HS increased the evaporative water loss and decreased the CO<sub>2</sub> exhalation in birds housed in the chamber (table 5). Nicarbazin did not exert any significant effect on either of these parameters, probably due to the short duration of respiration trial.

## HEAT STRESS AND NICARBAZIN ON ADULT ROOSTERS

TABLE 5. EFFECTS OF NICARBAZIN AND AMBIENT TEMPERATURE ON THE EVAPORATIVE WATER LOSS AND CO<sub>2</sub> PRODUCTION OF ADULT ROOSTERS HOUSED IN RESPIRATORY CHAMBER (MEAN + SE)

Items	Ambient temperature	Nicarbazin (mg/kg)		$\bar{X}$
		0	125	
Evaporative water loss (g/kg BW per h)	Normal	1.30 ± 0.06	1.37 ± 0.08	1.33 ± 0.05
	Hot	2.30 ± 0.03	2.41 ± 0.03	2.35* ± 0.03
	$\bar{X}$	1.79 ± 0.19	1.89 ± 0.20	NS
CO <sub>2</sub> production (L/kg BW per h)	Normal	0.78 ± 0.04	0.80 ± 0.04	0.79* ± 0.03
	Hot	0.67 ± 0.02	0.72 ± 0.02	0.69 ± 0.01
	$\bar{X}$	0.72 ± 0.03	0.76 ± 0.03	NS

\* Means with no common superscript among 4 treatments (2 × 2) differ significantly (p < .05).

The decrease in CO<sub>2</sub> exhalation and the increase in evaporative water loss in HS birds could be mostly explained by lowered pCO<sub>2</sub> (table 3) due to the reduced metabolic rate (Kleiber, 1961). Van Kampen (1981), and Belay and Teeter (1993) also reported that broilers subjected to hot temperature decreased their heat production. The reduced metabolic rate in HS birds may be the direct cause of decreased blood pCO<sub>2</sub>, not the hyperthermal panting itself as claimed so far.

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