# Evaluation of OmniGen-AF<sup>®</sup> in Lactating Heat-stressed Holstein Cows

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#### Introduction

There is now a strong scientific consensus that human-induced climate change is occurring (CCSP,2008). Projections are that this warming will continue throughout the 21st century with global average temperature rising an additional 1.1 to 5.4°C (CCSP 2008). These changes will have large and measurable impacts on dairy cattle worldwide (Klinedinst et al., 1993) through a variety of routes including changes in food availability and quality, changes in pest and pathogen populations, alteration in immunity and both direct and indirect impacts on animal performance such as growth, reproduction, and lactation.

Heat stress (HS) is a health and economic issue in every dairy-producing area of the world. The economic impact of HS on American animal agriculture is over \$2 billion annually and the dairy industry is one of the most susceptible. Conservative estimates have the impact at \$900 million/year, in the dairy industry alone (St. Pierre et al., 2003).

Production, reproduction, and animal health are all impaired by hyperthermia. The physiological and production responses to heat stress are well documented, but not fully understood. During HS, respiration rate and body temperature increase while feed intake, milk yield, and reproduction decrease.

Milk synthesis decreases are partially linked to reduced feed intake. There are additional losses in milk yield that are associated with metabolic changes (Rhoads et al., 2009). Specifically, metabolism of lactating dairy cows is shifted towards increased peripheral utilization of glucose. Countering the negative production effects of heat stress on lactating dairy cows requires improving feed intake and preventing the shift in energy metabolism associated with acclimation to thermal stress.

Immune function and health are also reduced with HS. The severity and occasion of disease are increased when immune and inflammatory responses are impaired (Sordillo, 2013). Impaired immune function during thermal stress may be associated with altered production rate of cortisol. Cortisol levels in heat stressed cows increase with exposure to heat within two hours (Christison and Johnson, 1972). After the first 12 hours of heat, cortisol levels return to normal.

The ruminal environment also can be altered during thermal stress. Increased respiration rates can cause respiratory alkalosis, ruminal acidosis, and eventually

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metabolic acidosis (Sanchez et al., 1994). Oxidative stress can be increased with heat stress (Ilanqovan et al., 2006) and can impair the heat shock response and increase cell damage and death (Adachi et al., 2009).

## **Experimental Objectives and Methods**

The purpose of this study was to evaluate the effects of feeding OmniGen-AF to lactating dairy cattle subjected to HS. Specifically, we tested whether OmniGen-AF would improve measures of immune function in heat stressed dairy cows.

The study consisted of two phases: 1) the commercial dairy and 2) the controlled environmental chambers. During the commercial dairy phase, multiparous lactating Holstein cows (n = 30) were balanced by days in milk (DIM), milk production, and parity (91  $\pm$  5.9 DIM, 36.2  $\pm$  2.5 kg/d, and 3.1  $\pm$  1.4). Cows were separated into one of two groups. The control group received the base TMR with no supplement. The treatment group was fed the base diet plus 56 g/head per day of OmniGen-AF mixed into the TMR. Daily milk production was measured. The commercial dairy phase lasted for 45 days. The dairy portion also was used to meet the manufacture's recommended 45 d feeding for OmniGen-AF to function.

After the commercial dairy phase was complete, 12 cows (6 control and 6 treatment) were housed in the environmentally controlled rooms at the University of Arizona, Agricultural Research Center (ARC). Cows continued within the same treatment group in the ARC and the commercial dairy phases.

The ARC portion lasted for 21 days (Figure 1). The diurnal cycle during thermoneutral (TN) and recovery maintained a temperature humidity index (THI) < 68. During HS, the THI was greater than 68 for 16 hours/day. Temperatures mimicked ambient temperatures at a southwest United States dairy during summer heat and TN conditions.

Environmental Rooms																				
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Thermoneutral							Heat Stress							Thermoneutral						

Figure 1. Environmental conditions in the environmental rooms during the 21-day study.

Fresh feed was provided twice daily and cows were individually fed. Control animals received base TMR and OmnigGen-AF cows received 56 g/head per day, split between two meals. Cows were subjected to 7 days of TN conditions, 10 days of HS, and 4 days of recovery (TN). Feed intake, milk production, and milk composition were measured daily. Rectal temperatures and respiration rates were recorded 3 times per day (600, 1400, and 1800 h).

Blood samples were collected by venipuncture from the tail (coccygeal) vein on days 1, 21, and 45 of the commercial dairy phase and on days 7, 8, 14, 17, and 18 in the controlled environmental chambers. Samples were collected 6 times per day (0400, 0800, 1200, 1600, 2000, and 2400 h) on days 7, 8, 17, and 18, and once per day on day 14 (0800 h). Blood was collected in Vacutainer (BD Vacutainer, Franklin Lakes, NJ) tubes containing sodium heparin for plasma, and in sterile blank tubes for serum.

Statistical analyses were performed using the PROC MIXED procedure (version 9.3, SAS Institute, Cary, NC). Cow was the experimental unit (ARC phase). Data are presented as least square means with significance declared with a P value  $\leq$  0.05.

#### **Experimental Results**

#### Commercial Farm Phase

There were no initial differences in milk yield (control = 38.6 kg/day and treatment = 38.6 kg/day) at the start of the commercial dairy phase of the study. There was a numerical advantage to feeding OmniGen-AF (Figure 2) of 1.5 kg of milk/day, but this was not significant (control = 36.8 kg/day and treatment = 38.3 kg/day).





### **Environmental Chamber Phase**

There was a period effect on milk yield (P < 0.01) during the environmental room (ARC) phase associated with a decline in milk yield in both groups during HS. Milk yield at the ARC (P < 0.23) did not differ between control and OmniGen-AF fed groups (Figure 3), however, there was a numerical advantage (1.1 kg/day) for cows fed OmniGen-AF during HS (P < 0.26) which was similar to the pattern in milk yield noted during the on-farm phase.



ARC milk yield

**Figure 3.** Effect of dietary OmniGen-AF on milk yield during thermoneutral (TN), heat stress (HS), and recovery period conditions in environmental chambers.

Feed intake was not measured during the on-farm phase of this study but was measured during the ARC phase. Feed intakes in the two groups did not differ during TN but was higher during HS in OmniGen-AF-fed cows (46.8 kg/d and 42.9 kg/d, P < 0.01, Figure 4; Table 1).

Milk protein (%) and fat (%) were lower in OmniGen-AF-fed cows (Table 1) during HS but not during TN. There was no difference in FCM or protein yield between treatments. Cows fed OmniGen-AF displayed decreased SCC compared to control cows (59.4 and 26.3 × 1000, P < 0.03; Table 1) with the greatest difference during the recovery period (Figure 5). There was a spike in SCC around day 5 (TN) and during recovery around day 17 (Figure 5).



**Figure 4.** Effect of dietary OmniGen-AF on feed intake by lactating cows exposed to thermoneutral and heat stress conditions in environmental chambers.

		Control						
Item	TN	HS	Recovery	TN	HS	Recovery	SEM	P -value
Feed intake (kg)	46.1	42.9	47.5	47.1	46.8*	49.1	1.04	0.01
Milk yield (kg)	33.1	30.3	30.4	33.9	31.4	31.3	1.02	0.23
Fat (%)	4.03	4.22	4.16	3.94	3.82	3.83	0.22	0.04
FCM (kg/d)	35.0	33.7	33.7	34.7	32.8	32.6	1.45	0.39
Protein (%)	2.95	2.98*	2.86	2.95	2.86	2.79	0.07	0.15
Protein (kg)	0.98	0.89	0.90	1.00	0.93	0.92	0.30	0.13
Lactose (%)	4.87	4.85	4.99	4.89	4.78	4.96	0.08	0.61
SCC	20.3	23.9	59.4*	19.6	22.9	26.3	9.12	0.03



**Figure 5.** Effect of dietary OmniGen-AF on somatic cell content in milk of lactating Holstein cows subjected to thermoneutral and heat stress conditions in environmental chambers.

Respiration rate and rectal temperatures did not differ between treatments during TN; however, during HS, OmniGen-AF reduced respiration rate, (Table 2, P < 0.01) in both environments at 1400 h and in HS animals at 1800 h when environmental heat load was greatest. Rectal temperatures were lower in cows fed Omnigen-AF at 1400 and 1800 h compared to controls when environmental heat loads were maximal.

	Control			OmniGen-				
TN	HS	Recovery	TN	HS	Recovery	SEM	P-value	
1								
26.9	31.9	28.3	26.6	30.4	27.9	1.40	0.40	
34.3	63.1*	35.3	30.1	58.3	35.5	2.99	0.20	
34.9*	60.8*	32.1	29.5	52.4	<b>29.</b> 7	2.62	0.01	
mp (°C)								
38.2	38.0	37.9	38.2	38.1	38.1	0.05	0.26	
38.0	38.7*	38.0	38.1	38.5	38.1	0.09	0.77	
38.2	39.1*	38.2	38.2	38.8	38.3	0.08	0.25	
	TN 26.9 34.3 34.9* mp (°C) 38.2 38.0 38.2	TN         HS           26.9         31.9           34.3         63.1*           34.9*         60.8*           mp (°C)         38.2         38.0           38.0         38.7*         38.2         39.1*	TN         HS         Recovery           26.9         31.9         28.3           34.3         63.1*         35.3           34.9*         60.8*         32.1           mp (°C)	TN         HS         Recovery         TN           26.9         31.9         28.3         26.6           34.3         63.1*         35.3         30.1           34.9*         60.8*         32.1         29.5           mp (°C)         38.2         38.0         37.9         38.2           38.0         38.7*         38.0         38.1         38.1           38.2         39.1*         38.2         38.2         38.2	TN         HS         Recovery         TN         HS           26.9         31.9         28.3         26.6         30.4           34.3         63.1*         35.3         30.1         58.3           34.9*         60.8*         32.1         29.5         52.4           mp (°C)	TN         HS         Recovery         TN         HS         Recovery           26.9         31.9         28.3         26.6         30.4         27.9           34.3         63.1*         35.3         30.1         58.3         35.5           34.9*         60.8*         32.1         29.5         52.4         29.7           mp (°C)	TN         HS         Recovery         TN         HS         Recovery         SEM           26.9         31.9         28.3         26.6         30.4         27.9         1.40           34.3         63.1*         35.3         30.1         58.3         35.5         2.99           34.9*         60.8*         32.1         29.5         52.4         29.7         2.62           mp (°C)	

 
 Table 2. Effects of OmniGen-AF supplementation and environment on respiration rate and rectal temperature in lactating dairy cows

\*  $P \le 0.05$  and indicates the higher value.

Hormones in plasma are important as potential indicators of the physiological status of a cow and reflect the physiological compensations a cow undergoes at various stages of lactation and exposure to HS. Serum cortisol levels were highest on day 8 (first day of HS, Figure 6). This is in agreement with prior reports that acute but not chronic HS is associated with increases in circulating cortisol concentrations (Christian and Johnson, 1972, Wise et al., 1988). OmniGen-AF treated cows had significantly lower serum cortisol on day 8 (0.8372 vs. 0.4838  $\mu$ g/dL for control and OmniGen-AF respectively, *P* < 0.006) and did not differ on other days. This suggests that Omnigen may reduce impact of acute stress on the cortisol response in lactating dairy cows.



**Figure 6.** Effect of environment and Omnigen-AF on serum cortisol concentrations in lactating dairy cows housed in environmental chambers.

Serum insulin and plasma glucose levels (Figures 7 and 8) were not different between groups (P = 0.8248 and 0.945). Serum insulin concentrations in both groups rose during the latter part of the HS period and during the recovery period. The reason for this pattern is unknown.



**Figure 7.** Effect of Omnigen-AF and environment on serum insulin concentrations in lactating dairy cows housed in environmental chambers.



**Figure 8.** Effect of Omnigen-AF and environment on plasma glucose concentrations of dairy cows housed in environmental chambers.

The immune function of cattle on this study was evaluated by looking at the expression of the interleukin-8 receptor (Figure 9) and expression of Regulated on Activation, Normal T Expressed and Secreted (RANTES) protein (Figure 10) which is a member of the interleukin-8 family of cytokines.



Figure 9. Effect of dietary Omnigen-AF on IL8R receptor gene expression in leukocytes in lactating dairy cows housed in environmental chambers.



Figure 10. Effect of Omnigen-AF and environment on RANTES gene expression in lactating dairy cows housed in environmental chambers.

# Summary

Heat stress exposure was mild to moderate in this study. The threshold for heat stress in lactating dairy cows is a THI > 68, respiration rates > 60 bpm, and rectal temperatures > 38.5°C (Zimbleman et al., 2009). OmniGen-AF reduced impact of thermal stress on stress of lactating dairy cows. Cows fed Omnigen-AF had reduced rectal temperatures and respiration rates during periods of peak thermal load. Respiration rates in treated cows did not exceed 60 bpm and mean rectal temperatures were 0.2 to 0.3°C cooler. OmniGen-AF fed cows displayed higher feed intakes during HS as well. Cows fed OmniGen-AF also displayed a lower cortisol spike on the first day of heat stress.

Milk yield decreased with heat stress in both control animals and the OmniGen-AF fed animals. However, feed intake was unchanged in cows fed Omnigen-AF and milk yields were numerically higher. Changes in SCC were consistent between groups. Cows fed OmniGen-AF displayed decreased SCC compared to control cows with the greatest difference during the recovery period.

Serum cortisol levels were similar to previous findings (Christison and Johnson, 1972) and increased within the first day of heat exposure. The animals in the ARC had higher cortisol levels compared to published levels, but the confinement and changes in surrounding from the dairy to the ARC may account for some of the changes.

Cytokine (RANTES) gene expression was higher in cows fed Omnigen-AF during the HS portion of the study but not during recovery. The elevated cytokine gene expression may be associated with improved immune function in cows fed Omnigen-AF.

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# **SESSION NOTES**