BARD

FINAL REPORT

PROJECT NO. US-2846-97

Hot Temperature Stress and Turkey Reproduction

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Project Title: Hot Temperature Stress and Turkey Reproduction

Investigators

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Collaborating Investigators:

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Vasoactive intestinal peptide, immunization, ovarian function, serotonin, gonadotropic hormone expression, inhibin

Abbreviations commonly used in the report, in alphabetical order:

Keyhole Limpet Hemocyanine, KLH; turkey inhibin, tINH; vasoactive intestinal peptide, VIP

Budget: IS: \$112,000

US: \$173,000

Total: \$285,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)	1	2	-	3
Submitted, in review, in preparation	1	-	2	3
Invited review papers	-	-	-	-
Book chapters	-	-	-	-
Books	-	-	-	-
Master theses	-	1	1	2
Ph.D. theses	-	-	1	1
Abstracts	-	-	-	-
Not refereed (proceedings, reports, etc.)	-	-	-	-

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Dr. Yupaporn Chaiseha -- SSN 472-25-5088

Dr. Renu Bhatt -- SSN 473-37-6827

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1	1		2
Longer Visits (Sabbaticals)	-	1		1

Description of Cooperation:

The working relationship between the co-investigators and their associates has been excellent. The laboratories of the co-investigators continue to cooperate in the renewal of this grant. Also, we were able to cooperate on other projects supported by the industry. The cooperation facilitated and made our work more efficient: samples were exchanged for hormone and antibody analysis. LED lambs are being manufactured in Israel and used in the US. Students came to the US to learn certain techniques and return to Israel. This has been a very enjoyable, efficient and productive cooperation.

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted	-	-		-
Issued (allowed)	-	-	-	-
Licensed	-	-	-	-

Abstract

BARD Grant No. US2846-97: Hot Temperature Stress and Reproductive Efficiency in the Domestic Turkey

High temperature stress is of major concern to turkey producers in Israel and the United States. The decline in the rate of egg production at high environmental temperature is well recognized, but the neuroendocrinological basis is not understood.

Our objectives were: 1) to characterize the hypothalamo-hypophyseal axis involvement in the mechanism(s) underlying the detrimental effect of heat stress on reproduction, and 2) to establish procedures that alleviate the damaging effect of heat stress on reproduction.

Heat stress (40°C, Israel; 32°C, U.S.) caused significant reduction in egg production, which was restored by VIP immunoneutralization. The decline in egg production did not appear to be entirely related to the expression of incubation behavior due to the rise in circulating PRL in stressed birds. Heat stress was found to increase circulating PRL in ovariectomized turkeys independent of the reproductive stage.

Active immunization against VIP was shown for the first time to up-regulate LH β and FSH β subunit mRNA contents. These findings taken together with the results that the heat stress-induced decline in egg production may not be dependent upon the reproductive stage, lead to the suggestion that the detrimental effect of heat stress on reproductive performance may be in part mediated by VIP acting directly on the GnRH/gonadotropin system.

Inhibin (INH) immunoneutralization has been shown to enhance FSH secretion and induces ovulation in mammals. It is hypothesized that immunization of heat-stressed turkeys against INH will increase levels of circulating FSH and the number of preovulating follicles which leads to improved reproductive performance. We have cloned and expressed turkey INH- α and INH- β A. Active immunization of turkey hens with rtINH- α increased pituitary FSH- β subunit mRNA and the number of non-graded preovulatory yellow follicles, but no significant increase in egg production was observed.

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United States

This work has resulted in three referred papers and one MS thesis.

In order to enhance the reproductive performance of breeder hen turkeys subjected to stress, and since inhibin immunoneutralization increases the level of circulating follicle stimulating hormone, it is hypothesized that active immunization of female turkeys with inhibin would neutralize endogenous inhibin, and increase levels of circulating follicle stimulating hormone (FSH), and the number of preovulatory follicles and subsequently enhance egg production of stressed female turkeys.

In order to achieve this objective, it was deemed essential to clone and express turkey inhibin. We isolated cDNA encoding turkey γ inhibin and β -subunits from the turkey ovary using reverse transcription-polymerase chain reaction (RT-PCR). The isolated γ subunit and β subunit included the entire open reading frames encoding 329 and 424 amino acids, respectively. Northern blot analysis revealed that the transcripts of turkey γ inhibin and β -subunits were approximately 1.7 and 8.4 kb, respectively.

The mature encoding region of recombinant turkey γ inhibin was subcloned into a thioredoxin expression vector system. This recombinant fusion protein was used for active immunization against endogenous inhibin in the turkey. Active immunization of female turkeys with recombinant γ inhibin induced antibodies against endogenous inhibin, elevated pituitary levels of FSHB subunit mRNA, and increased the number of nongraded preovulatory yellow follicles. Immunization against inhibin had no effect on egg production in turkeys.

Active immunizations with vasoactive intestinal peptide suppressed circulating prolactin, inhibited the expression of incubation behavior, and resulted in a substantial increase in egg production. Egg production of hens immunized with γ inhibin + VIP increased slightly compared with that of VIP immunized hens. FSHB subunit mRNA pituitary content of the γ inhibin-immunized group was similar to that of VIP-immunized birds, and both immunized groups showed significantly greater FSHB mRNA content than controls. LH mRNA pituitary content of VIP-immunized groups was also significantly increased over controls and those animals immunized with inhibin. These results clearly show for the first time that VIP acts as an antagonist toward both LH and FSH secretion.

In another study, the mechanism(s) by which ambient temperature affects prolactin secretion in female turkeys was investigated. Chronic exposure to low (10°C) or high (32°C) temperature had no effect on the increase in circulating prolactin levels that follow photostimulation. However, acute exposure to high temperature showed a significantly greater prolactin response to photostimulation than hens subjected to low temperature. These findings may implicate prolactin as the hormone mediating the effect of temperature on the turkey breeding cycle. Also, the results showed that ovariectomy

enhanced the increase in circulating prolactin that followed the exposure to gonadal, stimulatory photoperiod. These findings differ from previous results, where increased prolactin levels have been reported in response to ovarian steroid injections. These findings taken together suggest a dual role for ovarian hormones in regulating prolactin, engendering a permissive or suppressive effect dependent upon their ratios and levels.

Israel

Experiment 1: the effect of active immunization against VIP on reproductive failure associated with heat stress in turkey hens.

Material and Methods:

Forty-eight B.U.T. turkey hens at the end of their first reproductive season were purchased from commercial turkey breeder farm (Yefeh Hod, Israel). Upon arrival birds were randomly housed in 8 environmental and light control rooms (6 m² each, 6 birds/room) under non photostimulatory conditions (6h light, 18h dark). Birds were force molted according to the primary breeder recommendations.

Ten weeks after molting had been initiated, body weights were recorded and birds were divided according to their body weight to 4 treatment groups:

1. Actively immunized against VIP under normal temperature conditions (20-28°C) - NHI.
2. Actively immunized against VIP under high temperature conditions (36-40°C) - HI.
3. Actively immunized against KLH under normal temperature conditions (20-28°C) - NHNI.
4. Actively immunized against KLH under high temperature conditions (36-40°C) - HNI.

Birds in each room were equally divided into 2 groups, 3 birds per group. Birds in one group were immunized with VIP, and the birds in the second group with KLH. Half of the rooms were heated to the desired temperature by electrical heaters, and half of the rooms were maintained under normal conditions. Temperature was controlled by thermostat operated negative pressure fans located in each room.

Conjugated VIP was prepared by conjugating VIP with KLH using commercial conjugating kit (Pierce, USA).

Experimental procedure: birds were primary vaccinated at photostimulation with 125 µg of VIP-KLH conjugate or 125 µg of KLH in the control group in Freund's complete adjuvant. Booster injections were given every 4 weeks by using 25 µg of the immunogens in incomplete Freund's adjuvant.

Eight weeks after photostimulation birds were exposed to 8 hr per day of high temperatures according to their treatment groups until termination of the experiment. Daily recording of egg production, egg quality (egg weight, shell condition), nest visits as well as feed consumption were conducted.

Weekly blood sample were obtained for determination of plasma hormones levels and VIP antibody titer.

Plasma hormones: prolactin was measured by using RIA according to Proudman and Opel 1981. Plasma LH was conducted by using RIA according to Burke et al., 19. Plasma

steroids were measured using commercial kit (DPC, USA). VIP antibody titer were conducted by ELISA developed in our lab.

RESULTS:

Blood antibody titer against VIP is presented in figure 1. Birds immunized against VIP had high titer, in addition birds maintained under heat condition had significantly lower titer than birds reared under thermoneutral conditions.

Figure 1: VIP antibody titer of turkey hens, immunized against VIP-KLH conjugate and reared under hot temperature or thermoneutral conditions (legends are explained in text, each bar present mean $\pm$ S.E., bars marked with different letters are significantly different ($P < 0.05$)).

Heat stress caused a significant reduction in egg production (figure 2). The reduction was lower in the group actively immunized against VIP.

Figure 2: egg production of turkey hens, immunized against cVIP-KLH conjugation and reared under hot temperature or thermoneutral conditions (legends are explained in text, each line present mean $\pm$ S.E.)

Table 1 summarizes egg production data for whole reproduction season. A significant reduction in egg production was observed in groups reared under hot condition, in addition, birds immunized against VIP had higher egg production compare to the non immunized birds maintained under hot conditions. Egg weight was significantly heavier in the HI group compare to all other groups. Eggs collected from the NHNI group were significantly smaller than all other groups.

Treatment Group	NHNI	NHI	HI	HNI
EGGS/HEN	59.49 $\pm$ 2.3 ^{b*}	74.42 $\pm$ 3.1 ^a	58.88 $\pm$ 2.1 ^b	49.5 $\pm$ 2.1 ^c
Egg Weight (g)	96.21 $\pm$ 0.2 ^c	97.56 $\pm$ 0.3 ^b	98.55 $\pm$ 0.1 ^a	97.38 $\pm$ 0.1 ^b
Mean % egg production	32.87 $\pm$ 1.0 ^b	41.12 $\pm$ 2.1 ^a	32.53 $\pm$ 1.3 ^b	27.35 $\pm$ 2.0 ^b

Table 1: a summary of egg production and egg weight of white turkey hens immunized against cVIP-KLH conjugation and reared under hot temperature or thermo-neutral conditions (each number present mean $\pm$ S.E., numbers marked with different letters are significantly different (P<0.05)).

Experiment 2: the effect of active immunization against VIP, inhibin and combination of VIP + inhibin on reproductive failure associated with heat stress in turkey hens.

Experimental procedure:

40 white turkey hens at 28 weeks of age were divided into 5 groups (n=8 per group). The first group was immunized against VIP using the procedure presented in experiment one, the second group was immunized against Inhibin (Alon Chemicals Rehovot Israel), the third group was immunized against VIP and Inhibin, the fourth group was immunized against KLH (control KLH) and the fifth group was not injected and served as uninjected control.

Results:

Figure 3 summarizes the egg production data. Birds immunized against VIP had significantly higher egg production compared to all other experimental groups. In addition immunizations against both VIP and inhibin (alone and combined) had no positive effect on egg production.

Since no clear results on egg production were obtained in the second experiment a third experiment was design in order to try a different approach in eliminating the rise in prolactin level associated to heat stress.

Figure 3: egg production of large white turkey hens, reared under thermoneutral conditions or exposed to high temperature. Birds were actively immunized before photostimulation against cVIP, inhibin and cVIP + inhibin. Bars presents mean $\pm$ S.E., bars marked with different letters are significantly difference ($P < 0.05$).

Experiment 3: the effect of orally administration of parachlorophenylalanine (PCPA) on reproductive failure associated to heat stress in turkey hens.

Experimental procedure:

28 large white turkey hens at 28 weeks of age were housed in two environmentally controlled rooms ($n=14$) under 6 hr of light and thermoneutral conditions ($24-26^{\circ}\text{C}$). Birds were photostimulated at 30 weeks of age by providing 15 hr of light. At 36 weeks of age the temperature in one room was elevated gradually to 40°C for 12 hr/day. One week after initiation of heat stress half of the birds in each room were orally administered with 50mg/kgBw of parachlorophenylalanine (PCPA) for three consecutive days. This treatment was provided again after 7 days at the age of 38 weeks. Body weight was measured at the beginning and the end of the experiment, egg production and nesting activity were measured 4 times/day. At the end of the experiment at 39 weeks of age birds were killed ovaries and oviducts were removed and weight, and selected preovulatory follicles were stored in liquid nitrogen for the determination of mRNA gene expression of steroidogenic enzymes.

Results

Body weight results were significantly reduced in birds exposed to heat stress lower although not significant in the group that did not receive PCPA (Figure 4).

Figure 4: Body weight results of large white turkey hens reared under thermoneutral (control) or 40°C for 12 hr/day (heat) and treated orally with PCPA (for details see text). Bars presents mean $\pm$ S.E., bars marked with different letters is significantly difference ($P < 0.05$).

After initiation of heat stress egg production significantly dropped in both groups, however, the decline was significantly greater in birds that did not receive PCPA treatment (Figure 5).

Figure 5: Egg production (%) results of large white turkey hens reared under thermoneutral (control) or 40°C for 12 hr/day (heat) and treated orally with PCPA (for details see text). Lines presents mean $\pm$ S.E., values marked with different letters are significantly difference ($P < 0.05$).

Egg weight was significantly reduced in birds exposed to high temperature (Figure 6). The reduction was greater in control heated birds compare to PCPA heated birds.

Figure 5: Egg weight (gm) results of large white turkey hens reared under thermoneutral (control) or 40°C for 12 hr/day (heat) and treated orally with PCPA (for details see text). Lines presents mean $\pm$ S.E., values marked with different letters are significantly difference ($P < 0.05$).

Nesting activity was elevated in both untreated groups reaching significance in the control heated birds at 37 weeks of age (one week after initiation of heat stress). PCPA blocked nesting activity associated with high temperature (Figure 6).

Figure 6: nesting activity of large white turkey hens reared under thermoneutral (control) or 40°C for 12 hr/day (heat) and treated orally with PCPA (for details see text). Lines presents mean $\pm$ S.E., values marked with different letters are significantly difference ($P < 0.05$).

Description of Cooperation

The working relation between the co-investigators and their associates has been excellent. The laboratories of the co-investigators continue to cooperate in the renewal of this grant. Also, we were able to cooperate on other projects supported by the industry. The cooperation facilitated and made our work more efficient: samples were exchanged for hormone and antibody analysis. LED lambs are being manufactured in Israel and used in the US. Students came to the US to learn certain techniques and return to Israel. This has been a very enjoyable, efficient and productive cooperation.

Publications

1. Ahn, J.; You, S., Kim, H.; Foster, D.N.; El Halawani, M.E. 2001. Molecular cloning and expression of turkey inhibin- α and - β A subunits. Poultry Science 80: 1690-1694.

2. Ahn, J.; You, S.; Kim, H.; Chaiseha, Y.; El Halawani, M. 2001. Effects of active immunization with inhibin α subunit on reproductive characteristics of turkey hens. Biol Reprod 65: 1594-1600.
3. Gahali, K.; El Halawani, M.; Rosenboim, I. 2001. Photo stimulated Prolactin release in the turkey hen: Effect of ovariectomy and environmental temperature. Gen Comp Endocrinol 124: 166-172.

In preparation

1. Effect of active immunization against cVIP of reproductive failure associated to heat stress in turkey hens.
2. Effect of heat stress on ovarian function of laying hens.
3. The effect of PCPA on reproductive failure associated to heat stress in turkey hens.