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Heat Shock Proteins as Modifying Factors in Physiological Stress Responses

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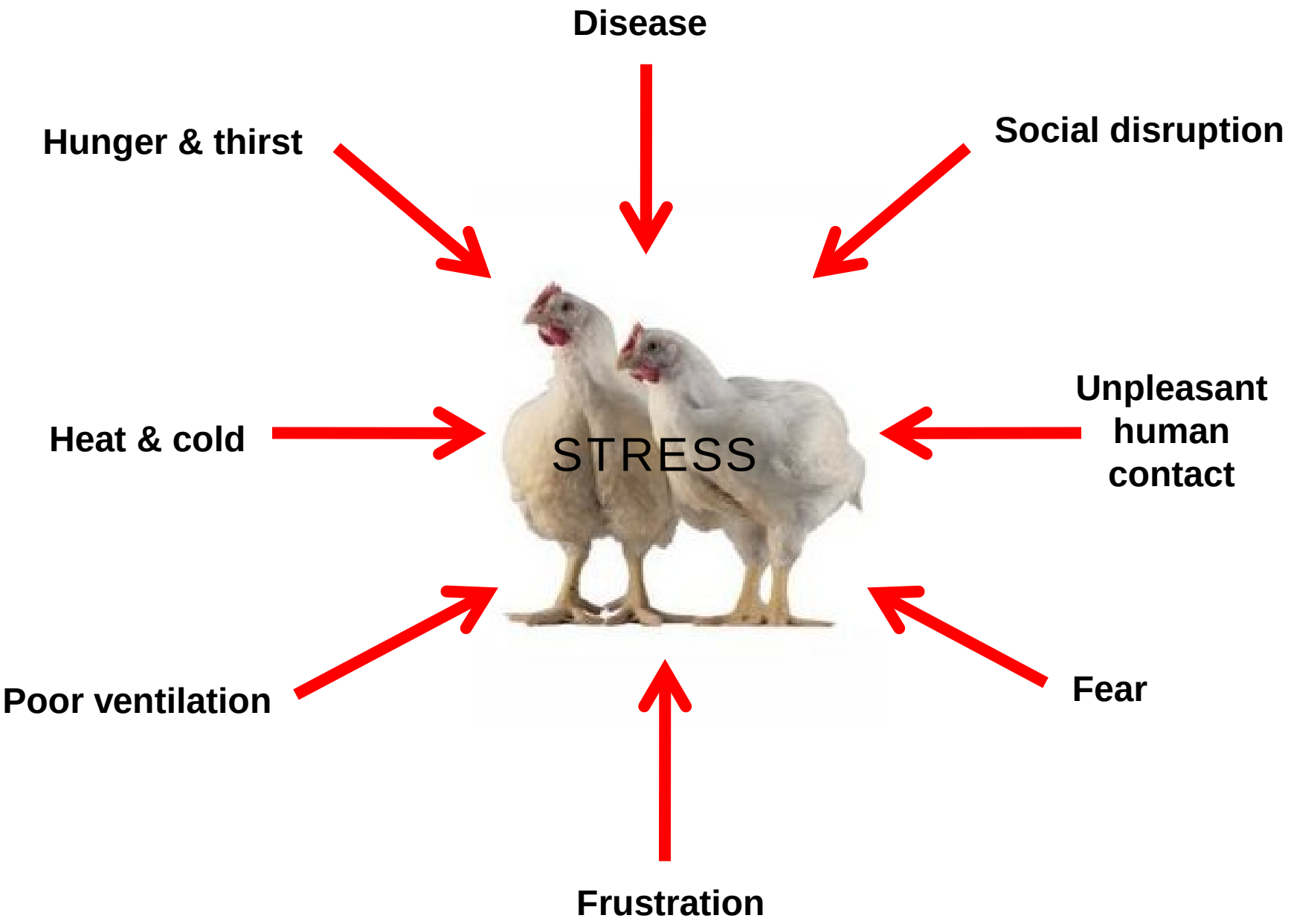
Farm animals are constantly challenged by imperfection

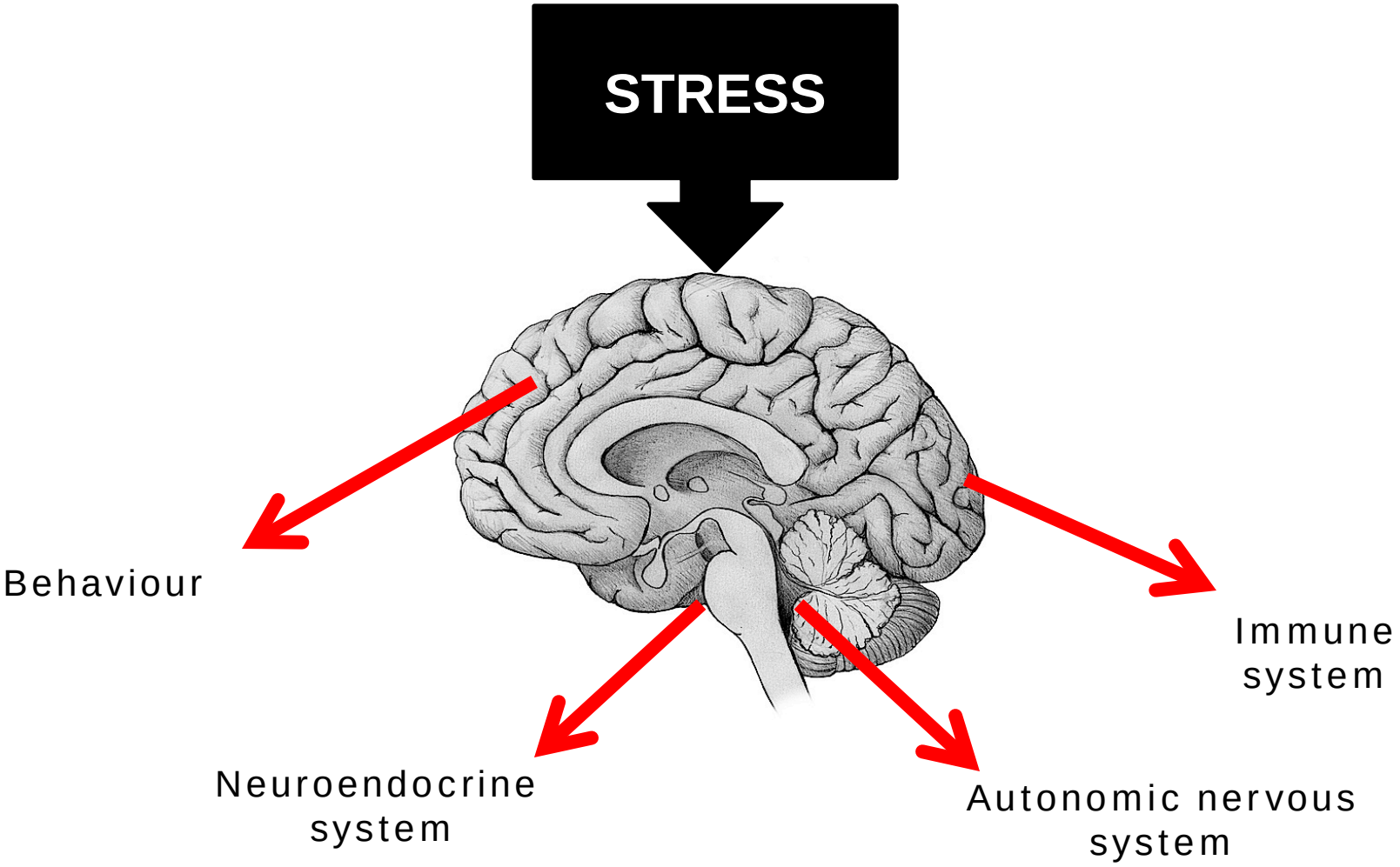


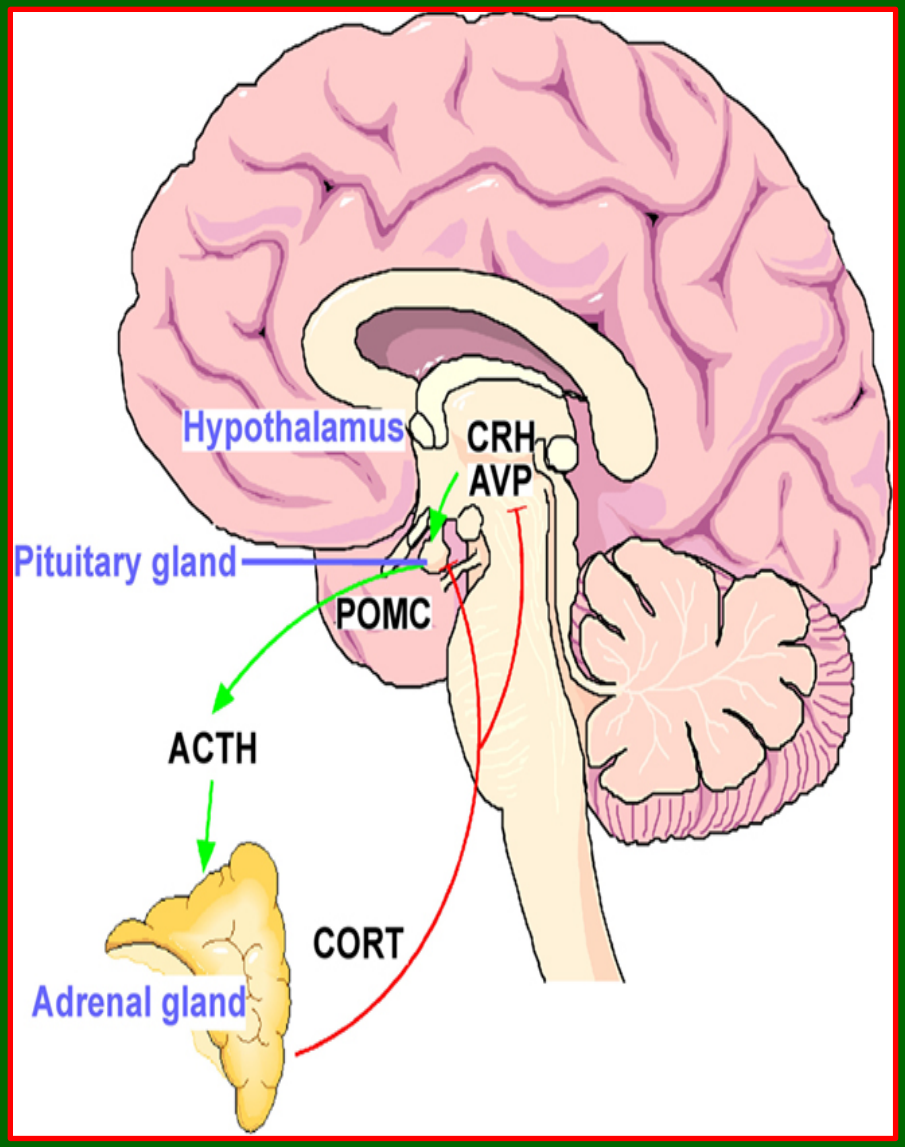
It is rare for an animal – in the wild or on a farm – to not to be responding to several stressors at once (Curtis, 1982).

Since stress in life is unavoidable, we can never expect to develop conditions that will always keep our animals stress free (Moberg, 2000).

Stress is the rule, not the exception, in an animals life, and nature has endowed animals with a marvelous array of physiological, anatomical, immunological, and behavioural responses to these impingements (Curtis, 1985).

The general types of biological responses available to the animal for coping with stress





Hypothalamic-pituitary-adrenal axis response

Organisms ranging from bacteria to humans and plants respond to stress by synthesizing a group of proteins known as heat shock proteins (HSPs).

HSPs were first reported by F.M. Ritossa (1962) in *Drosophilia Melanogaster* larvae that were exposed to "heat shock".

HSPs have been divided into families, which have been named according to their approximate molecular weight. The five main groups are Hsp100, Hsp90, Hsp70, Hsp60 and the small Hsps (Knowlton 1997).

When living organisms are exposed to thermal and non-thermal stressors the synthesis of most proteins is retarded but HSPs are rapidly synthesised (Etches et al., 1995)

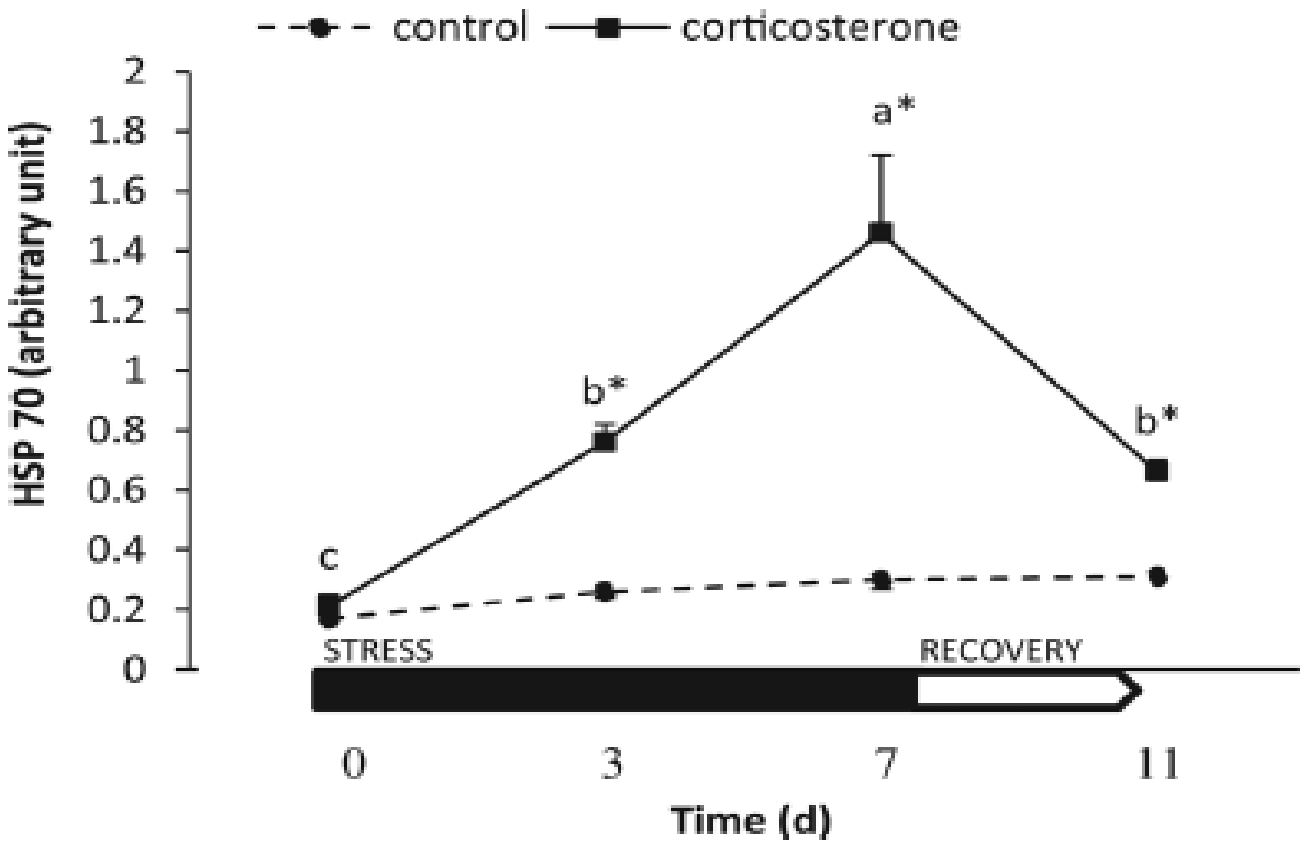
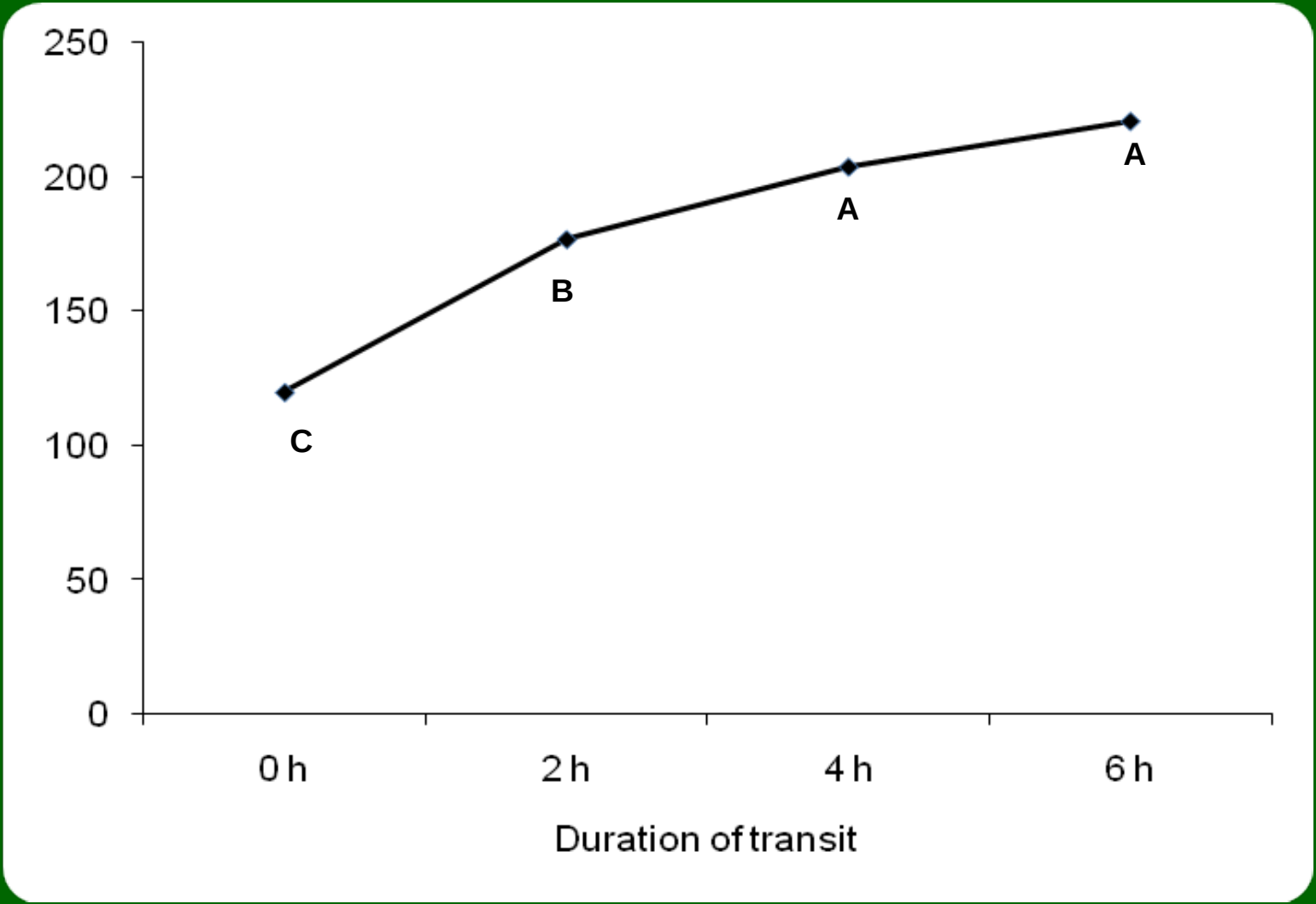


Figure 5. Time course changes in brain heat shock protein 70 (HSP 70) following daily saline (control) or corticosterone administration for d 0 (14 d of age), d 3 (17 d of age), d 7 (20 d of age), and d 11 (24 d of age). Means (n = 16) within a treatment group with no common letters (a–c) differ at $P < 0.05$. *Significant difference between treatment groups ($P < 0.05$).

(Zulkifli et al., 2014)

Mean heat shock protein 70 densities at various durations of transit in broiler chickens



Means with different letters differ at $P<0.05$

(Al-Aqil and Zulkifli, 2009)

The effect of road transportation on goats

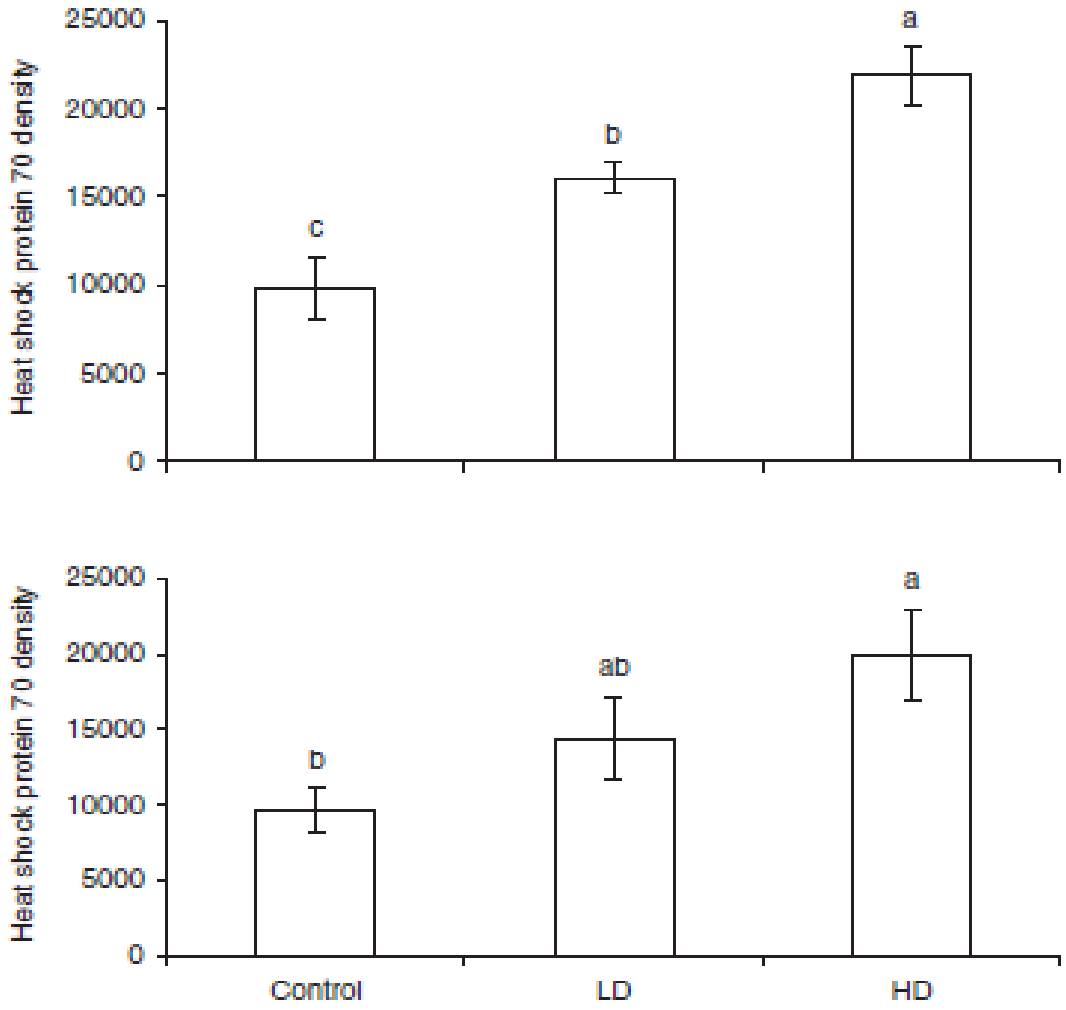


Figure 1 The effect of transportation on heat shock protein 70 density of kidney (top) and liver (bottom) tissues. (a) to (c) Means with no common letters differ ($P < 0.05$). LD = transport floor space of 0.40 m^2/animal; HD = transport floor space of 0.20 m^2/animal.

(Zulkifli et al., 2010)

The effect of crating on broiler chickens

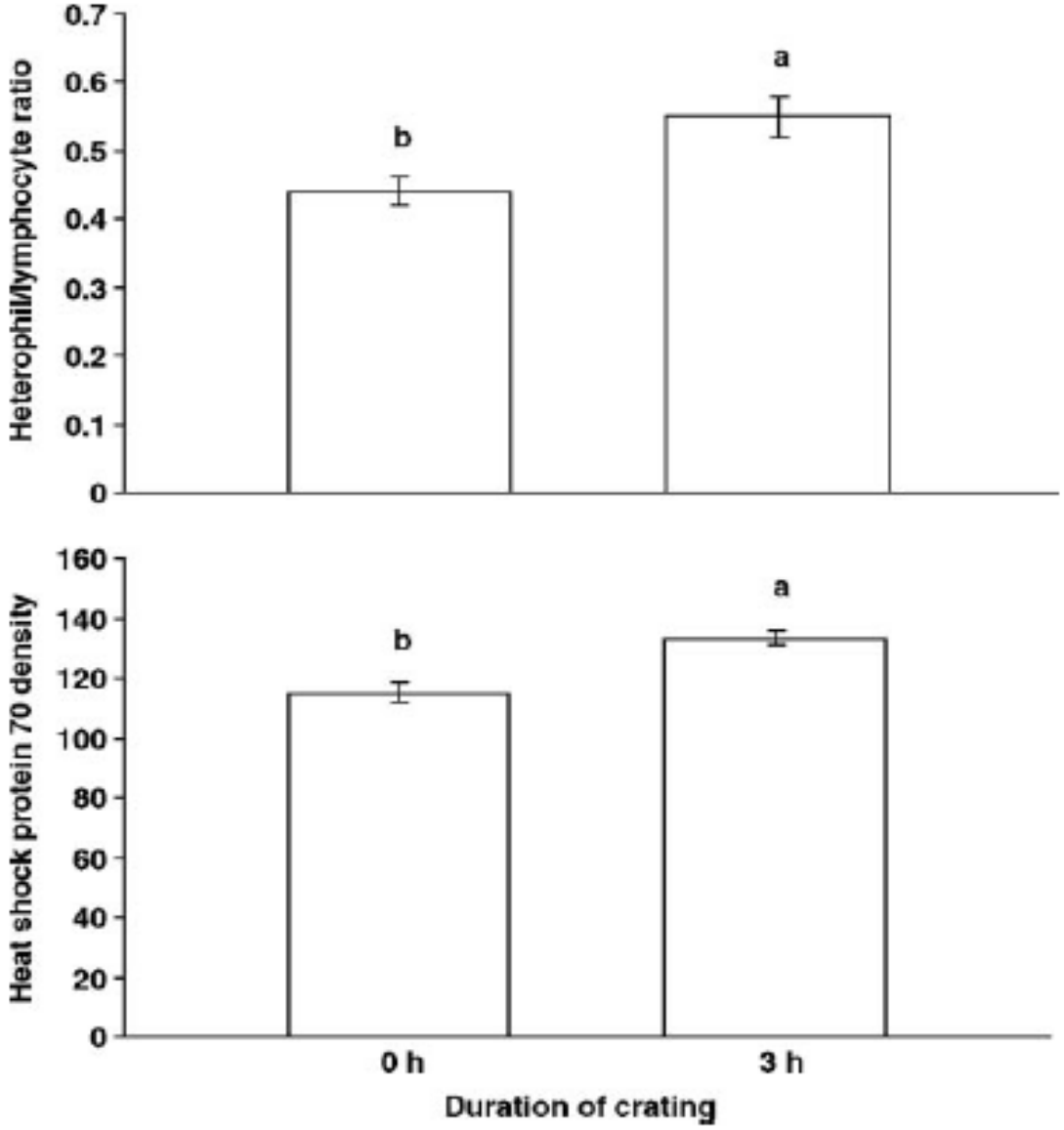
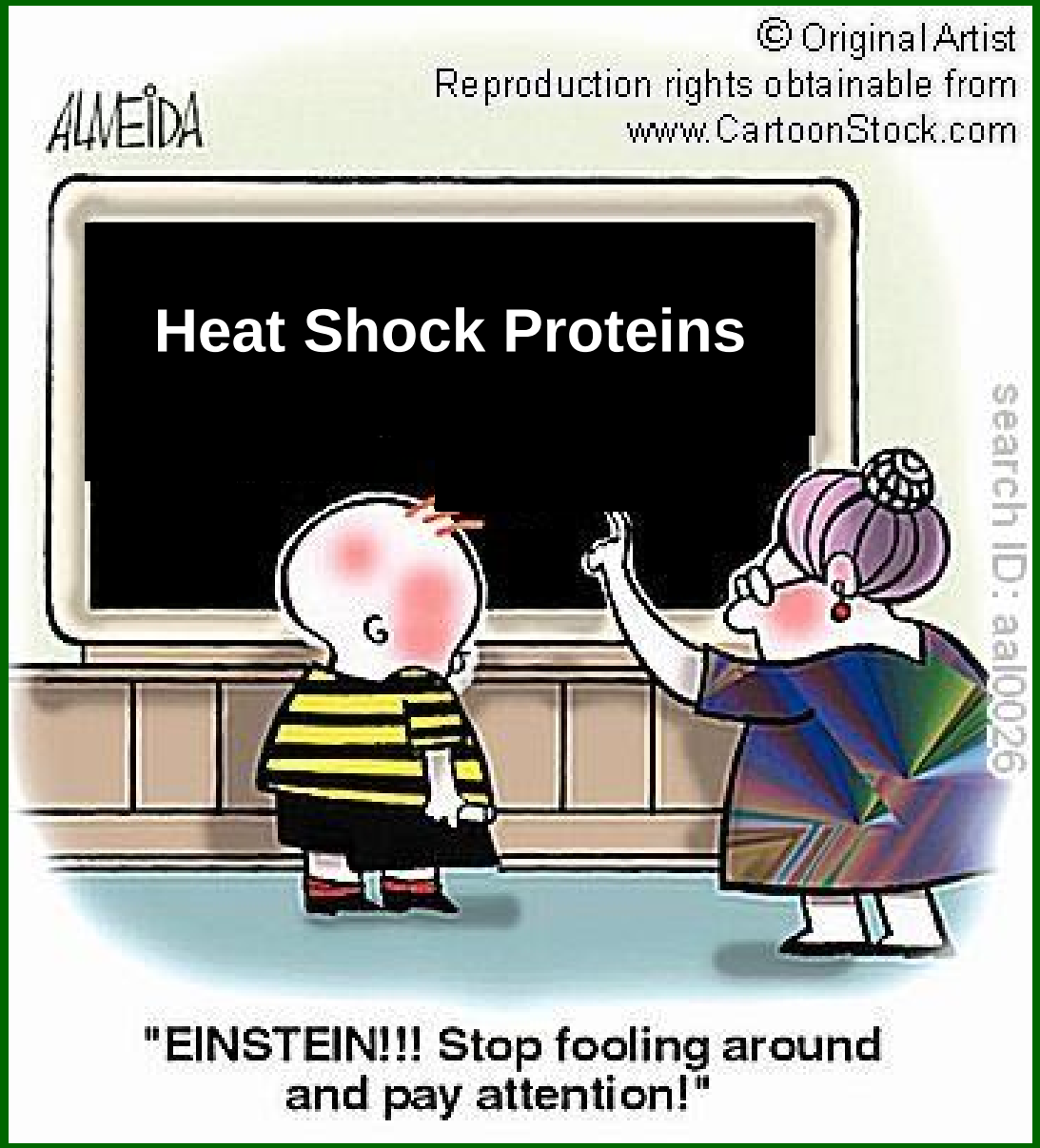


Figure 2. The effect of crating on heterophil/lymphocyte ratios (top) and heat shock protein 70 densities (bottom) by duration. [a,b]Means with no common letters differ ($P < 0.05$).

(Zulkifli et al., 2009)



Why there is a lot of interests in HSPs?

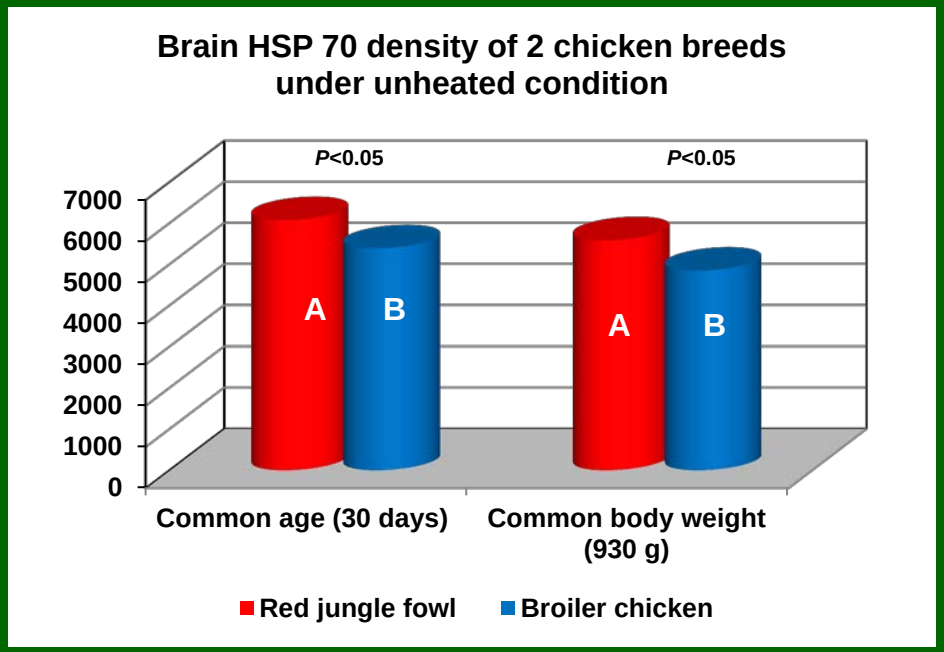
HSPs appear to play a critical role in protecting cells against the adverse effects of stress

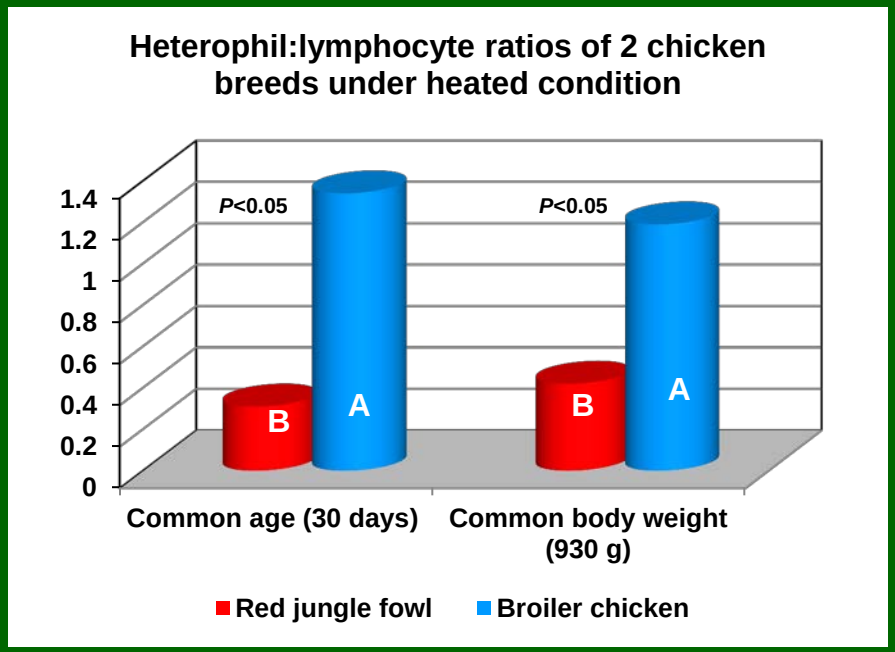
In a heat-shocked cell, the HSP may bind to the heat-sensitive proteins and protect them from degradation, or may prevent damaged proteins from immediately precipitating and permanently affecting cell viability (Etches et al., 1995)

Of the many expressed HSPs, those with a molecular weight of approximately 70 kDa appear to correlate best with heat tolerance (Craig and Gross, 1999)

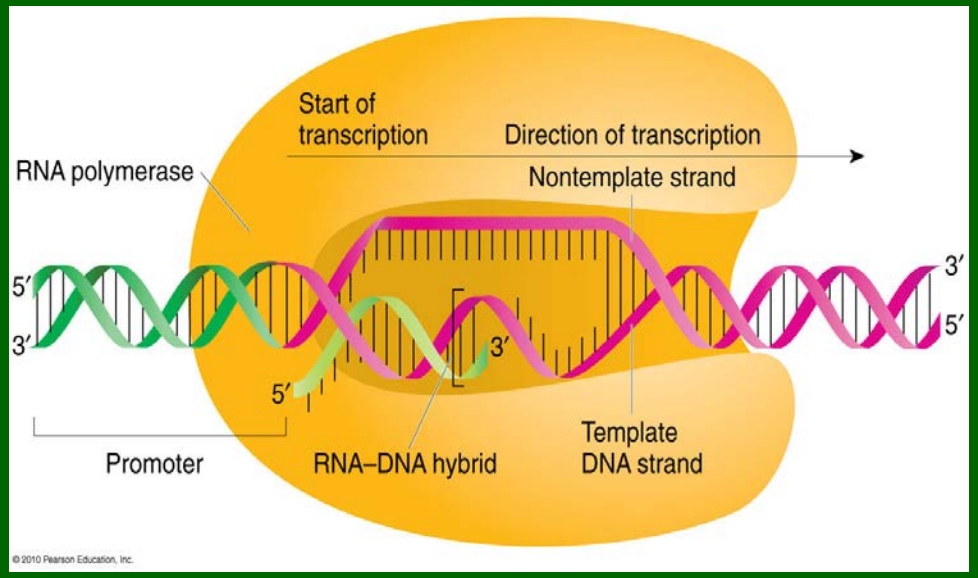
Heat shock proteins are cellular lifeguards

Chicken breeds with higher basal HSP 70 expression at normal physiological temperatures were better able to tolerate high temperatures (Soleimani et al., 2011)

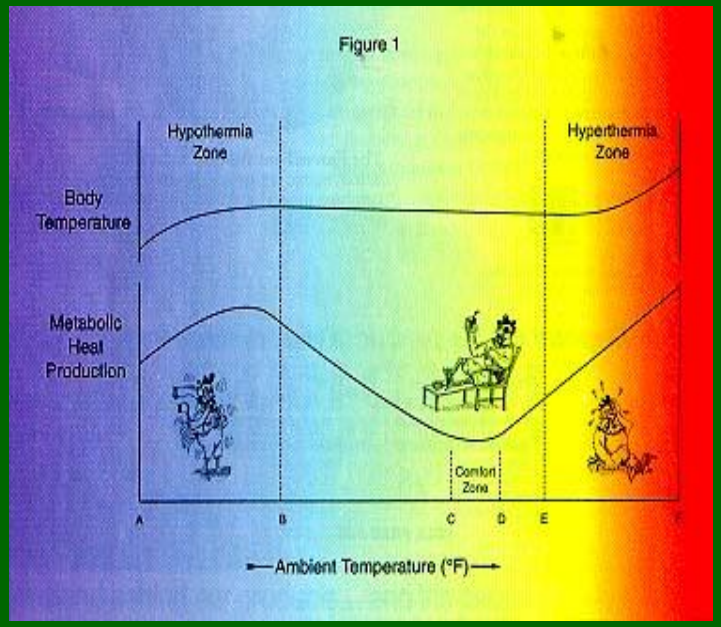




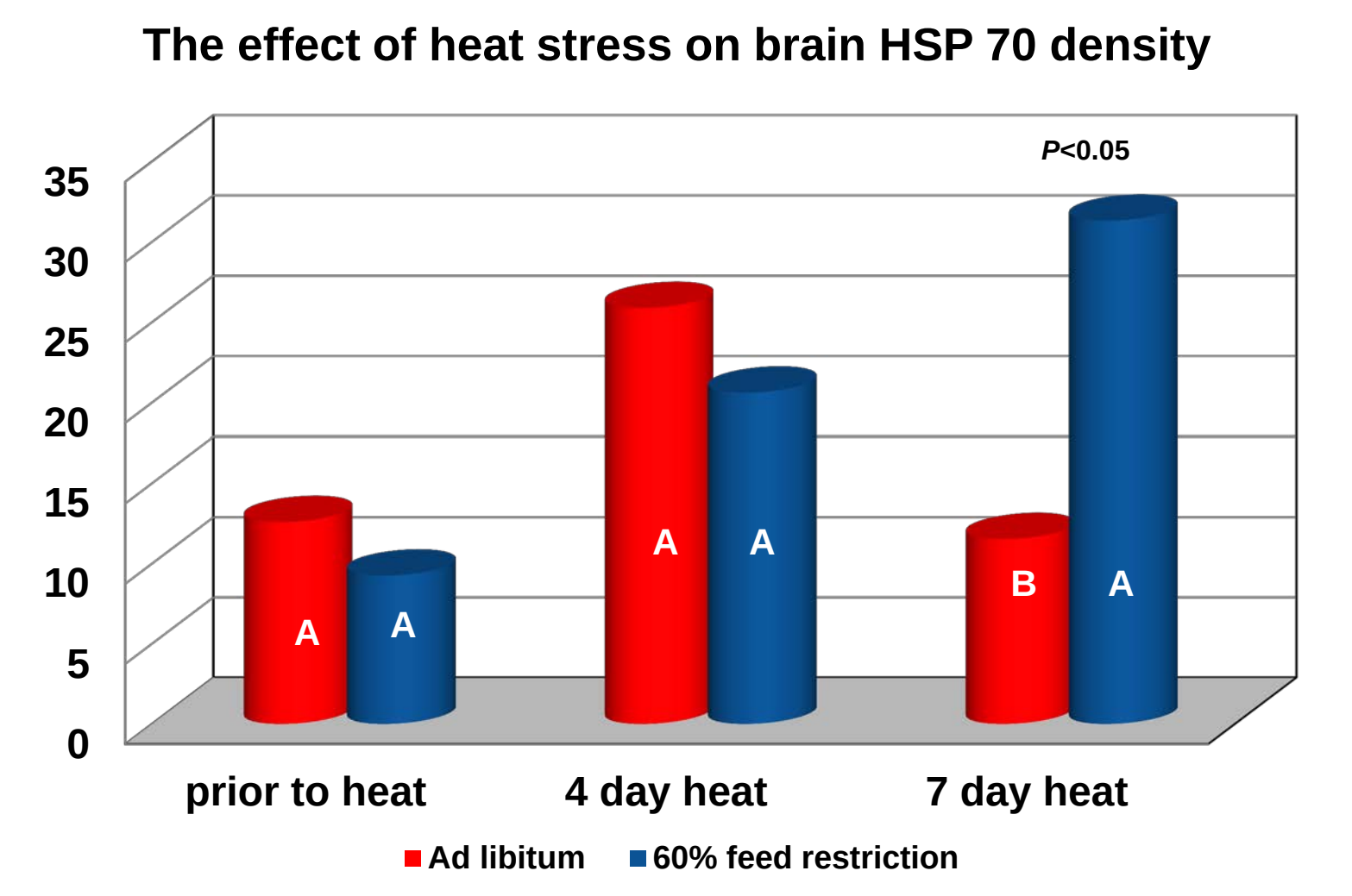
HSPs are closely associated with cellular homeostasis and acquisition of thermal tolerance in laboratory animals (Moseley, 1997).



Neonatal stresses may evoke HSPs mRNA transcription but the RNA could be 'sequestered' and not translated until exposure to subsequent challenge later in life (Craig, 1995)



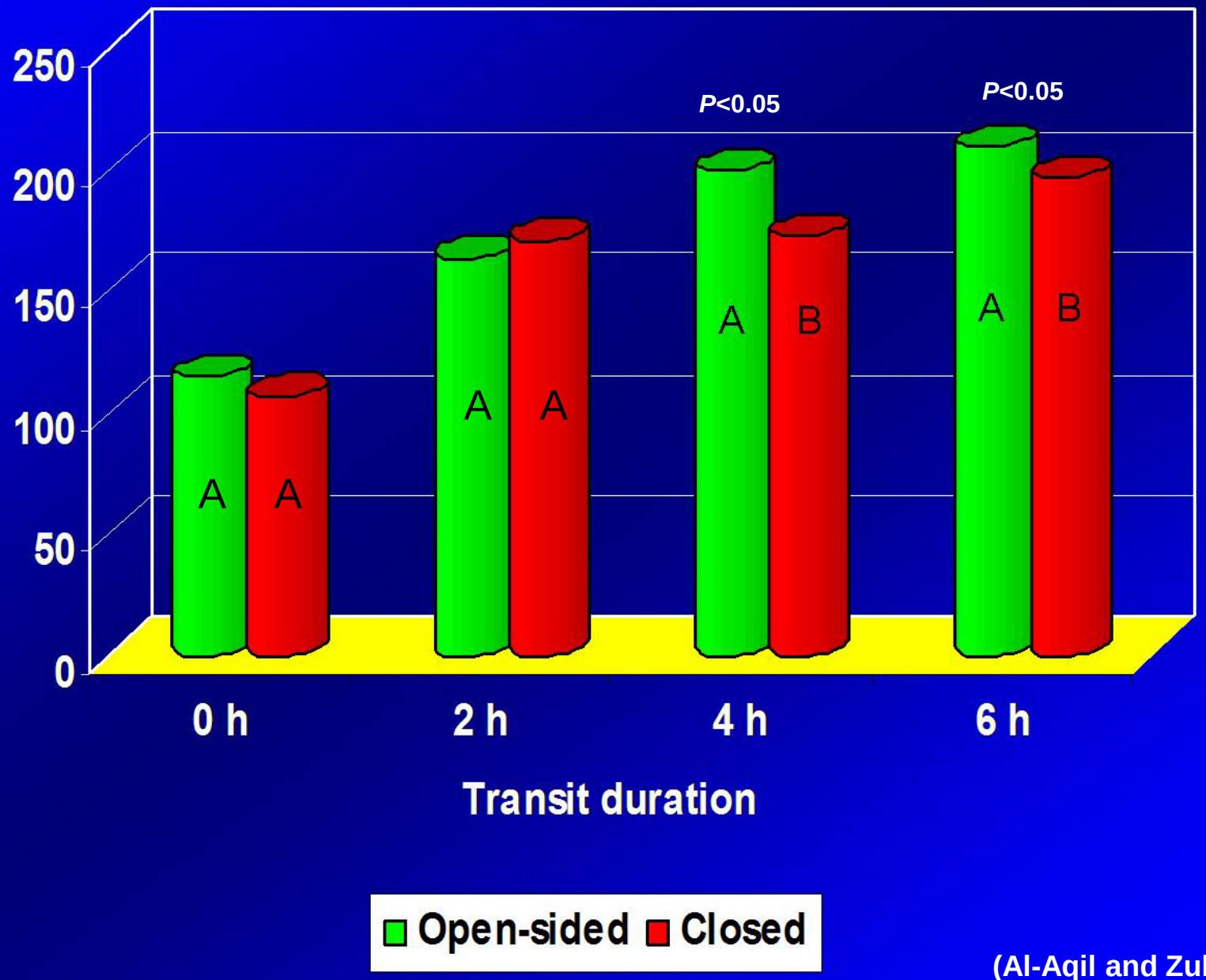
Enhanced heat tolerance as a result of neonatal feed restriction (Zulkifli et al., 2002; 2003), heat conditioning (Wang and Edens, 1998) and combination of both (Liew et al., 2003) in poultry has been attributed to greater HSP 70 response.



Mortality rate following heat exposure
Ad libitum = 18%
60% feed restriction = 0%

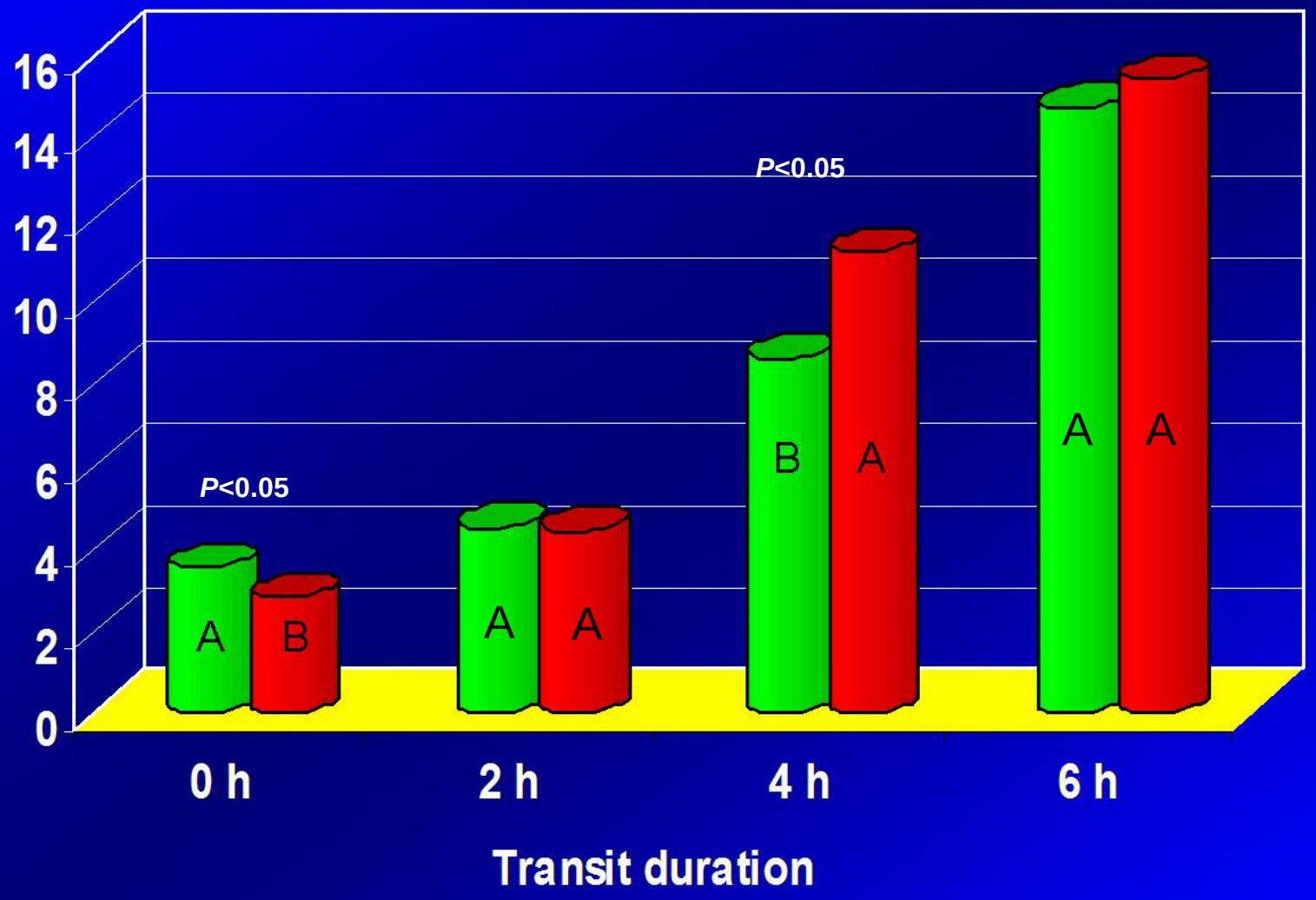
(Zulkifli et al., 2000; 2002)

The effect of transportation on brain HSP 70 density in chickens raised in open-sided and closed houses



(Al-Aqil and Zulkifli, 2009)

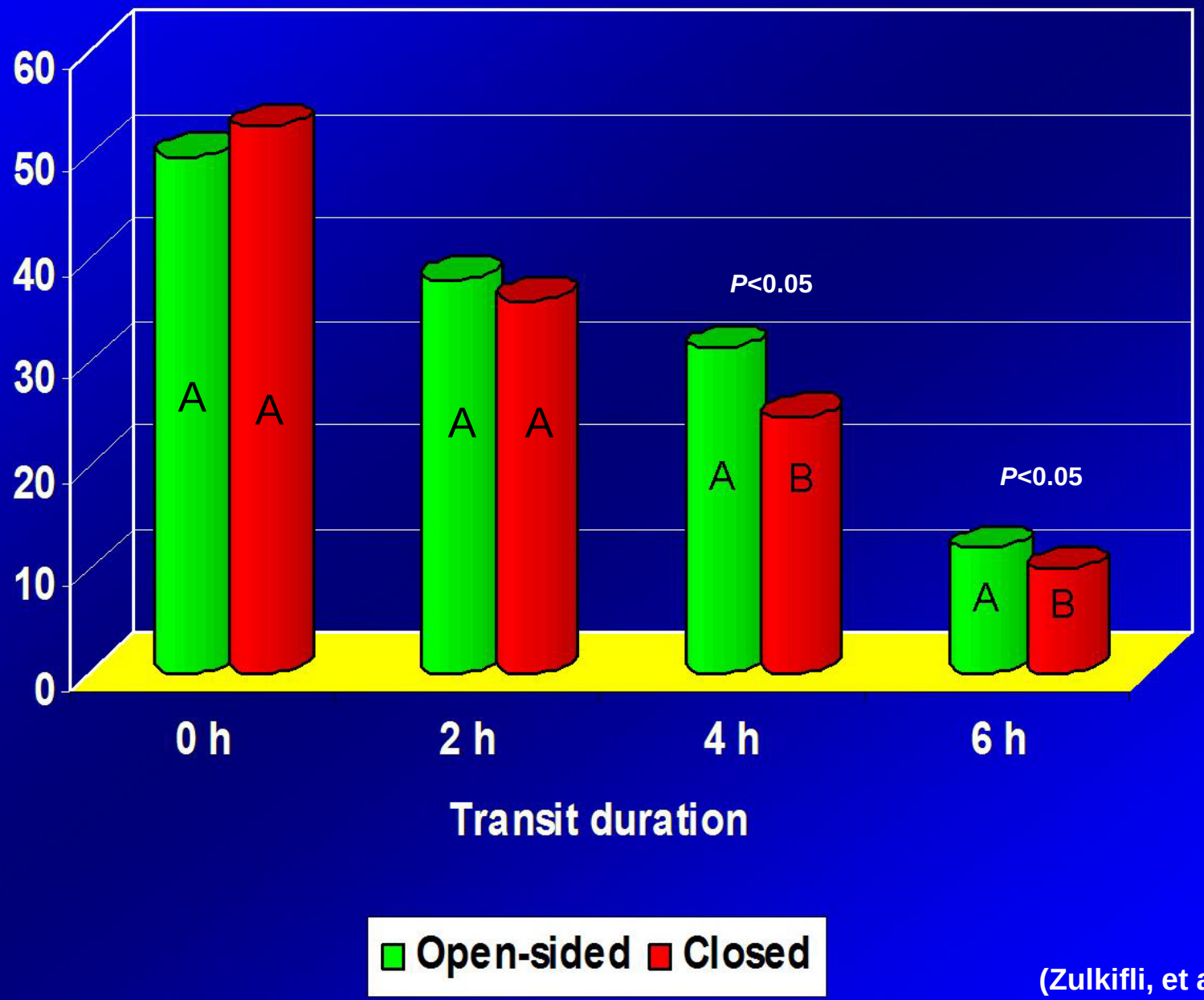
The effect of transportation on plasma levels of corticosterone (ng/ml) in chickens raised in open-sided and closed houses



Open-sided Closed

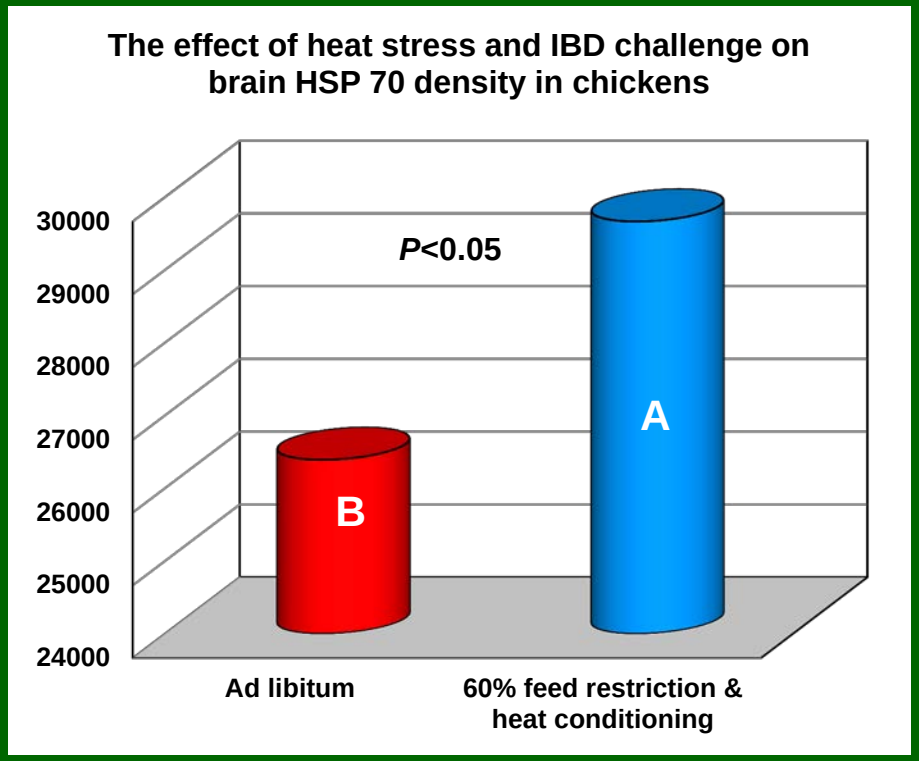
(Al-Aqil and Zulkifli, 2009)

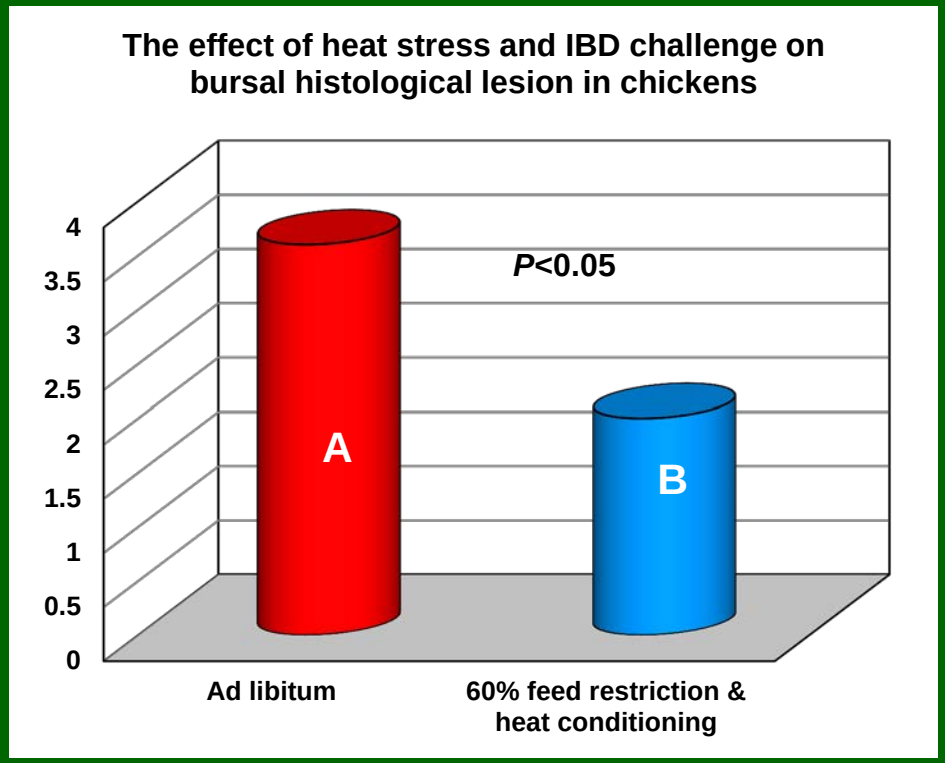
The effect of transportation on breast muscle glycogen content (mg/g) in chickens raised in open-sided and closed houses



(Zulkifli, et al., 2011)

Stress may impede antibody production and effective cell-mediated immunity thereby increasing susceptibility to viral diseases (Siegel, 1995)





HSP 70 response can be beneficial in enhancing resistance to infectious bursal disease in chickens under heat stress (Liew et al., 2003)

Induction of HSP 70 response in poultry appeared to be associated with underlying fearfulness (Zulkifli et al., 2009)

Table 3. Mean (±SEM) heat shock protein 70 densities in which fear responder group × duration of heat exposure interactions were significant

	Duration of heat exposure (h)	
Fear responder group[1]	0	3
LF	$114.20^{b} \pm 6.27$	$166.85^{b} \pm 1.62$ *
HF	$116.29^{b} \pm 2.61$	$206.93^{a} \pm 8.65$

[a,b]Means within a row-subgroup with no common superscripts differ ($P \leq 0.05$).

*Difference between fear responder groups ($P \leq 0.05$).

[1]LF = low fear group; HF = high fear group.

The quality of human-animal interactions can have a profound impact on many facets of animal's physiology and behaviour (Hemsworth and Coleman, 1998; 2004; Zulkifli , 2014)

Changes in heat shock protein 70, blood parameters, and fear-related behavior in broiler chickens as affected by pleasant and unpleasant human contact

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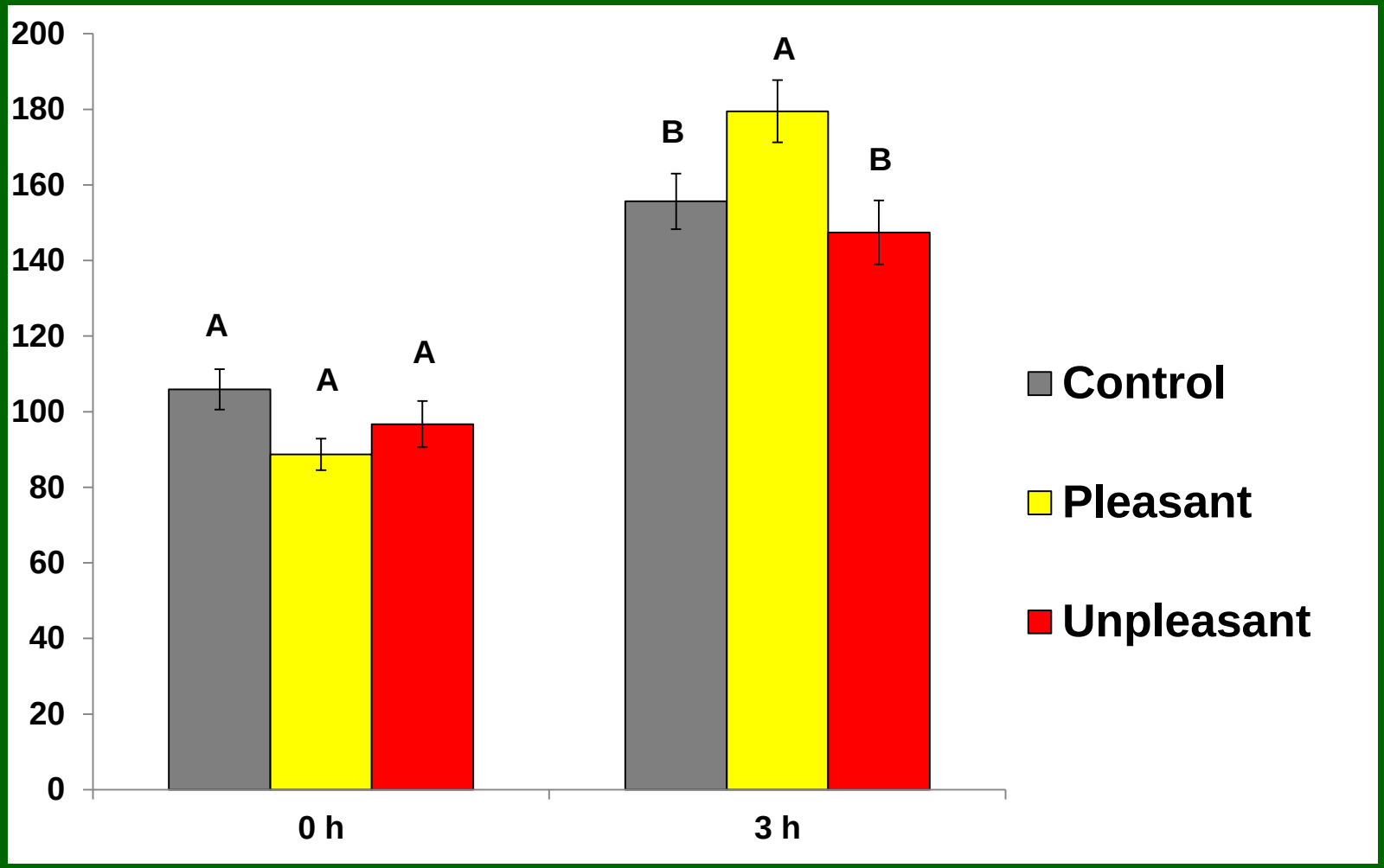
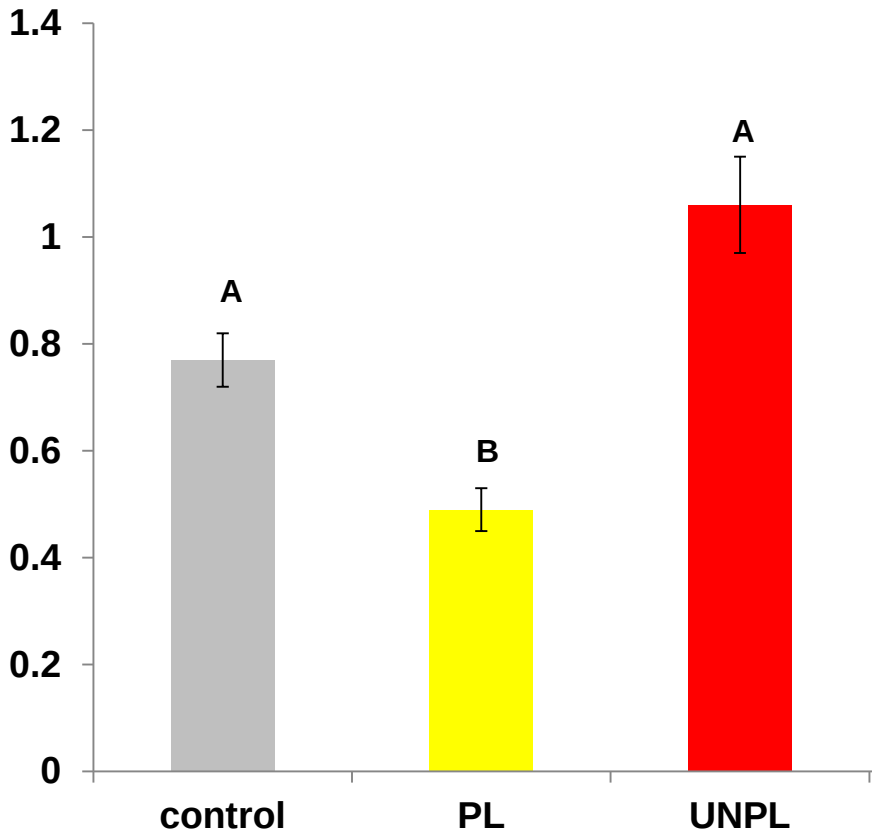
Pleasant human contact enhanced heat shock protein 70 expression in chicks following road transportation (Al-Aqil et al., 2013)



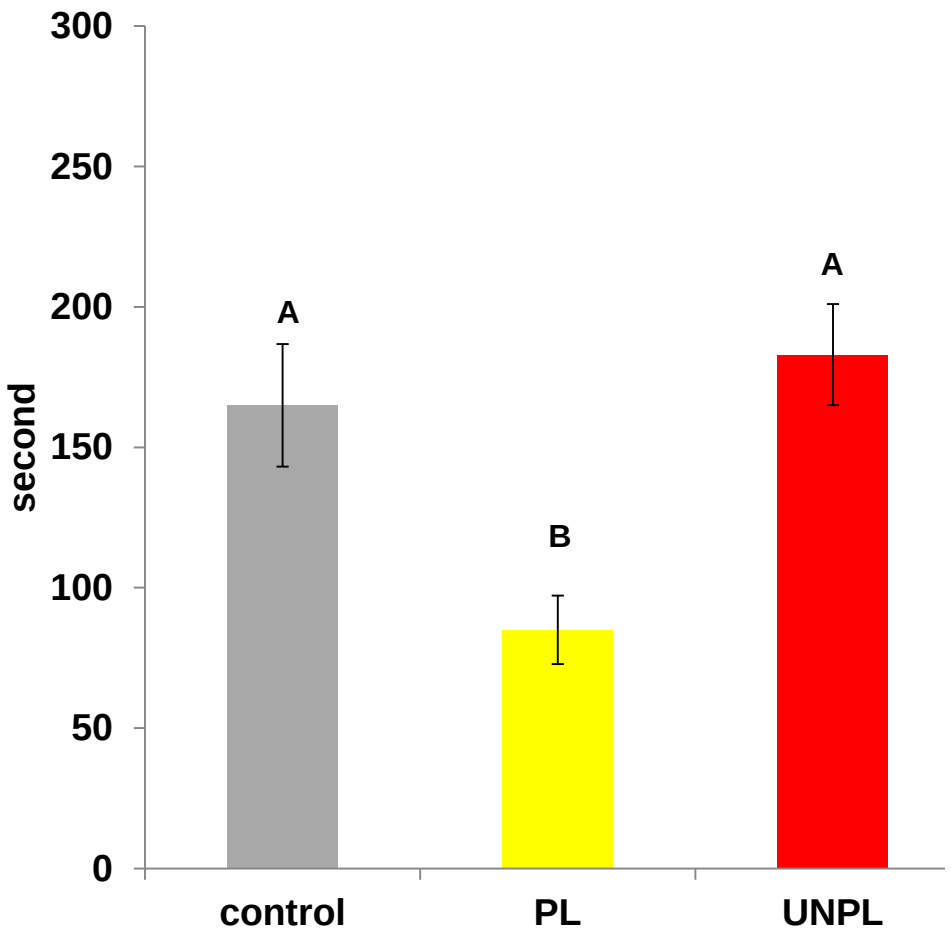
Means with no common letters differ at P<0.05

Pleasant physical human contact reduced heterophil / lymphocyte ratios following 3 h of road transportation in broiler chickens (Al-Aqil et al., 2013)



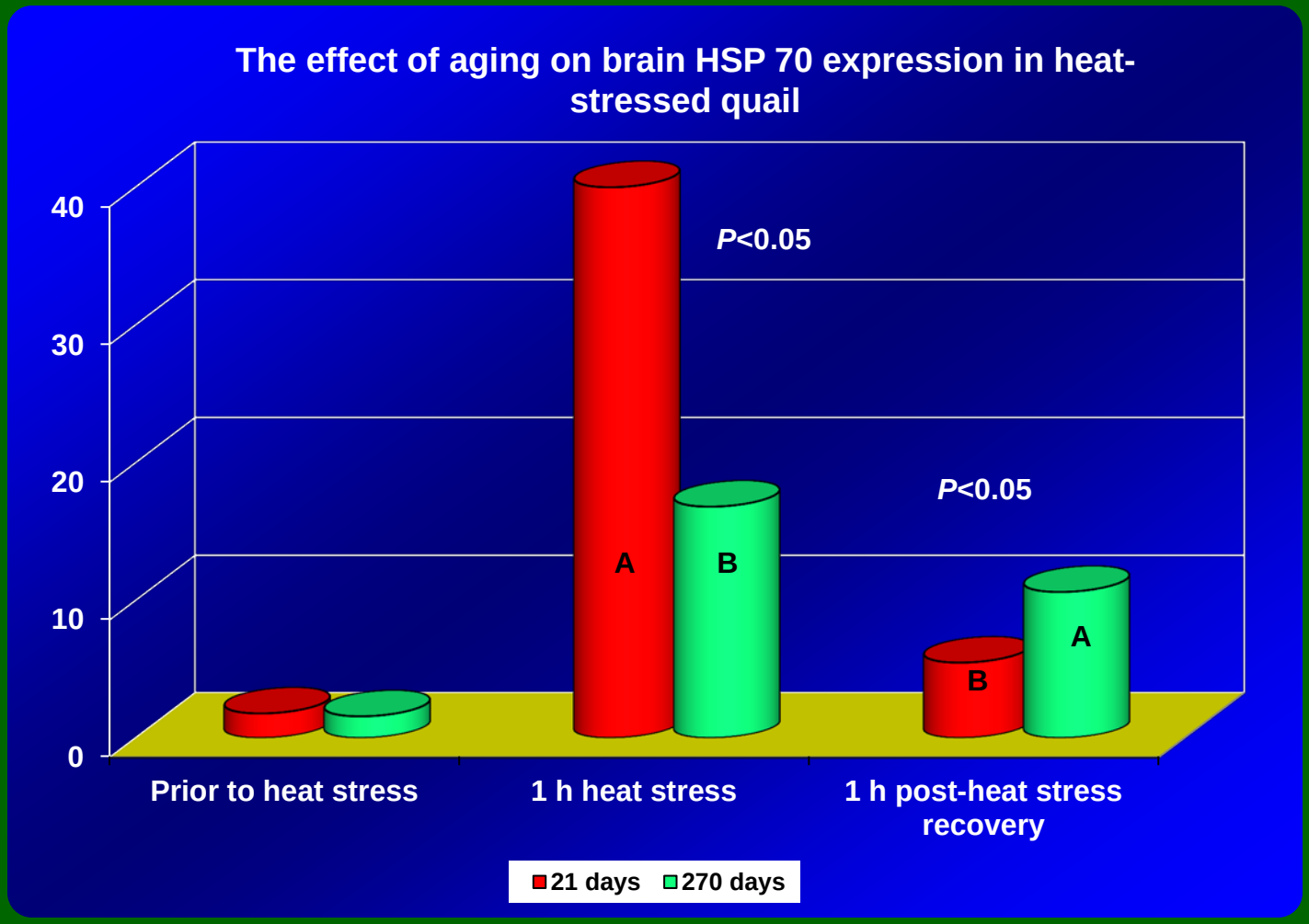
Means with different letters differ at $p < 0.05$

Pleasant l human contact reduced tonic immobility duration following 3 h of road transportation in broiler chickens (Al-Aqil et al., 2013)



Means with different letters differ at $p < 0.05$

Aging can impair the capacity of poultry to express HSP 70 under stressful conditions (Soleimani et al., 2011)



CONCLUSIONS

- HSPs are essential for cellular survival from heat stress and other types of physiological challenge.
- The mechanisms by which HSPs elicit adaptation and provide protection against cellular stress in poultry and other farm animals are still not thoroughly understood.
- Elucidating the role of HSPs as modifying factors in physiological stress response have opened up new avenues in the molecular and cellular mechanisms of adaptation and coping to environmental insults
- A thorough molecular characterisation of the heat-shock response in a range of farm animals would provide the basis for future genetic manipulation of the heat-shock response in a way that has not been possible before.

Acute phase proteins, interleukin 6, and heat shock protein 70 in broiler chickens administered with corticosterone

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ABSTRACT An experiment was conducted to determine the effect of corticosterone (CORT) administration on serum ovotransferrin (OVT), α1-acid glycoprotein (AGP), ceruloplasmin (CPN), and IL-6 concentrations, and brain heat shock protein (HSP) 70 expression in broiler chickens. From 14 to 20 d of age, equal numbers of birds were subjected to either (i) daily intramuscular injection with CORT in ethanol:saline (1:1, vol/vol) at 6 mg/kg of BW, or (ii) daily intramuscular injection with 0.5 mL ethanol:saline (1:1, vol/vol; control). Blood samples were collected before CORT treatment (14 d old), 3 and 7 d after CORT injections, and 4 d after cessation of CORT administration for determination of serum levels of CORT, OVT, AGP, CPN, and IL-6. Brain samples (whole cerebrum) were collected to measure HSP 70 density. Although CORT administration significantly increased feed intake, weight gain was significantly depressed. Administration of CORT also increased CORT, OVT, CPN, AGP, IL-6, and HSP 70 expression. Four days following cessation of CORT administration, OVT declined to the basal level but not CPN and AGP. In conclusion, an elevation in CORT can induce an acute-phase response and HSP 70 expression. Thus, APP and HSP 70 may be of value as indicators of stress in poultry.

Key words: acute phase protein, interleukin 6, heat shock protein 70, corticosterone, broiler

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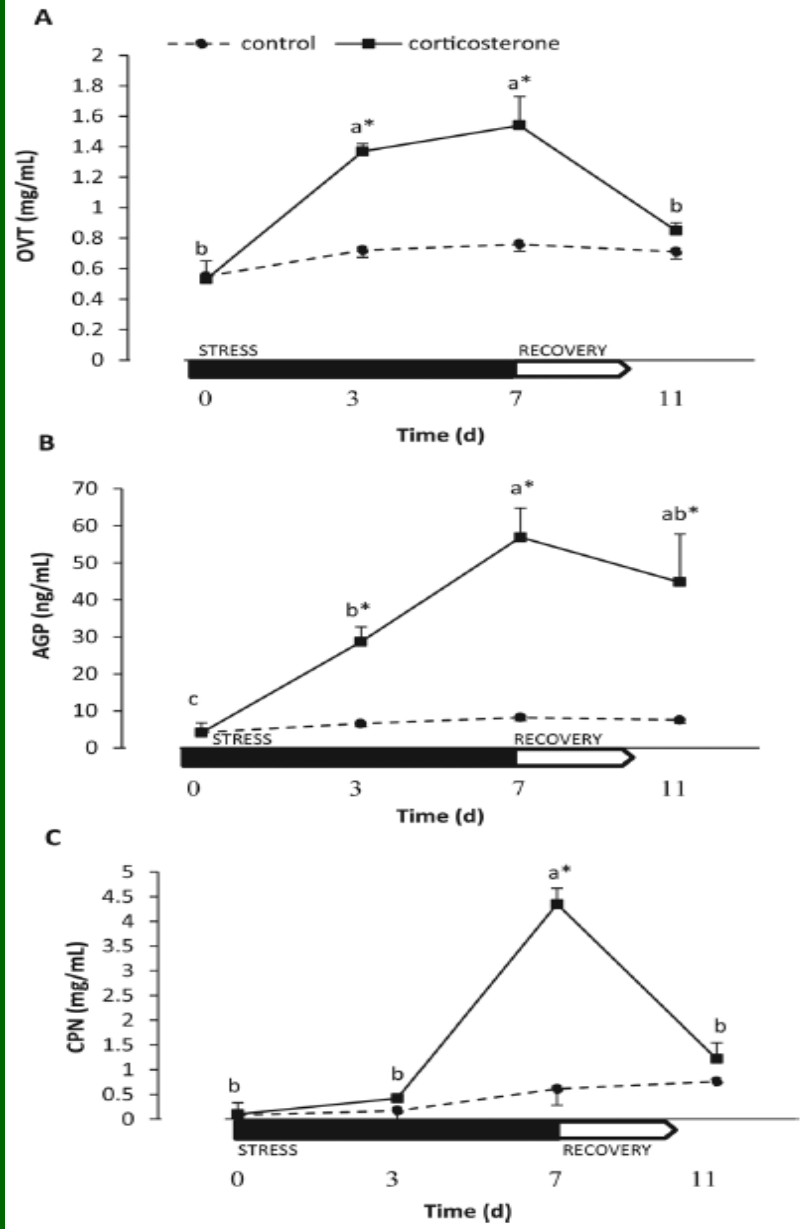

Figure 3. Time course changes in serum ovotransferin (A; OVT), α_1-acid glycoprotein (B; AGP), and ceruloplasmin (C; CPN) concentrations following daily saline (control) or corticosterone administration for d 0 (14 d of age), d 3 (17 d of age), d 7 (20 d of age), and d 11 (24 d of age). Means (n = 16) within a treatment group with no common letters (a–c) differ at $P < 0.05$. *Significant difference between treatment groups ($P < 0.05$).

Response to dietary supplementation of L-glutamine and L-glutamate in broiler chickens reared at different stocking densities under hot, humid tropical conditions

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ABSTRACT A study was conducted to determine whether supplementing AminoGut (a commercial dietary supplement containing a mixture of L-glutamine and L-glutamic acid) to broiler chickens stocked at 2 different densities affected performance, physiological stress responses, foot pad dermatitis incidence, and intestinal morphology and microflora. A randomized design in a factorial arrangement with 4 diets [basal diet, basal diet + 0.5% AminoGut from d 1 to 21, basal diet + 0.5% AminoGut from d 1 to 42, and basal diet + virginiamycin (0.02%) for d 1 to 42] and 2 stocking densities [0.100 m^2/bird (23 birds/pen; LD) or 0.067 m^2/bird (35 birds/pen; HD)]. Results showed that villi length and crypt depth were not changed by different dietary treatments. However, birds in the HD group had smaller villi ($P = 0.03$) compared with those of the LD group. Regardless of diet, HD consistently increased the serum concentrations of ceruloplasmin, α-1 acid glycoprotein, ovotransferin, and corticosterone ($P = 0.0007$), and elevated heterophil to lymphocyte ratio (0.0005). Neither AminoGut supplementation nor stocking density affected cecal microflora counts. In conclusion, under the conditions of this study, dietary supplementation of AminoGut, irrespective of stocking density, had no beneficial effect on growth performance, intestinal morphology, and physiological adaptive responses of broiler chickens raised under hot and humid tropical conditions. However, AminoGut supplementation from d 1 to 42 was beneficial in reducing mortality rate. Also, the increased serum concentrations of a wide range of acute phase proteins together with elevated corticosterone and heterophil to lymphocyte ratio suggested that high stocking density induced an acute phase response either indirectly as a result of increased incidence of inflammatory diseases such as foot pad dermatitis or possibly as a direct physiological response to the stress of high stocking density.

Key words: L-glutamine, L-glutamic acid, stocking density, acute phase protein, well-being

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Table 5. Mean (±SEM) intestinal *Escherichia coli* and lactobacilli counts, and foot pad dermatitis (FPD) scores by diet and stocking density

Item	*E. coli* (log cfu/g of digesta)	Lactobacilli (log cfu/g of digesta)	FPD
Diet[1]			
Basal	11.36 ± 0.11^{a}	8.24 ± 0.54	1.81 ± 0.12^{a}
AG3	10.82 ± 0.37^{a}	6.86 ± 0.36	1.42 ± 0.11^{b}
AG6	11.48 ± 0.28^{a}	8.02 ± 0.58	1.67 ± 0.11^{ab}
VM	6.48 ± 0.82^{b}	7.78 ± 0.57	1.59 ± 0.11^{ab}
Density[2]			
LD	9.73 ± 0.73	7.44 ± 0.36	1.15 ± 0.06^{b}
HD	10.37 ± 0.47	8.05 ± 0.38	2.09 ± 0.06^{a}
ANOVA (P-value)			
Diet	0.0001	0.31	0.01
Density	0.11	0.23	0.0001
Diet × density	0.30	0.74	0.35

[a,b]Means ± SEM within a column-subgroup with no common superscripts are different at $P < 0.05$.

[1]AG3: AminoGut d 1 to 21; AG6: AminoGut d 1 to 42; VM: virginiamycin.

[2]LD: low stocking density; HD: high stocking density.


“THANK YOU”
“TERIMA KASIH”
(Fernando Armosolo, 1938)