Variation in Some Blood Parameters of Geese Subjected to Feather Gathering

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Abstract: Two experiments were conducted on 50 (25♂, 25♀) geese of Babat Hungarian Upgraded breed during their post-breeding molting period to measure stress response to feather gathering. They were assigned to five treatment groups in both experiments: (1) control, (2) gathered, (3) given anti-stress supplement before gathered, (4) sham gathered and (5) given anti-stress supplement before sham gathered. In Experiment 1 blood samples were taken one hour before and one hour after the procedures and plasma levels of thyroxin (T₄) and triiodothyronine (T₃) were measured by radioimmunoassay. The concentrations of both T₄ and T₃ decreased post-exposure indicating a mild to moderate distress. In Experiment 2 blood samples were taken 24 h and 7 days post-procedures for determining total white blood cell counts and the heterophil granulocyte to lymphocyte (H/L) ratios. Feather gathering caused a mild stress that affected circulating white blood cells. However, the H/L method on its own is not a reliable stress indicator of the feather gathering procedure. The anti-stress supplement seemed to have little effect on the thyroid hormones levels or the haematological variables.

Key words: Goose, heterophil to lymphocyte ratio, thyroxin, triiodothyronine

INTRODUCTION
Gathering feathers from live geese is a traditional practice in Hungary regulated by the MARD (178/2009). (XII.29.), yet animal rights activists campaigned lately against this practice in the media for causing to goose pain and distress (Kozak et al., 2010).

Feathers are gathered from geese during their natural molting when they shed and re-grow feathers. Young geese at 10-11 weeks of age and breeder geese after the egg-laying season undergo one complete molt followed by three partial molts every 6-7 weeks (Kozak, 2011). Both feather growth and the shedding of feathers (moult) are under endocrine control. Thyroxin (T₄) released by the thyroid gland stimulates the growth of young feathers and the actual shedding of feathers may result from a fall in the sex steroids at the end of the breeding season, when thyroxin secretion is above normal (Campbell and Lack, 2013).

Although, geese are more stress-prone during molting than at other times the feathers can be gathered painless, without causing lesions to the skin. The new generation of feathers pushes the old out of the living tissue, so that the old feathers contain no living tissue and are not linked to the circulation or innervations. Any intimate connection between the base of the feather and the feather follicle is interrupted. The goose skin is relatively insensitive but the timing of gathering is crucial since feathers need to have “maturity” (Rauch et al., 1993).

The hypothalamic-pituitary-adrenal (HPA) axis plays a pivotal role in triggering the stress response. Physiological indicators of acute stress include the rapid secretion of catecholamines (adrenalin, noradrenalin) from the adrenal medulla and glucocorticoids from the adrenal cortex (Sapolsky, 1992). In birds, plasma levels of catecholamines can increase within seconds of exposure to stress and glucocorticoids rise within minutes (Le Maho et al., 1992). Elevation of adrenalin leads to increased vasoconstriction, heart rate, blood pressure and blood glucose (Freeman, 1985; Goldstein, 1987; Wittmann, 1984). Elevation of corticosterone leads to a series of events that can enhance short-term survival, including redirected behaviour and mobilization of energy reserves (Wingfield et al., 1998).

Similarly to the HPA axis the hypothalamic-pituitary-thyroid (HPT) axis is also stress-responsive. Its principal components are the hypothalamic thyrotropin-releasing hormone (TRH), pituitary thyroid-stimulating hormone (TSH) and the thyroid hormones: thyroxin and triiodothyronine (T₃). Thyroid hormones influence major processes such as growth, differentiation and metabolism (Norris and Carr, 2013). Stress regulation of the HPT axis has received little attention. Thyroid function is usually down-regulated during stressful conditions; triiodothyronine and thyroxin levels decrease with stress. Namely, stress inhibits TSH secretion through the action of glucocorticoids (end product of HPA axis) on the central nervous system (Helmreich et al., 2005).

Another indicator of stress is the ratio of heterophil granulocytes to lymphocytes (H/L) in the blood (Gross and Siegel, 1983; Maxwell, 1983). Stressors (e.g., food or water deprivation, temperature extremes, constant...
light, or exposure to novel social situations) elevate the number of heterophil granulocytes and depress the number of lymphocytes (Gross and Siegel, 1986; Gross, 1989; McFarlane and Curtis, 1989). Leukocyte numbers change more slowly (30 min to 20 h) in response to stress than does corticosterone (Dein, 1988; Maxwell, 1993; Cunnick et al., 1994). Further, these changes are less variable and longer lasting than the corticosterone response and multiple stressors usually have an additive effect (McFarlane and Curtis, 1989; McKee and Harrison, 1995). The mechanisms mediating these cellular changes are poorly defined in birds (Dohms and Metz, 1991), but may include changes in adrenal corticotrophin hormone and corticosterone (Gross and Siegel, 1983; Gross, 1989) and/or altered production of and responsiveness to cytokines (Cunnick et al., 1994). Previous examination of plasma glucose and corticosterone levels revealed that gathering feathers caused not more distress to geese than the handling or catching (Janan et al., 2001; Toth et al., 2012). This has prompted us to examine the thyroid hormones’ levels and the heterophil granulocyte to lymphocyte ratio as a stress/welfare indicator for geese subjected to feather gathering with or without given an anti-stress supplement.

MATERIALS AND METHODS

Two experiments were conducted on 50 (25♂, 25♀) geese of Babat White Hungarian Upgraded breed during their post-breeding moulting period in early July. They were housed in pens on deep litter system with yard access and fed ad libitum a maintenance ration appropriate to the EU regulation (Council of Europe, 1999).

The geese in both experiments were assigned to one of five groups of 10 (5♂, 5♀) according to the following treatment protocol:

- **Group 1**: Geese moulted naturally (control)
- **Group 2**: Geese were subjected to feather gathering
- **Group 3**: Geese given the anti-stress supplement from 5 days before gathering feathers
- **Group 4**: Geese were subjected to sham feather gathering
- **Group 5**: Geese given the anti-stress supplement from 5 days before sham gathering

The feather gathering procedure was performed on geese between 9:00 and 10:00 am. The geese were positioned dorsally with the head downwards, while feathers and down were removed from the lower belly, the flanks and the areas not covered by the wings. Subsequently, geese were turned on their ventral side and feathers were removed from the back (Szentirmay, 1988). The duration of this procedure including catching was about 10 min per goose. Sham feather gathering was done similarly without removing any feathers and it lasted for 4-5 min. The anti-stress supplement containing all essential amino acids and vitamins was given in the drinking water at a dose of 1 ml/goose/day. In Experiment 1, the effect of feather gathering on the thyroid hormones’ level was examined. Blood was taken from the wing vein into heparinized tubes one hour before and one hour after the said procedure during the forenoon to eliminate any diurnal variation in thyroid hormones (Newcomer, 1974); thyroxin (T4) is highest during the day, triiodothyronine (T3) is at its highest during the day (Klandorf et al., 1978). Plasma samples were obtained by centrifugation and stored at -20°C until analyzed. Plasma levels of thyroxin (T4) and triiodothyronine (T3) were determined by radioimmunoassay according Peteh et al. (1978).

In Experiment 2, the effect of feather gathering on total and differential white blood cell count was examined. Blood was taken from the wing vein into native collection tubes 24 h and 7 days post-procedures taking into consideration that exposure to a short-term physical stressor resulted fluctuations in heterophil to lymphocyte (H/L) ratio within 18 h; the response peaked about at 24 h and returned to normal at about 30 h in chickens (Gross, 1990). Total white blood cell (WBC) count was determined with a Bürker chamber using a WBC diluting fluid described by Horvath (1979). Differential count (relative percentage of each type of white blood cells) was appraised from 100 cells of May-Grunwald and Giemsa stained blood smears prefixed in methanol. The H/L ratio was calculated by dividing the sum of heterophil types by the sum of lymphocyte types (Gross and Siegel, 1983).

The blood variables were statistically processed using Microsoft Office Excel 2007 employing analysis of Variance (ANOVA) and the Student’s t-test to reveal the significance of between or within-group differences in the means (Svab, 1981).

RESULTS

There were no significant between-sex differences in the means of the blood variables, therefore the group means are presented to give an overall view.

Mean plasma levels of thyroid hormones are shown by group and sampling time in Table 1. Mean plasma T4 levels showed no significant between-group difference one hour before the feather gathering or sham gathering procedure (p>0.1). The values decreased in all five groups one hour post-procedures that was significant in group 3 and 5 (p<0.10, p<0.05, respectively). The decrease was lowest in group 1 (4%) comparable in groups 4 and 2 (7.4 and 7.5%, respectively) and highest in groups 3 and 5 (10.3 and 12.5%, respectively).

Mean plasma T3 levels varied non-significantly across all the five groups one hour before the procedures. The
values decreased in all five groups one hour post- 
procedures that was significant in groups 2, 4 and 5
(p<0.05). The decrease was lower in groups 1 and 2
(18.5 and 20.8%, respectively), while it was higher in
groups 4, 3 and 5 (25.9 and 27.3, 27.3%, respectively).
Mean total WBC counts, percentage of heterophil 
granulocytes and lymphocytes and the H/L ratios are 
shown by group and sampling time in Table 2. Mean 
total WBC counts showed non-significant variation by 
either groups or sampling time (p>0.10). Mean 
percentage of heterophil granulocytes was identical in 
groups 1, 2 and 3, respectively and slightly higher in 
groups 4 and 5 at the 24 h post-procedure sampling. It 
was higher in group 1 compared to the other groups 7 
days later. Mean percentage of lymphocytes varied 
non-significantly at the 24 h post-procedure sampling. 
However, 7 days later it was significantly lower in group 
1 (p<0.10) than in the other groups, except group 4. The 
H/L ratio ranged between 0.63-0.70 at the first and 0.60- 
0.67 at the second sampling time.

**DISCUSSION**

The avian hypothalamus secretes TRH during stress 
and periods of decreased circulating hormones 
(Engelking, 2012). Thyroid hormones are also involved 
in stress regulation (Klandorf et al., 1978).

Previous studies suggested that stress causes a 
decrease in thyroid hormones, but the results are 

inconclusive. Immobilization has been shown to both 
increase and decrease thyroid hormone levels 
(Turakulov et al., 1994; Langer et al., 1983). In force-fed 
goose plasma level of both T4 and T3 was lower, than in 
birds fed naturally due to a possible metabolic 
adaptation to energy consumption of the body (Janan 
unpublished). The stress-induced decrease in thyroid 
hormones appears to be mediated, in part, by changes 
in hypothalamic drive to the axis (Helmerich et al., 2005).

In this study, the consistent decreases in plasma T4 and 
T3 levels across all the five goose groups suggested a 
stress response. The small decrease in the control 
group possibly indicated a mild distress due to blood 
sampling, while the higher degree of decreases in the 
other groups may have caused a moderate distress 
associated with the uneasiness of feather gathering or 
sham gathering beyond blood sampling.

The application of heterophil to lymphocyte (H/L) ratios 
to assess stress has its origin in the 1980s. However, 
the inherent variation in leukocyte cell counts among 
individuals limits the utility of this method. Research with 
birds also demonstrated a relatively slow leukocyte 
response time that makes difficult obtaining baseline 
samples convenient (Davis et al., 2008).

The experimental treatments used in the Gross 
(year=1989) study altered the H/L ratios without having much 
effect on the white blood cell count. In our experiment total 
WBC counts, percentage of heterophil
granulocytes and lymphocytes varied relatively little 24 h pre- and 7 day post-procedures. A low H/L accompanied by a high total leukocyte count, a frank indicator of stress (Cotter, 2015), could not be detected in our sample. In birds, the H/L ratio is normally less than 1. Gross and Siegel (1993) suggested that reference values for the H/L ratio is about 0.2, 0.5 and 0.8 are characteristic of low, optimal and high degree of stress in chickens. Based on this affirmation, geese in our experiment showed H/L values above the optimal (0.60) and below the high degree of stress (0.70). However, these figures corresponded to the physiological H/L ratios reported for domestic geese of 0.78 (Bardos, 2000) and for adult Babat Hungarian Upgraded geese of 0.55 (Nikodemusz et al., 1991). Thus the application of the H/L ratio of chickens (Gross and Siegel, 1983) may not be appropriate for domestic geese.

The results obtained here indicate the inadequacies of the H/L method, used alone, as a reliable stress indicator of gathering feathers from geese. This procedure resulted in a milder stress response that affected the circulating white blood cells.

The anti-stress supplement seemed to have little effect on the thyroid hormones levels or the haematological variables. The study of Carew et al. (1998) also showed that amino acid excesses had little effect on plasma levels of thyroid hormones in broiler chicks. A similar anti-stress supplement also showed no effect on plasma corticosterone levels in growing geese subjected to the feather gathering or shame gathering procedure (Toth et al., 2012).

ACKNOWLEDGEMENTS

We appreciate the comments made by Dr. Attila Salamon (Ballyrichard Farm, Ireland) on an earlier version of this manuscript.

REFERENCES


