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STUDY ON THE METABOLIC IMPLICATION OF SUPPLEMENTAL TRYPTOPHAN IN EXPOSED TO STRESS CHICKENS

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Abstract

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The effect of supplemental tryptophan (5g/1Kg^{-1} diet) on some indices of stress, heterophil-to-lymphocyte ratios (H:L), leukocyte and erythrocyte numbers in chickens under stress induced by alternating periods of normal feeding with periods of feed and water withdrawal was investigated. Twenty chickens at the age of six weeks were randomly allocated into two groups- control and experimental. Experimental birds were deprived of feed and water and their legs tied for seventeen hours each day in four consecutive days. Body weight was registered on d 0 (baseline value), d 7 (preliminary period-free of stress) and on d 11 (experimental period). Blood samples were taken on d 0 and d 11. Relative weights of some immunobiological organs were also measured at the end of the experimental period. Supplemental tryptophan alleviated body weight decline, decreased spleen ($P>0.05$) and liver ($P<0.05$) relative weights, but had no significant effect on those of adrenal glands and bursa of Fabricius. Plasma urea levels were not influenced by supplemental tryptophan but plasma glucose levels declined by 14h and cholesterol levels by 17h in comparison with control group. Tryptophan supplementation prevented H: L ratio increment caused by stressor treatment. Leukocyte numbers and hematocrit values were not significantly influenced by tryptophan. Interleukin-1α response to stress declined in triptophan supplemented chickens by 14 h following the start of stressor treatment but corticosterone response was not influenced. Our results indicate that tryptophan is implicated in white blood cells dynamics and influences the pattern of the observed stress indices in chickens.

Key words: tryptophan, stress, H: L ratio, IL-1α, white blood cells, corticosterone, chickens

Introduction

Tryptophan is one of the limiting essential amino acids in protein metabolism. The metabolism of the amino acid L-tryptophan is a highly regulated physiological process leading to the generation of several neuroactive compounds within the central nervous system. These include the aminergic neurotransmitter serotonin (5-hydroxytryptamine, 5-HT), products of the kynurenine pathway of tryptophan metabolism (including 3-hydroxykynurenine, 3-hydroxyanthranilic acid, quinolinic acid and kynurenic acid), the neurohormone

melatonin, several neuroactive kynuramine metabolites of melatonin, and the trace amine tryptamine (Ruddick et al., 2006). Because of its involvement with brain serotonin, tryptophan has been shown to be responsible for affecting mood regulation, feed intake, behavior, and sleep patterns (Baraniyova,1991). Psychological stress activates the serotonergic neurons in the hippocampus and the amygdale through the cortical association areas and through ascending catecholaminergic neurons from the brain stem (Koob and Heinrichs, 1999). Stress in general often results in changes of serotonin (5-HT) metabolism, but large variations are found in the literature with different kinds of stress, different measurements and different time schedules. In contrast of other stress factors, restrain stress increases the content of 5-HT in the dorsal ralphe nucleus (DRN), but does not increase the metabolism of 5-HT and does not induce changes in hypothalamic levels of 5-HT (Jorgensen, 2007). Serotonin does not pass the blood-brain-barrier and the only way to increase the concentration of serotonin is treatment with precursors, 5-hydroxytryptophan or tryptophan, which act to increase the cerebral serotonin state. The depressive effect of tryptophan deficiency (Uttrecht et al., 1991) is aggravated in the presence of excess protein in the form of large neutral amino acids.

In poultry tryptophan has been previously shown to alter behaviour of birds and the activity of certain neurotransmitters (Shea et al., 1991; Denbow et al.,1993; Shea-More et al.,1996; Corzo et al.,2005b), but results about tryptophan association with the physiological stress were inconsistent (Corzo et al., 2005a; MacKenzie al., 2004).

The purpose of this study was to investigate the effect of supplemental tryptophan on some indices of physiological stress, interleukin-1α, heterophil-to-lymphocyte ratios (H: L) and leukocyte and erythrocyte numbers in chickens under stress.

Materials and Methods

Twenty chickens (White Plymouth rock pure initial line from the National Genetic Resources) at the age of six weeks were randomly allocated into two groups- control and experimental and were raised in pens (5 chicks per cage). The experimental design included preliminary period (0-7d) and experimental period (7-11d). During the preliminary period the chickens were raised in stress free conditions and then were deprived from food and water and their legs were tied for 14 h each day in four consecutive days (from 15h till 08 h the next morning) under the conditions of the experimental period.

Both control and experimental chickens were fed *at libitum* on a diet adequate to support chickens growth. Supplemental tryptophan (5g/1Kg^{-1} diet) was added to the diet of experimental chickens from day 0 to day 14. Body weight was registered on d 0 (baseline value), d 7 (preliminary, free of stress period) and d 11 (experimental period). Blood samples were taken on d 0 and d 11. On d 11 blood samples were collected twice –at 14 h and 17 h after the beginning of stress exposure. On 13 h after the exposure to stress the bird's legs were untied but they were still hungry and thirsty. Following the last sampling (17h) all birds were decapitated and the relative weights (g/kg BW) of adrenal glands, bursa of Fabricius, spleen and liver, were measured.

Plasma glucose level was determined by the method of Ceriotti as modified by Profirov (1990) and plasma total cholesterol and urea levels were measured by the method of Watson (1960) and Rerat et al. (1979), respectively.

Plasma corticosterone and Intelreukin-1α were determined using enzyme immunoassay kits (IBL, Gesellschaft fur immunchemie und immunbiologie, MBH, D 22335 Hamburg, Germany).

Leukocyte and erythrocyte numbers were counted by the classical method of Ibrishimov and Lalov (1984).

Peripheral blood leukocytes subpopulations were counted microscopically in smears (Giemsa-Romanovsky-stain) made by 17h following the start of stressor episode.

The results of one factor statistical analysis are expressed as means ±S.E.M. and were analyzed by ANOVA.

Results and Discussion

Stress exposure caused decline of body weight ($P<0.05$) in control chickens. Supplemental tryptophan alleviated body weight (BW) decline (Figure 1) in experimental chickens. The rapid loss of BW during the stressor episode may indicate that birds were under extreme stress (Thaxton and Puvadolpirod, 2000). Tryptophan inadequacy was shown (Corzo et al., 2005b) to depress body weight gain, feed intake and feed conversion. Consequently, the observed body weight decline in control birds could be related to possible stress-induced insufficiency of dietary tryptophan.

Plasma glucose level (Figure 2) in experimental chickens declined significantly ($P<0.05$) at the end of stressor treatment. The metabolic mechanism, which is meant to provide energy necessary for the homeostatic condition is gluconeogenesis (Puvadolpirod and Thaxton, 2000, a.b.c). This mechanism in our experiment was probably activated in control, but not in tryptophan supplemented chickens. Our data come to show that supplemental tryptophan was probably implicated in glucose metabolism under the imposed stress conditions.

Plasma cholesterol level (Figure 3) declined too in tryptophan supplemented chickens at the end of stressor treatment relative to those in control group ($P<0.05$). Plasma cholesterol level has been accepted as one of the promising indicators of stress in human and animals (Puvadolpirod and Thaxton, 2000a). Stress-induced plasma cholesterol enhancement is

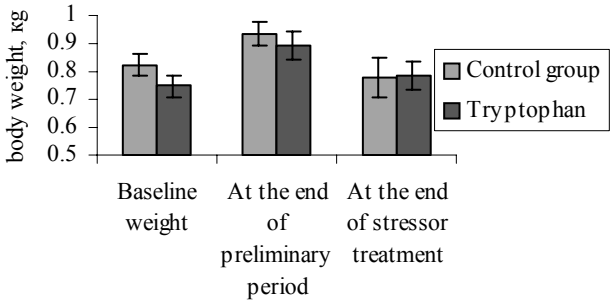

Fig.1. Effect of supplemental tryptophan on body weight of chickens under stress

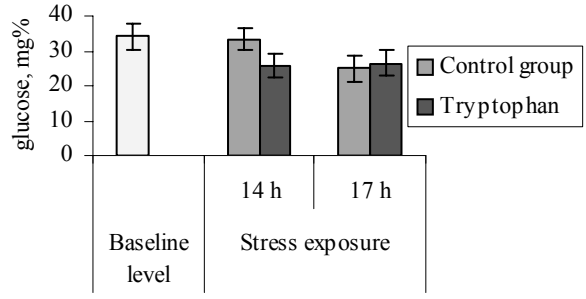

Fig. 2. Effect of supplemental tryptophan on plasma glucose levels in chickens under stress

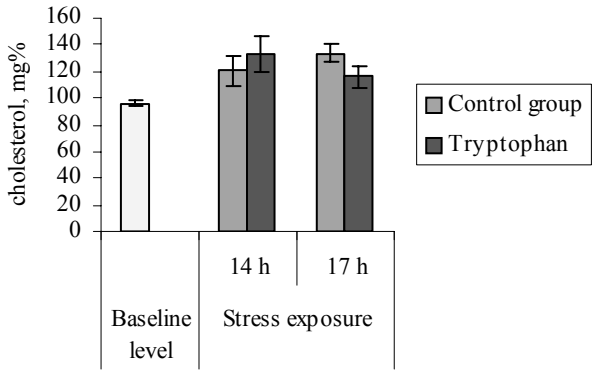

Fig. 3. Effect of supplemental tryptophan on plasma cholesterol levels in chickens under stress

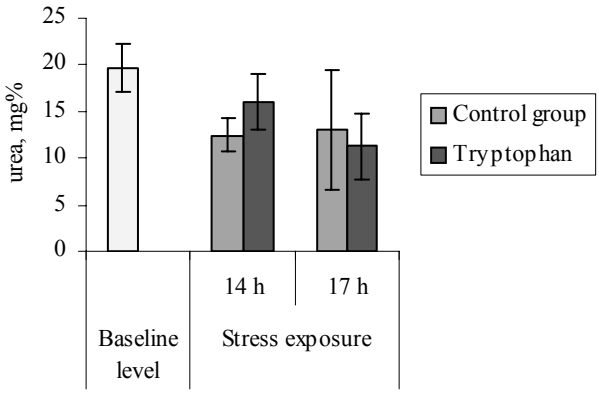

Fig. 4. Effect of supplemental tryptophan on plasma urea levels in chickens under stress

currently not well understood (Sahin and Kusuk, 2001). The very fact that cholesterol level declined in experimental chickens supports our view that supplemental tryptophan counteracts the dynamics of classical metabolites which are widely used as reliable stress indicators. Plasma urea levels were not influenced by supplemental tryptophan (Figure 4) but were significantly lower ($P<0.05$) by 14h in control chickens and by 17h in experimental chickens as compared to basal urea level. Plasma urea level is influenced by many factors but the main ones in our case were most probably the lack of food and dietary protein respectively in either group of chickens during the experimental period.

Tryptophan decreased spleen ($P>0.05$) and liver relative weights ($P<0.05$), but had no significant effect on those of adrenal glands and bursa of Fabricius (Table 1). The unchanged relative weights of adrenal glands and bursa of Fabricius in tryptophan supplemented chickens coincide with the relatively slight (twofold) enhancement of adrenal activity as compared to that reported by Puvadolpirod and Thaxton (2000a) in adrenocorticotropin treated chickens (more than 20 times increase of plasma corticosterone). Food withdrawal in our experiment did not cause similar changes of the studied stress indices as those observed under various stress conditions in chickens. The decline of liver relative weight is not consistent with the reported higher liver weight in chickens under continuous administration of adrenocorticotropin (Puvadolpirod and Thaxton, 2000a, c). It was obviously due to the specific metabolic involvement of tryptophan. Liver is unique in utilizing tryptophan dioxygenase (TDO), which is principally regulated by tryptophan concentration and general regulators of metabolism, such as corticosteroids and insulin (Moffett et al., 1998). However we don't know whether the implication of tryptophan in liver metabolic processes had a certain stress protecting effect.

Table 1
Relative weight of some lymphoid organs in chickens under stress

Lymphoid organs	Relative weights, g/kg BW	
	Control group	Experimental (tryptophan) group
Adrenal glands	0.067 ± 0.008	0.04 ± 0.01
Bursa of fabricius	0.698 ± 0.059	0.618 ± 0.062
Spleen	2.112 ± 0.165	1.657± 0.23
Liver	30.134 ± 2.2415*	23.13 ± 0.796*

* $P<0.05$

Tryptophan resulted in insignificant decline of spleen relative weight. Numerous investigations have shown that tryptophan depletion and tryptophan utilization are related to immune response modulation (Saito et al, 1993; Widner et al., 2000; Moffet and Namboodiri, 2003). However, many aspects of immune-related tryptophan metabolism remain to be elucidated and our data do not afford us to present complete explanation of the observed decline of spleen weight, since it was not related with higher corticosterone level.

Plasma corticosterone levels increased significantly ($P<0.05$) by 14h in either group and declined by 17h in control chickens as compared to basal level, while in experimental chickens corticosterone level at that time was still significantly higher relative to basal level (Figure 5). The rate of corticosterone enhancement by 14 h in both groups was relatively low given the extreme nature of the applied stress-load. It is well

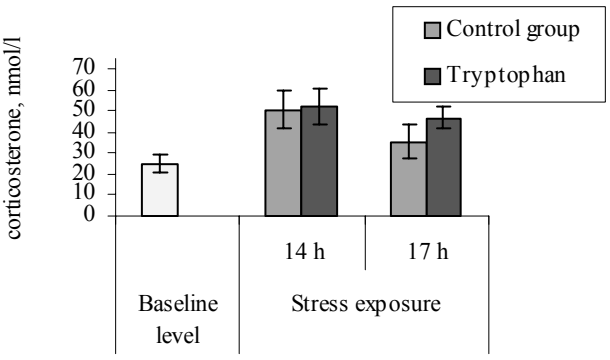

Fig. 5. Effect of supplemental tryptophan on plasma corticosterone levels in chickens under stress

known that adrenocorticotropin induces about 20 fold increase of corticosterone level in chickens (Puvadolpirod and Thaxton, 2000a). The lower rate of corticosterone enhancement in our case could be due either to adaptation to the applied stressors during the previous stressor episodes thus reducing the effect of the psychological component of the stressors during the last stressor episode or to the homeostatic control of energy metabolism under the conditions of feed withdrawal. Under such conditions it is reasonable to expect coordinated efforts of central nervous system directed toward preservation of energy stores rather than their quick exhaustion by increasing plasma glucocorticoid levels. Our data are consistent with those reported by Maxwell (1993) in his review, where he claims that H/L ratio change was inadequate during severe food restriction studies despite the widely accepted view that heterophils to lymphocytes ratio is less variable indicator of avian stress than plasma corticosterone level. The lack of change of H/L ratio in tryptophan supplemented chickens (Figure 6) is in agreement with the lower rate of corticosterone enhancement in the chickens under stress. The observed effect of supplemental tryptrophan on H/L ratio could be explained with the established immunomodulatory role of tryptophan (Moffet and Namboodiri, 2003).

According to the existing theories tryptophan breakdown suppresses T cell proliferation by dramatically reducing the supply of this crucial amino acid. The other version is that the downstream metabolites of tryptophan catabolism act to suppress certain immune cells, probably by pro-apoptotic mechanisms. The fact that H/L ratio was unchanged under the conditions of our experiment is in agreement with the dynamics of the other stress-indices used in our study and demonstrates that the intermittent food withdrawal reduces adrenal response.

Hematocrit (Figure 7) and erythrocyte numbers (Figure 8) tended to be higher in both groups after the last stress exposure relative to baseline values. Unchanged erythrocyte numbers accompanied with increased values of hematocrit and hemoglobin values have been reported by 4 and 7h after adrenocorticotropin administration in chickens (Puvadolpirod and Thaxton, 2000b) which the authors attributed to the increased metabolic activity during extreme stress as a compensatory reaction to the lack of oxygen in the tissues. In similar experiments Olanrewaju et al. (2006) have found elevated hematocrit and erythrocyte values following continuous adrenocorticotropin delivery. These data suggest that hematocrit and erythrocyte numbers in animals under stress have fluctuating pattern of their dynamics. Consequently, the unchanged erythrocyte values in our experiment could be related with the relatively lower enhancement of adrenal activity.

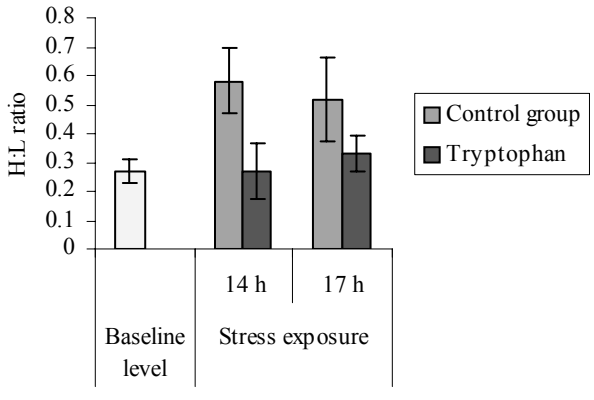

Fig. 6. Effect of supplemental tryptophan on heterophil to lymphocyte ratios in chickens under stress

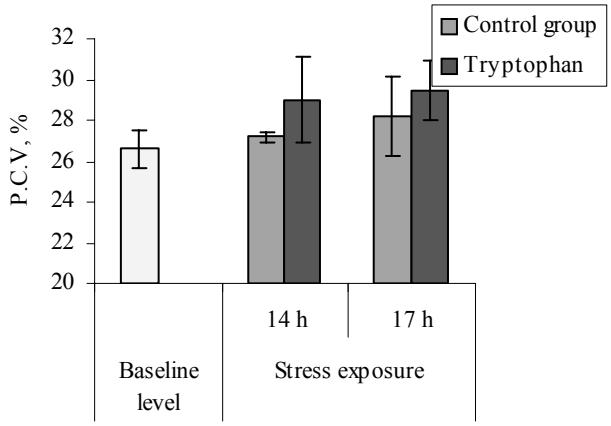

Fig. 7. Effect of supplemental tryptophan on P.V.C. level in chickens‘ blood under stress

Blood leukocyte numbers in both groups (Figure 9) increased significantly ($P<0.05$) in comparison with the baseline levels but tended to be higher in tryptophan supplemented chickens. The inconsistent results reported by Puvadolpirod and Thaxton (2000b) and Dhabhar et al. (1995),showing leukocytes elevation and decline respectively could be reconciled by the fact that blood samples were taken at different time spells relative to the beginning of each stress episode in the corresponding experimental designs. Our data concerning leukocyte numbers are consistent with those reported by Puvadolpirod and Thaxton (2000b) and demonstrate that fasting has specific stimulatory effect on leukocyte numbers despite the fact that most of the other stress indices were not influenced by the severe stress as judged by the sharp decline of body weight.

Interleukin- 1α response to stress declined significantly ($P<0.05$) by 14h in tryptophan supplemented chickens following the start of stressor treatment (Figure 10), while in the control chickens it tended to decline by 17h relative to the baseline levels. The observed decline in experimental group could be due to the higher body stores of tryptophan, since quinolinate, a tryptophan catabolite, has been suggested as a possible means of replenishing NAD in leukocytes which

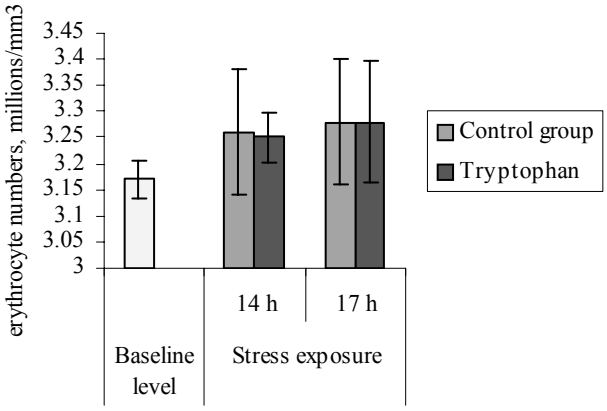

Fig. 8. Effect of supplemental tryptophan on blood erythrocyte numbers in chickens under stress

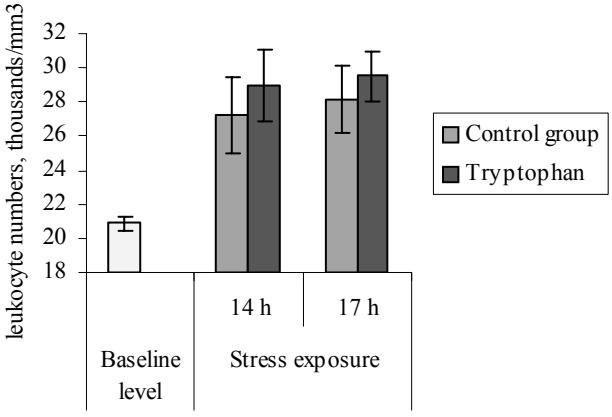

Fig. 9. Effect of supplemental tryptophan on blood leucocyte numbers in chickens under stress

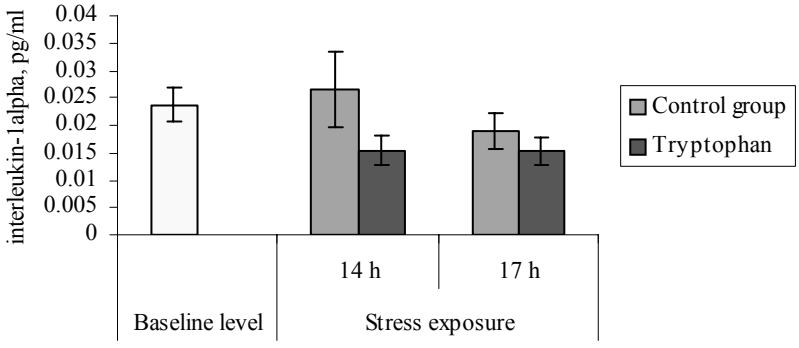

Fig. 10. Effect of supplemental tryptophan on plasma Interleukin-1α in chickens under stress

is depleted by oxidative stress (Moffet and Namboodiri, 2003). This assumption coincides with the established higher leukocyte numbers elevation in tryptophan supplemented chickens.

It is known that variety of stressors as well as interleukin-1-α itself increase brain concentration of interleukin-1-α (Lenard and Dunn, 2005). Besides, interleukin-1α and various stressors elevate brain tryptophan presumably by increasing brain tryptophan uptake. Consequently, the observed decline of plasma interleukin-1α in tryptophan supplemented chickens suggests a possible decline of brain uptake of tryptophan.

Conclusion

The intermittent starvation reduced adrenal response to stress, despite the loss of body weight. Supplemental tryptophan alleviated body weight decline, decreased plasma glucose and cholesterol levels and the relative weight of liver, but had no effect on the relative weights of adrenal glands and *bursa of Fabricius*. Taken as whole these results indicate that supplemental tryptophan counteracts the expected stress induced changes in the most of the studied indices.

Acknowledgements

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