

## EFFECTS OF RUMEN-PROTECTED NIACIN (RPN) ON MILK PRODUCTION, SOME BIOCHEMICAL PARAMETERS AND METABOLIC PROFILE INDICES IN DAIRY COWS DURING SUMMER SEASON

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### ABSTRACT

The purpose of the current study was to examine the effects of dietary supplements of Holstein dairy cows with rumen-protected niacin on colostrum quality, milk yield and composition, some blood biochemical parameters, antioxidant status, immunity and incidence of some metabolic disorders during the summer season. A total of 50 pregnant multiparous lactating Holstein cows ( $3 \pm 1$  parity) with average body weights of  $650 \pm 15$  kg. The experiment was done from the last month of pregnancy and continued for four months after calving during the summer season (starting from June to September). The cows were assigned randomly into two experimental groups (25 cows each), as control and treated and fed the same TMR (total mixed ration) that was formulated to meet cows' requirements (NRC, 2001). All cows were fed similarly, except the treated group received 5 gm of rumen-protected niacin (NiaShure™) / head/ day. Values of blood biochemical parameters showed a significant ( $p < 0.05$ ) rise in glucose, albumin and lysozyme levels for the niacin group, compared to the control during July and August. In contrast, Malondialdehyde (MDA) values showed a significant ( $p < 0.05$ ) decrease during July, August and September, while haptoglobins and Oxidative Hemolysis of Peroxidase-Treated Red Blood Cells (OHdG-8) recorded a significant ( $p < 0.05$ ) decrease during July and August only. However, there were non-significant differences between the two groups for dry matter intake (DMI), milk yield, milk fat, protein and solids non fat values. However, the niacin group noted a significant decrease in % of metabolic disorders accompanied by parturition, such as endometritis, mastitis, ketosis and retained placenta, in addition to improvement of colostrum quality and quantity during all summer season. The findings suggested that niacin supplementation could potentially play a role in alleviating the adverse effects of heat stress on lactating animals.

**Keywords words:** Heat stress, rumen-protected niacin, antioxidant status, dairy cattle, milk yield.

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## INTRODUCTION

In recent times, heat stress and climate change have become major issues for Egyptian dairy cattle. Heat stress results in annual losses for the dairy sector due to reduced reproductive performance, higher mortality and decreased production capacity (Nardone *et al.*, 2010). It negatively affects the production, well-being, and health of dairy cows, which has a substantial financial cost to the worldwide dairy cattle industry. Also, it sets off a chain of events that impair dairy cattle's health and productivity as decreases in milk output which may reach up to 25%, depending on the intensity and length of the heat stress (West, 2003 and Sammad *et al.*, 2020). Furthermore, stress-related immune changes can harm health, leading to increased susceptibility to infectious agents and weaken immune responses (West, 2003). Potential markers of thermal stress include heat shock proteins (HSPs), innate immune markers such as acute-phase proteins (APPs), and oxidative stress markers (Alicka, M., and Marycz, K. (2018) Bagath and Rashamol 2019, Arthington *et al.*, 2003). Dairy cows with high productivity are more susceptible to heat stress, especially for higher milk yield, milk protein and fat (Kadzere *et al.*, 2002). We face challenges when high output dairy cows are exposed to heat stress. Therefore, dairy animals are given heat stress relievers like niacin to help them overcome this issue. Niacin is also known as nicotinic acid (NA) or vitamin B3, which is considered one of the water-soluble B-complex (Goldsmith *et al.*, 1952) and plays a significant role in metabolism as a result of their conversion into the coenzymes NAD and NADP, which are necessary for a number of oxidation events in energy metabolism. (Nelson *et al.*, 2008), including lipid and glucose metabolism (Pires *et al.*, 2016). Supplemented (NA) has unsteadiness in the rumen, and only 5% is estimated to be bioavailable, making its supplementation inefficient. So, a rumen-protected form of NA (encapsulated niacin; EN) is a

commercially accessible form of NA that is rumen-protected and offers a more efficient dietary supplementation option for NA. (Morey *et al.*, 2011). Colostrum is a vital source of immune protection, which contains sufficient amount of immunoglobulin as well as giving the calf passive immunity. Furthermore, colostrum must be given to newborn calves as soon as possible after birth, to develop normally (Bielmann *et al.*, 2010). Many studies have been conducted on the effects of supplementary (EN) on metabolism during the pre- and postpartum period. (Neihoff *et al.*, 2009), However, little is known about how it might affect the quality of colostrum or calf immunity. Which are the key factors for dairy farm success and profitability.

The present study was designed to study the effects of niacin supplementation on milk yield, milk composition, colostrum quality, immunity, some blood biochemical parameters, and blood antioxidant activities in heat stressed dairy cow.

## MATERIALS AND METHODS

### 1. Ethics Statement

This present study was approved by the institutional Animal Care and Welfare Committee Ethics (Approval No. ARC ARRI 75/21), Faculty of Veterinary Medicine, Cairo University.

### 2. Experimental Design

The experiment was done on a private farm of dairy cattle in Wadi Elnatroun, governorate, Egypt on fifty pregnant multiparous lactating Holstein cows ( $3 \pm 1$  parity) with average body weights  $650 \pm 15$  kg. The experiment began from the last month of pregnancy and continued for four months during the summer season. The cows were randomly blocked into 2 groups of control and treated groups (25 cows each) and fed the same TMR (total mixed ration) that was formulated to meet requirements (NRC, 2001). Each group was housed in open yards with free access to water and

feed. All cows were fed similarly, except that the treated group received 5gm of rumen-protected niacin (NiaShure™) / head/day (Yanxia *et al.* 2008).

The experiment period was extended from June to September (2022). Representative feed samples were taken at the beginning of the experiment for chemical analysis.

**Table 1:** Composition of experimental group diet.

Item	Control Diet As-fed basis (kg/head /day)
<b>Ingredients</b>	
Corn grain, ground , dry	5
Flax , crushed	1
Rice bran	1
Soybean meal 46%	3
Alfalfa hay mid- maturity	4
DDGS, low fat	1
Sodium Bicarbonate	0.15
Wheat straw	0.5
white Corn silage , 28 % DM	22
Lupine , coarse ground	1
Mono- basic calcium phosphate	0.05
Fractionated palm oil 99%	0.3
Glycerol	0.05
Salt	0.07
Lithithamnium calcarum	0.1
Premix <sup>1</sup>	0.025
Toxisorb premium	0.02
<b>Chemical analysis:</b>	
CP (% of DM) <sup>2</sup>	17.53
NEL (M. Cal. /kg. DM) <sup>3</sup>	1.73
NDF (% DM) <sup>4</sup>	30.45
ADF (% DM) <sup>5</sup>	19.6
Forage NDF (% DM)	22.84
Starch	26.68
E.E (% of DM) <sup>6</sup>	5.69
Diet RUP <sup>7</sup>	5.69

1= (Vit. A 9000000 IU ,Vit D3 2500000 IU, Vit. E 35000 mg , Biotin 1000 mg , Zinc 100000 mg, Mn 80000 mg, Cu 30000 mg, I 800 mg, Co 400 mg , Se 400 mg.

2 = crude protein, 3= net energy for lactation, 4= neutral detergent fiber, 5= acid detergent fiber, 6 = ether extract, 7= rumen un-degradable protein

### 3. Milk analysis:

#### 3.1. Colostrum sample collection

Fifty ml of colostrum samples were collected at the first milking between 1 and 15 h after calving and stored at  $-20^{\circ}\text{C}$  until tested (Elsohaby *et al.*, 2017).

#### 3.2. Brix Refractometer Indirect Measurement Methods

An indirect on-farm measurement method to determine the immunoglobulins concentration in colostrum was done to assess colostrum quality (Bartens *et al.*, 2016).

A Brix Refractometer expressed results as % Brix, (Quigley *et al.*, 2013), a Brix score (or density) of  $\geq 22$  percent is the cut off for detecting good quality colostrum (Bielmann, 2010).

#### 3.3. Method for using a brix refractometer to measure colostrum quality

An optical Brix refractometer (model 300001; SPER Scientific, Scottsdale, AZ), with a scale from 0 to 32% Brix. Approximately 50 ml of colostrum was placed on the prism and the sample cover was lowered. The refractometer was then held up to a light source, and the Brix score was read through the view finder at the interface between light and dark areas. The refractometers were cleaned and calibrated with distilled water at room temperature before each analysis (Elsohaby *et al.*, 2017).

The **Interpreting the reading:** (Weaver *et al.*, 2000)

- $\geq 22\%$  means good quality colostrum. (IgG concentration  $\geq 50$  mg/ml)
- 18-21% means poor quality colostrum.
- $<18\%$  means unsuitable colostrum.

#### 3.4. Measurement of milk composition:

Tank milk samples were collected weekly and analyzed using Lactoscan Ultrasonic Milk Analyzer (Bulgaria-25010) (APHA, 2004 “American Public Health Association”).

This milk analyzer can analyze the percentage of fat, solid non fat SNF, protein,

of the same sample directly after milking, without the need to prepare samples.

#### **4. Blood sampling for biochemical, antioxidants' and immunological analysis:**

Twenty-five cows in each group were randomly selected to study the effect of each treatment on various blood biochemical markers, antioxidant levels, and some immunological responses

**4.1. Sampling:** Ten ml of blood samples (with anticoagulant for separation of plasma and without anticoagulant for separation of serum) were collected monthly from the jugular vein 4 h after feeding during the trial period. Blood samples were centrifuged to separate the serum and plasma (3000 rpm for 30 min) that was preserved at -20 °C even conducting analysis.

**4.2. Biochemical analysis:** Samples were analyzed for biochemical analysis (Science & Technology Center STC). Total proteins (TP) and albumin (alb) according to Randall Krohn (2011), while globulin was calculated by subtracting the albumin values from the total protein values (Jolles *et al.*, 2014). Cholesterol was analyzed according to Allain (1974). Glucose level was determined according to Trinder (1969). Calcium was analyzed according to Burtis *et al.* (1999) and phosphorus was analyzed according to El-Merzabani *et al.* (1977).

**4.3. The antioxidants activities:** The antioxidants activities were spectrophotometrically analyzed by using standardized commercial test-kits (Biodiagnostic); total antioxidant capacity (TAC) (Koracevic *et al.*, 2001), reduced glutathione (GSH) (Anderson, *et al.*, 1989), superoxide dismutase (SOD) (Marklund, 1980), catalase enzyme activity (CAT) (Fossati, 1980), malondialdehyde (MDA) (Ohkawa *et al.*, 1979).

#### **4.4. Immunological parameters:**

Immunological parameters were carried out as follows: Estimation of Nitric oxide levels was done according to Rajarman *et al.*

(1998) using an ELISA plate reader (Epoch, BioTek, Germany). The rumen-protected niacin supplementation of dairy cows. Detection of lysozyme concentration was carried out according to Schultz (1987). Determination of haptoglobins level was carried out using standard kits of (Cloud-Clone Corp (PRC) Shanghai) and ELISA reader (Rayto, PRC, Germany). Determination of Oxidative Hemolysis of Peroxidase-Treated Red Blood Cells (OHdG-8): It was measured by commercial ELISA kits (SunRed Shanghai).

**5. Diagnosis of metabolic disorders:** Cows were assessed daily for health by rectal thermometer, stethoscope, and physical examination.

#### **5.1 Diagnosis of subclinical mastitis by using California milk test (CMT).**

For detection of subclinical mastitis as a field test recommended by the American Public Health Association (A.P.H.A., 1992) according to Schalm *et al.* (1971)

#### **5.2 Diagnosis of endometritis by using ultrasonography.**

Ultrasonography was performed by using real-time B-mode scanners (Sonoscape-China) equipped with 4-7.5 MHz frequency linear-array rectal transducer for diagnosis of endometritis; determine the thickness of the uterine wall and accumulation of uterine fluid.

**5.3 Diagnosis of retained placenta** (failure to expel fetal membranes within 24 h post-parturition (Qu *et al.*, 2014).

**5.4 Diagnosis of ketosis** on urinary acetoacetate concentration >40 mg/dl, as measured with Ketostix strips (Bayer Corp., Elkhart, IN) (Qu *et al.*, 2014).

#### **6. Measurement of body temperature**

The rectal body temperature was recorded daily with an electronic thermometer once a day during the whole experiment period.

#### **7. Statistical analysis:**

Analysis of variance (ANOVA) was used to examine statistical differences across groups, and then, Duncan's test, using SPSS for Windows version 16.0 (SPSS 16.0, SPSS

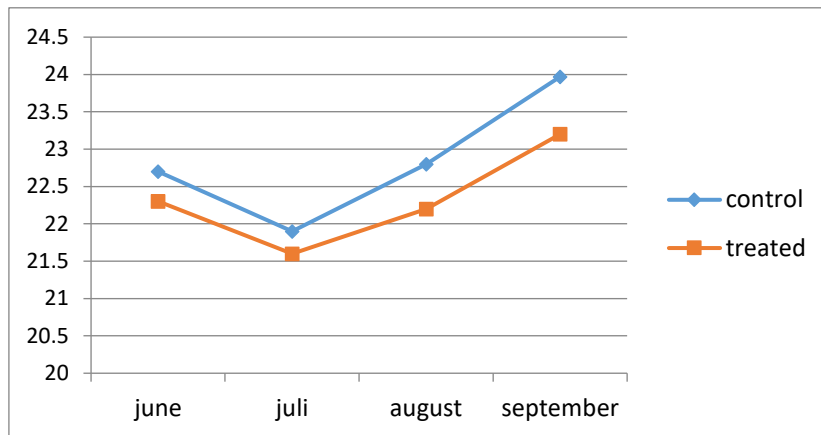
Inc., 2007), at (<0.05) significant levels were considered. A percentage test was used for analysis of the results of metabolic disorders.

**RESULTS**

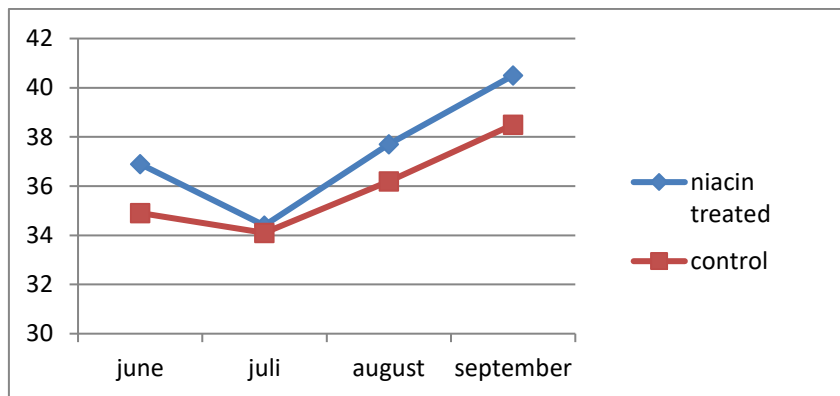
**DMI, milk yield and composition:**

The results of DMI (Fig.1), milk yield (Fig.2) and milk composition are

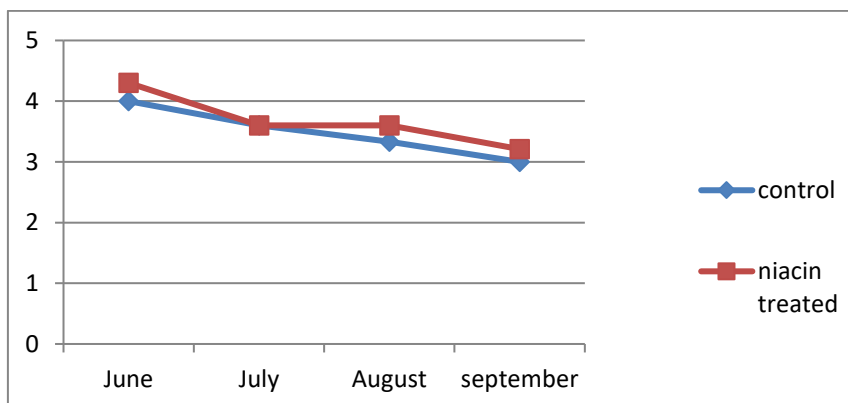
presented in Table (2). The results showed non-significant differences (P<0.05) between the two groups.



**Fig. 1:** The effect of rumen-protected niacin supplementation on dry matter intake (DMI) of dairy cows:



**Fig. 2:** The effect of rumen-protected niacin supplementation on milk yield of dairy cows



**Fig. 3:** The effect of rumen-protected niacin supplementation on milk fat (%) of dairy cows.

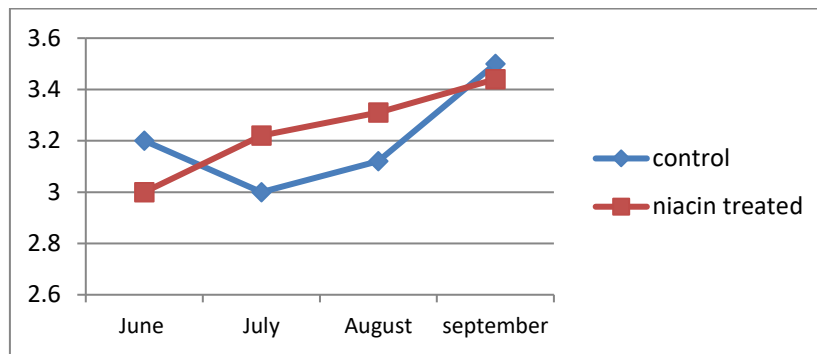


Fig. 4: The effect of rumen-protected niacin supplementation on Milk protein (%) of dairy cows:

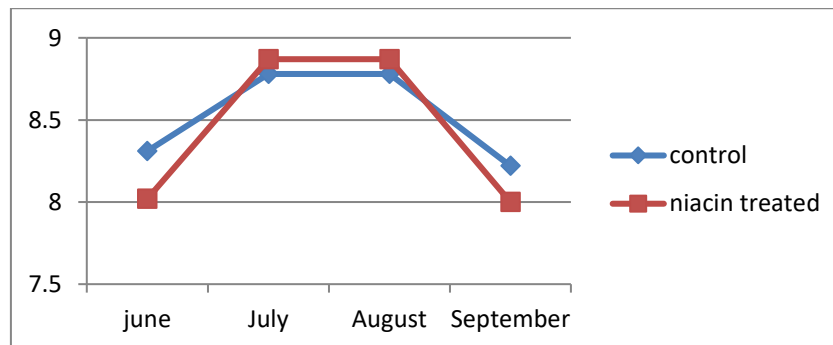


Fig. 5: The effect of rumen-protected niacin supplementation on solids non fat (%) of dairy cows

Table 2: The effect of rumen-protected niacin supplementation of dairy cows on milk yield and composition

Month	Milk yield (kg/d)		Milk fat (%)		Milk protein (%)		Solids non fat (%)	
	control	treated	control	treated	control	treated	control	treated
June(n=25)	34.9±0.8	36.9±1.2	4.0±0.54	4.3±0.332	3.2±0.51	3.0±0.09	8.31±0.76	8.02±0.85
July(n=25)	34.1± 0.4	34.4± 0.6	3.60± 0.125	3.61 ±0.234	3.09 ±0.143	3.22 ± 0.136	8.78 ± 0.198	8.87 ± 0.201
August(n=25)	36.2±0.1.2	37.7±0.1.7	3.33±0.65	3.6±0.11	3.12±0.06	3.31±0.02	8.78 ± 0.198	8.87 ± 0.201
Sep. (n=25)	38.5±0.6	40.5±0.4	3.0±0.11	3.21±0.15	3.5±0.32	3.44±0.05	8.22±0.98	8.0±1.0

m ± SE, n=25.

Our results recorded in Table (3) revealed a significant decrease ( $P<0.05$ ) in body temperature in the niacin treated group during August and September ( $38.8\pm0.07$ ,  $39.5\pm0.09$  &  $38.6\pm0.09$ ,  $39.3\pm0.06$ ) respectively, while in June and July recorded a slight decrease by 3 fractions ( $38.8\pm0.03$ ,  $38.5\pm0.02$  &  $38.8\pm0.04$ ,  $39.1\pm0.07$ ), respectively, compared to the control group.

On the other hand, colostrum quality recorded a significant increase ( $P<0.05$ ) in the niacin treated group, compared to the

control group during the whole summer season ( $30.4\pm0.33$ ,  $24.4\pm0.58$  &  $30.2\pm0.33$ ,  $21.0\pm0.16$  &  $30.8\pm0.33$ ,  $22.8\pm0.62$  &  $31\pm0.29$ ,  $23.6\pm0.45\%$ ), respectively. On the same pattern, colostrum quantity recorded a significant increase ( $P<0.05$ ) in niacin treated group during the same period ( $5.6\pm0.43$ ,  $2.8\pm0.25$  &  $5.8\pm0.25$ ,  $2.7\pm0.35$  &  $8\pm0.52$ ,  $3.2\pm0.25$  &  $5\pm0.21$ ,  $3.4\pm0.49\text{kg}$ ), respectively. This finding is accompanied by a high HI index, especially during July (Max.85, Min.76) and August (Max.83, Min.77).

**Table 3:** Effect of rumen-protected niacin supplementation of dairy cows on rectal temperature and colostrum quality & quantity: m ± SE, n=25.

Month	HI	Rectal temp.		colostrum quality%		colostrum quantity (kg/d)	
		control	treated	control	treated	control	treated
<b>June</b> (n=25)	Max.80	38.5±0.02°	38.8±0.03 °	24.4±0.58	30.4±0.33*	2.8±0.25	5.6±0.34**
	Min.74						
<b>July</b> (n=25)	Max.85	39.1±0.07 °	38.8±0.04 °	21±0.16	30.2±0.33*	2.7±0.35	5.8±0.25**
	Min.76						
<b>August</b> (n=25)	Max.83	39.5±0.09 °	38.8±0.07 ° *	22.8±0.62	30.8±0.33*	3.2±0.25	8±0.52***
	Min.77						
<b>Sep.</b> (n=25)	Max.77	39.3±0.06 °	38.66±0.09 ° *	23.6±0.45	31±0.29**	3.4±0.49	5±0.21**
	Min.70						

The obtained results from the present study (Table 4) showed that albumin level in niacin treated group was significantly ( $P<0.05$ ) increased than the control group level in July ( $5.169 \pm 0.079$  and  $4.873 \pm 0.107$  g/dl,) and in August ( $5.87 \pm 0.31$  and  $4.32 \pm 0.20$ ), respectively. Similar trend was also noticed in glucose level of the niacin treated group, compared to the control one during July, August and

September ( $83.268 \pm 3.980$ ,  $69.61 \pm 1.66$  &  $88.2 \pm 5.32$ ,  $74.8 \pm 1.98$  &  $80.268 \pm 3.980$ ,  $74.61 \pm 5.66$  mg/dl), respectively. However, supplementing diets with rumen-protected niacin did not significantly affect ( $P<0.05$ ) concentrations of serum total protein, globulin, cholesterol, calcium, and phosphorus (Table 4).

**Table 4:** Effect of rumen-protected niacin supplementation of dairy cows on different blood parameters during the summer season: m ± SE, n=25.

	June (n=25)		July (n=25)		August (n=25)		September (n=25)	
	control	treated	control	treated	control	treated	control	treated
<b>Total protein (g/dl)</b>	10.9±0.51	11.2±0.3	10.0±0.344	10.502±0.313	10.4±0.24	11.1±0.45	11±0.56	11.5±0.53
<b>Albumin (g/dl)</b>	5.55±0.22	6.02±0.83	4.873±0.107	5.169±0.079*	4.32±0.20	5.87±0.31*	6.01±0.36	5.99±0.22
<b>Globulin (g/dl)</b>	5.35±0.096	4.78±0.42	5.240±0.25	5.333±0.292	6.54±0.65	5.23±0.54	4.99±0.43	5.51±0.62
<b>Cholesterol (mg/dl)</b>	203 ± 5.87	200± 6.34	202.25± 13.34	199.699± 7.89	196.8± 8.88	189± 5.87	202.25± 13.34	199.699± 7.89
<b>Glucose (mg/dl)</b>	76.7± 3.32 b	80.4± 3.98	69.61± 1.66	83.268± 3.980*	74.8± 1.98	88.2± 5.32 *	74.61± 5.66	80.268± 3.980 *
<b>Calcium (mg/dl)</b>	9.03± 0.4	9± 0.54	8.77± 0.405	9.062± 0.291	9.03± 0.25	9.43± 0.451	8.77± 0.405	9.062± 0.291
<b>Phosphorus (mg/dl)</b>	4.98± 0.37	5.09± 0.032	5.07± 0.168	5.3± 0.080	5.3± 0.4	5.54± 0.21	5.07± 0.168	4.968± 0.23

In Table 5, our study demonstrated that MDA values among all antioxidant parameters showed a significant ( $p<0.05$ ) reduction for the niacin treated group, compared to the control group during July,

August & September ( $4.12 \pm 0.42$ ,  $6.850 \pm 0.603$  &  $4.09 \pm 0.45$ ,  $6.99 \pm 0.7$  &  $4.53 \pm 0.18$ ,  $5.99 \pm 0.88$  nmol/ml), respectively.

**Table 5:** Effect of rumen-protected niacin supplementation of dairy cows on some antioxidant parameters during summer season.  $m \pm SE$ ,  $n=25$ .

	June(n=25)		July(n=25)		August(n=25)		September(n=25)	
	control	treated	control	treated	control	treated	control	treated
<b>TAC</b> (mM\L)	1.23± 0.054	1.45± 0.027	1.364± 0.097	1.2280± 0.087	1.66± 0.033	1.87± 0.05	1.69± 0.1	1.89± 0.022
<b>CAT</b> (U\L)	269.9± 10.5	265± 14.89	272.7± 12.852	273.68± 7.578	278.7± 16.05	280.6± 7.59	278.7± 13.9	276.6± 6.66
<b>SOD</b> (U\mL)	33.53± 4.76	30.99± 3.33	35.498± 4.606	34.558± 2.081	36.6± 2.8	37.5± 3.05	35.99± 3.1	37.7± 4.35
<b>GSH</b> (mU\mL)	5.42± 0.32	5.65± 0.55	5.130± 0.419	4.508± 0.233	5.13± 0.67	5.07± 0.55	5.56± 0.98	5.09± 0.55
<b>MDA</b> (nmol\mL)	5.01± 0.8	4.76± 0.55	6.850± 0.603	4.12± 0.42 <sup>**</sup>	6.99± 0.7	4.09± 0.45 <sup>**</sup>	5.99± 0.88	4.53± 0.18 <sup>*</sup>

Table (6) showed a significant decrease ( $P < 0.05$ ) in haptoglobins level during June, July and August ( $21.5 \pm 2.4b$ ,  $28.5 \pm 2.0$  &  $22.22 \pm 2.314$ ,  $37.600 \pm 3.429$  &  $20.56 \pm 4.34$ ,  $33.98 \pm 5.59$   $\mu\text{g/ml}$ ), respectively. While

(OHdG-8) recorded a significant decrease ( $P < 0.05$ ) during July and August only ( $0.8368 \pm 0.105$ ,  $1.9340 \pm 0.069$  &  $1.0 \pm 0.05$ ,  $1.96 \pm 0.055$   $\text{ng/ml}$ ), respectively.

**Table 6:** Effect of rumen-protected niacin supplementation of dairy cows on Haptoglobins and (OHdG-8) measurements:  $m \pm SE$ ,  $n=25$ .

	June(n=25)		July(n=25)		August(n=25)		September(n=25)	
	control	treated	control	treated	control	treated	control	treated
<b>Haptoglobins</b> ( $\mu\text{g/ml}$ )	28.5± 2.0	21.5± 2.4 <sup>*</sup>	37.600± 3.429	22.22± 2.314 <sup>*</sup>	33.98± 5.59	20.56± 4.34 <sup>*</sup>	30.7± 1.9	29.22± 0.914
<b>(OHdG-8)</b> (ng/ml)	1.97± 0.022	0.94± 0.066	1.9340± 0.069	0.8368± 0.105 <sup>*</sup>	1.96± 0.055	1.0± 0.056 <sup>*</sup>	1.0± 0.098	0.989± 0.085

Data of some immunological status showed non-significant changes ( $P < 0.05$ ) in Nitric oxide (NO) values in the niacin treated group against the control group, while lysozyme level showed a significant

increase ( $P < 0.05$ ) in the treated group, compared with the control group during July and August ( $79.644 \pm 7.739$ ,  $50.450 \pm 3.619$  &  $66.4 \pm 3.4$ ,  $51.3 \pm 2.1$   $\mu\text{g/ml}$ ), respectively.

**Table 7:** Effect of rumen-protected niacin supplementation of dairy cows on some immunological measurements:  $m \pm SE$ ,  $n=25$ .

	June (n=25)		July (n=25)		August (n=25)		September (n=25)	
	control	treated	control	treated	control	treated	control	treated
<b>Nitric oxide</b> (uM\ml)	32.8± 7.77	30.9± 2.9	31.410± 2.651	33.733± 1.466	28.99± 6.4	27.2± 2.22	31.410± 2.651	34.733± 1.466
<b>Lysozyme</b> (ug/ml)	48.9±4	54.7± 8.33	50.450± 3.619	79.644± 7.739 <sup>*</sup>	51.3± 2.1	66.4± 3.4 <sup>*</sup>	48.350± 5.19	52.04± 3.39

Ketosis % showed a significant decrease ( $P < 0.05$ ) in the niacin-treated group, compared to the control one during the whole experimental period (7.4, 10 & 14.15, 6 & 1.4, 3 & 0, 4.8, 2.2%), respectively. On the other hand, mastitis % revealed a significant decrease ( $P < 0.05$ ) during June,

August and September (3.7, 6.7 & 0.4, 5 & 2.5, 5%), respectively. Both retained placenta and endometritis % recorded a significant decrease ( $P < 0.05$ ) during the whole experimental period (3.7, 10 & 4.7, 8.9 & 2.5, 13.4 & 0, 14.3), and (14.8, 26.6 & 16.3, 20 & 8, 9 & 0, 9.5), respectively.



**Table 8:** Effect of rumen-protected niacin supplementation of dairy cows on metabolic disorders  $m \pm SE$ ,  $n=25$ .

month	Ketosis%		Mastitis%		Retained placenta%		Endometritis%	
	control	treated	control	treated	control	treated	control	treated
<b>June</b> (n=25)	10	7.4*	6.7	3.7*	10	3.7*	26.7	14.8*
<b>July.</b> (n=25)	15.6	14*	2.2	2.7	8.9	4.7*	20	16.3*
<b>August.</b> (n=25)	3	1.4*	4.5	0	13.4	2.5*	9	8*
<b>Sep.</b> (n=25)	4.8	0	5.5	2*	14.3	0	9.5	0

## DISCUSSION

This study aimed to alleviate the adverse effects of heat stress on dairy cows by supplementing their diet with rumen-protected niacin and to evaluate its effect on various parameters, including feed intake, milk yield, immunity, some biochemical parameters, production and reproduction performance in dairy cows during the summer season. The rumen-protected form of niacin is commonly referred to as encapsulated niacin. These items are essentially little pellets with multiple layers of lipids covering the niacin in the middle. Because encapsulation increases niacin's bioavailability in the small intestine, the lipid layers in the pellet are relatively undegradable in the rumen, preventing the rumen microbes from degrading niacin (Morey *et al.*, 2011). Our study showed non-significant changes in the niacin-treated group, compared to the control group in DMI and milk yield values. This finding was in contrast with the common assumption that niacin supplementation can positively impact milk production (Naren *et al.*, 2021). This result may be contributed to different factors, such as the duration of supplementation, niacin dosage, and the specific physiological state of the cows. The present result was consistent with that of Yuan *et al.* (2011). Concerning milk composition, there were marginal differences observed between the control and niacin-supplemented groups. Milk fat content exhibited a negligible change between the two groups; this finding was in agreement with Zimbelman *et al.* (2013). Milk protein content showed a slight increase

in the niacin-supplemented group, suggesting that niacin supplementation might have a subtle effect on milk components. Further studies for exploring the underlying mechanisms and the potential impacts on milk quality are warranted. This outcome indicates that niacin supplementation might have a limited influence on milk composition. This finding agreed with Dufva *et al.* (1983), Skaar *et al.* (1989), Campbell *et al.* (1994) and Gorniak *et al.* (2014), who stated that feeding niacin did not increase the milk yield, but reduced milk fat rate and they pointed out that the niacin reduced the yield of plasma triglycerides. Further research, potentially considering factors such as cow health, stage of lactation, and dietary interactions, could show a more comprehensive understanding of the relationship between niacin supplementation and dairy cow productive performance (Arash *et al.*, 2016).

The present results revealed an increase in colostrum quality and quantity in the niacin supplemented group, compared to the control group, especially in July and August, when the HI index was at its highest level. Since the placental barrier's construction prevents immunity from being transferred from the mother's circulatory system to the foetus, managing colostrum in cows is crucial (Chucuri *et al.*, 2010). Therefore, immunity needs to be obtained passively from high-quality colostrum (IgG concentration  $\geq 50$  mg/ml (Weaver *et al.*, 2000, McGuirk and Collins, 2004). Niacin can alter the composition of milk and the

ruminal environment, in addition to improving blood flow to the mammary gland during the colostrum process. This could lead to an increase in the nutrients and immunoglobulin concentration in the colostrum. (Panel *et al.*, 2016).

Body temperature recorded a decrease in the niacin-treated group by a few fractions, compared to the control group. Niacin can promote both internal and peripheral vasodilatation, which enhances heat transfer from the body to skin sites and produces a temperature gradient that encourages heat escape from the skin into the surrounding air (Rungruang *et al.*, 2014). When exposed to mild or severe heat stress, the niacin-supplemented group showed a significant drop in skin and body temperatures compared to the control group, according to several studies. (Zimbelman *et al.*, 2013). Moreover, Pineda *et al.* (2016) indicated that rumen-protected niacin could reduce dairy cow body core temperature in high-temperature environments, which improves animal conditions in general, in addition to providing potential beneficial effects when cows are under heat stress (Yuan, 2012).

The obtained results of serum biochemical in the niacin supplementing group exhibited a non-significant increase in total protein level, compared to the control group. It suggests a possible influence of niacin supplementation on protein metabolism or protein synthesis. The increase in total protein could refer to alterations in amino acid metabolism, which could be explored in future studies. Albumin levels in the niacin-treated group were significantly increased in July and August, compared with the control group. This result might indicate improved protein synthesis or enhanced liver function (Kumar and Dass, 2006 and Niehoff *et al.*, 2009). A similar trend was also noticed in the glucose level of niacin niacin-supplemented group, compared to the control one during July, August and September. This elevation in glucose level might indicate improved glucose homeostasis or altered glucose utilization,

possibly linked to niacin's impact on energy metabolism. This result was in agreement with Ghorbani *et al.* (2008). He indicated that because niacin is integrated into the coenzymes NAD and NADP, it has a major effect on metabolism, which is necessary for a number of oxidation processes in the energy metabolism. (Nelson *et al.* 2008), including lipid and glucose metabolism (Pires *et al.*, 2016).

The data in the present study provided valuable insights into the differences between the niacin-treated group and the control group in terms of total antioxidant capacity (TAC), catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA) levels. Moreover, the present study recorded a nonsignificant change in all oxidant-antioxidant profiles except MDA. This finding was in agreement with the results of Bernabucci *et al.* (2002), who did not observe any association between niacin status and antioxidant enzyme activity, and disagreed with other experimental studies which indicated that niacin can increase SOD, CAT and GSH activities in hepatic tissue in rats (Perumal *et al.*, 2005, Varella *et al.*, 2006, and Tupe *et al.*, 2011). Malondialdehyde (MDA) values showed a significant reduction in the niacin group, compared to the control group during July, August & September. MDA is a marker of lipid peroxidation, and the lower levels in the niacin-treated group might indicate reduced oxidative damage to lipids. This finding is consistent with the findings of Talija *et al.* (2018) and Tupe *et al.* (2011) who mentioned that the concentration of MDA linearly decreased in the cows receiving niacin. Decreased lipid peroxidation during niacin supplementation may be due to its antilipolytic effect. Furthermore, our study's findings supported niacin's antioxidant properties and showed that it can protect the body from oxidative stress, particularly lipid peroxidation.

The data in the present study provided insights into the differences between the

niacin-supplemented group and the control group in terms of haptoglobin, oxidative hemolysis of peroxidase-treated red blood cells (OHdG-8), nitric oxide (NO) levels, and lysozyme activity. The niacin-treated group displayed lower levels of haptoglobin, compared to the control group during June, July and August. Haptoglobin is an acute-phase protein that increases in response to inflammation or infection. Stressors such as heat stress have been demonstrated to cause elevated blood levels of acute-phase proteins (APP), including haptoglobin, which is considered mirror stress in cattle. The lower haptoglobin levels in the niacin-treated group suggested a potential modulation of the acute-phase response (Arthington *et al.*, 2003), which indicates that niacin supplementation decreases stress proteins which reflect on animal body temperature. For (OHdG-8), the values were lower in the niacin-treated group, compared to the control group. (OHdG-8) is an indicator of oxidative damage to red blood cells. The decreased (OHdG-8) values in the niacin-treated group suggest a potential reduction in oxidative stress-induced hemolysis. This finding aligns with niacin's role as an antioxidant and its ability to mitigate oxidative damage. Heat stress can have a negative impact on immunity and health. It can weaken immune responses, increase vulnerability to infectious agents, and affect how severe an infectious disease is (West, 2003). Heat stress also has direct effects on immune function in cattle during the dry period, and those impacts decline after calving. However, lysozyme activity was higher in the niacin-treated group, compared to the control group. Lysozyme is an enzyme involved in innate immunity, contributing to bacterial cell wall degradation. It is a protein found in many animal and plant tissues as well as mucosal secretions like tears, saliva, and mucus. It is necessary for innate immunity, since it protects against bacteria, viruses, and fungi (Patrizia *et al.*, 2021). The increased lysozyme activity in the niacin-treated group suggests a potential enhancement of innate immune responses,

possibly indicating improved resistance to bacterial infections (Lin Feng *et al.*, 2016).

Dairy cows with high productivity are more susceptible to heat stress and more prone to metabolic stress during parturition due to increased demands for milk production, which is accompanied by decreased feed intake and an unfavorable energy balance. As a result, the organism enters a state of oxidative stress. The periparturient period in cows is associated with metabolic stress and a state of negative energy balance. These metabolic changes may have a negative impact on the health and milk yield of lactating cows (Kosta *et al.*, 2020). In our study, Ketosis % showed a significant decrease in the niacin treated group, compared to the control one during the whole experimental period, as Niacin is involved in the metabolism of body fat during early lactation and may lower the incidence of ketosis. Niacin also plays a critical role in oxidation and synthesis of glucose forming VFAs and prevents the formation of ketosis. So it lowers the level of ketone bodies and keeps cows from developing fatty livers or going into ketosis (Bulent and Seda 2018). Retained placenta and endometritis recorded a significant decrease during the experimental period. While mastitis % recorded a significant decrease during June, August and September in niacin treated cows, as niacin has anti-lipolytic and anti-inflammatory effects (Kosta *et al.*, 2020) and can increase the blood flow to the mammary gland, which can improve the udder health immunity (Panel *et al.*, 2016).

## CONCLUSION

In conclusion, adding 5 g /head /day of rumen-protected niacin during the summer season acts as a heat stress ameliorator and implies a positive impact on the animal's ability to cope with heat stress. Niacin can enhance the performance and reduce body temperature. The modulation of oxidative stress as (OHdG-8) and malondialdehyde support the niacin's role in mitigating heat-

induced oxidative damage. Also, niacin has an effective role in improving colostrum quality and quantity and decreasing metabolic disorders. The observed differences in haptoglobins, (OHdG-8) and lysozyme activity imply potential effects of niacin on the acute phase protein, oxidative stress, and innate immunity.

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## دراسة تأثير اضافة النياسين المحمي على الاداء الانتاجي والتكاثرى في الأبقار الحلابة خلال موسم الصيف

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تهدف الدراسة الحالية إلى دراسة تأثير اضافة النياسين المحمي الي أبقار الهولشتاين الحلوب على جودة الكولوسترم وإنتاج الحليب وتركيبه وبعض القياسات البيوكيميائية والمناعية بالاضافة الى مستوى مضادات الأكسدة ونسبة حدوث بعض الاضطرابات الأيضية المصاحبة للولادة خلال فصل الصيف.

تم إجراء هذه التجربة على مجموعة من ٥٠ بقرة هولشتاين حامل متعددة الولادات (عدد الولادات  $3 \pm 1$ ) بمتوسط وزن جسم يبلغ ( $650 \pm 10$ ) كجم. بدأت التجربة في الشهر الأخير من الحمل واستمرت لمدة أربعة أشهر خلال فصل الصيف (تبدأ الدراسة من يونيو إلى سبتمبر). تم تقسيم الأبقار عشوائياً إلى مجموعتين مجموعة ضابطة ومجموعة معالجة بالنياسين المحمي من الكرش (٢٥ بقرة لكل منهما)، وتم تغذية ابقار كلا منهنما بنفس العليقة والتي تمت صياغتها لتلبية متطلبات الأبقار (تي أم آر)، مع اضافة ٥ جرام من النياسين المحمي /للراس/اليوم الي المجموعة المعالجة.

أظهرت النتائج ان اضافة النياسين المحمي الي العليقة يؤدي الي زيادة معنوية في مستوى الجلوكوز والألبومين بلاضافة الي دوره في تحفيز المناعة من خلال الزيادة المعنوية في مستوى الليزوزيم والهابتوجلوبين. من ناحية أخرى سجلت مجموعة النياسين نقص معنوي في نسبة الاضطرابات الأيضية المصاحبة للولادة كالتهاب بطانة الرحم والتهاب الضرع والكيروزية والمشيمة المحتبسة بالاضافة إلى تحسن نوعية وكمية الكولوستروم. تشير النتائج ايضاً إلى أن مكملات النياسين يمكن أن تلعب دوراً في التخفيف من الآثار الضارة للإجهاد الحراري علي الأبقار خلال فصل الصيف.