STUDY ON METABOLIC IMPLICATION OF SUPPLEMENTAL VITAMINS B_3+B_6 IN EXPOSED TO STRESS CHICKENS

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Abstract

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This study was undertaken to investigate the effect of supplemental vitamin B_3 (40 mg.kg^{-1} diet) and vitamin B_6 (7 mg. kg^{-1} diet) on some indices of stress in chickens under stress, induced by alternating periods of normal feeding with periods of feed and water withdrawal. 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. Blood samples were taken by 14h and 17h following the start of the last stressor episode. Supplemental vitamins B_3+B_6 alleviated body weight decline, decreased spleen ($P<0.05$) and liver ($P<0.001$) relative weights, increased relative weights of adrenal glands ($P<0.05$) but had no significant effect on that of bursa of Fabricius. Plasma urea levels were not influenced by supplemental vitamins B_3+B_6 but plasma glucose levels declined by 17h ($P<0.05$) in control group. Cholesterol levels ($P<0.05$) was significantly lower by 17h in experimental group relative to control group. Vitamins B_3+B_6 supplementation did not prevent leukocyte numbers and H:L ratio increment caused by stressor treatment. Hematocrit values were significantly higher ($P<0.01$) by 14h in experimental birds in comparison with control chickens and baseline levels. Interleukin-1б response to stress declined in vitamins B_3+B_6 supplemented broilers by 14 h and 17h while that of control group declined by 17h only. Corticosterone response to the applied stress stimuli was not influenced by supplemental B_3+B_6. Our results indicate that vitamins B_3+B_6 are implicated in white blood cells dynamics and influence the pattern of the observed stress indices in chickens.

Key words: vitamins, B_3, B_6, stress, H:L ratio, IL-1б, white blood cells, corticosterone, chickens

Introduction

Vitamin B_3, also called Niacin, Niacinamide, or Nicotinic Acid, is an essential nutrient required by body for the proper metabolism of carbohydrates, fats, and proteins, as well as for the production of hydrochloric acid for digestion. B_3 also supports proper blood cir-culation, healthy skin, and aids in the functioning of the central nervous system. As many as 200 enzymes require the niacin coenzymes, nicotinamide adenine dinucleotide (NAD) and nicotine adenine dinucle-otide phosphate (NADP). NAD functions most often in energy producing reactions involving the degrada-tion (catabolism) of carbohydrates, fats, proteins, and

alcohol. NADP functions more often in biosynthetic (anabolic) reactions, such as in the synthesis of all macromolecules, including fatty acids and cholesterol (Brody, 1999; Cervantes-Laurean et al., 1999)

Vitamin B$_6$, also called Pyridoxine, refers also like vitamin B$_3$ to a family of water soluble substances - including pyridoxine, pyridoxal, and pyridoxamine, that are closely related in form and function. Vitamin B$_6$ that cannot be stored in the body must be obtained daily from either dietary sources or supplemental Vitamin B$_6$ is an important nutrient that supports more vital bodily functions than any other vitamin. This is due to its role as a coenzyme involved in the metabolism of carbohydrates, fats, and proteins. Vitamin B$_6$ is also responsible for the manufacture of hormones, red blood cells, neurotransmitters, enzymes and prostaglandins. The phosphate ester derivative pyridoxal 5'-phosphate (PLP) is the principal coenzyme form and has the most importance in metabolism (Dakshinamurti and Dakshinamurti, 2007). The poultry requirement for niacin, can be met in part by the conversion (45:1) of the essential amino acid tryptophan to niacin (Chen and Austic, 1989), as well as through dietary intake. PLP is a coenzyme for a critical reaction in the synthesis of niacin from tryptophan; thus, adequate vitamin B$_6$ decreases the requirement for dietary niacin (Leklem, 1999). In the brain, the synthesis of the neurotransmitter, serotonin, from the amino acid, tryptophan, is catalyzed by a PLP-dependent enzyme. Other neurotransmitters, such as dopamine, norepinephrine and gamma-aminobutyric acid (GABA), are also synthesized using PLP-dependent enzymes.

There are no data about the metabolic implication of supplemental vitamins B$_3$+B$_6$ in the experimental chickens exposed to repetitive fasting and immobilization stress. Food deprivation has been adopted by the commercial poultry industry to induce molt because it is the easiest method to apply and produces the best results (Webster, 2003). Feed deprivation, however, raises concerns about animal welfare.

The purpose of this study was to investigate the effect of supplemental vitamins B$_3$+B$_6$ on some indices of physiological stress, interleukin-1δ, heterophil-to-lymphocyte ratios (H:L) and leukocyte and erythrocyte numbers in chickens under stress.

Materials and Methods

The experiment was conducted with twenty chickens (White Plymouth rock - pure initial line from the National Genetic Resource) at the age of six weeks which were randomly allocated into two groups- control and experimental and were raised in pens (5 chicks in a pen). The chickens were exposed to repeated fasting and immobilization stress. 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 17 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 vitamin B$_3$ (40 mg/1kg diet) and vitamin B$_6$ (7 mg/1 kg diet) were 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 14 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).

Leucocyte 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

Body weight in control chickens was significantly (P<0.05) declined by the repeated stress exposure.

Supplemental vitamins $B_3 + B_6$ alleviated body weight (BW) decline (Figure 1) in experimental chickens. These results are similar to those obtained in our previous work with tryptophan supplemented chickens under identical experimental design (Moneva et al., 2008). It is known that there is a metabolic relationship between tryptophan and niacin on the one hand (Moffett and Namboodiri, 2003) and tryptophan and serotonin on the other hand (Ruddick et al, 2006) and that's why the similar results are logical. According to Oduho and Baker (1993) weight gain and voluntary feed intake responded linearly to nicotinamide addition up to 12 mg/kg, but 12 mg/kg of the added nicotinamide was still unadequate. The observed body
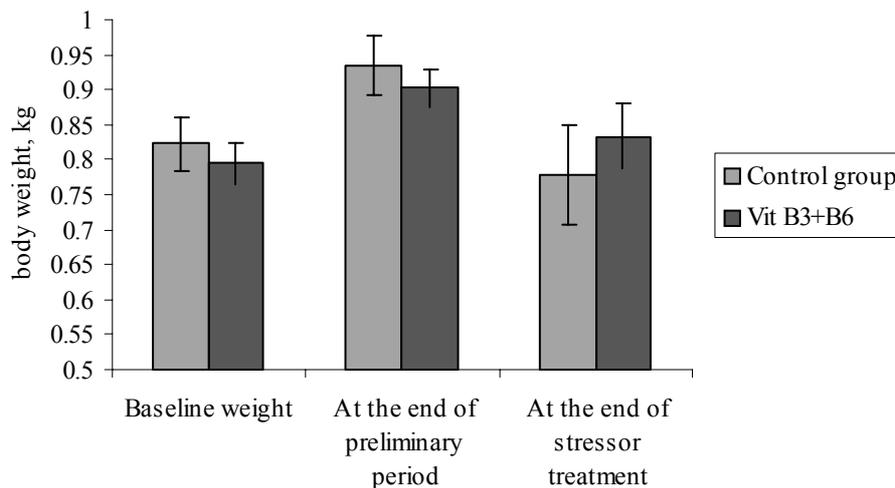

Fig.1. Effect of supplemental Vitamins $B_3 + B_6$ on body
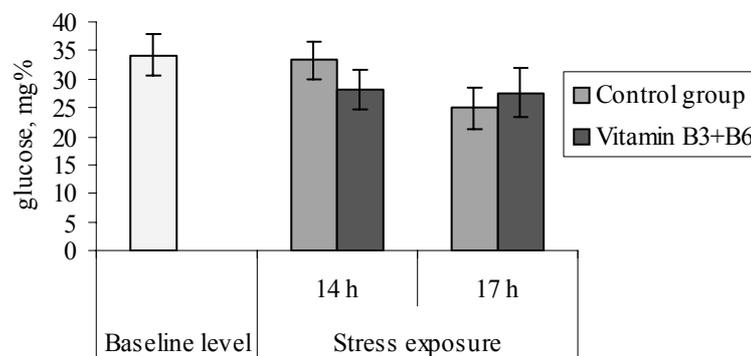

Fig.2. Effect of supplemental Vitamins $B_3 + B_6$ on plasma glucose levels in chickens under stress

weight decline in control birds could be related to possible stress-induced insufficiency of dietary tryptophan and niacin, because of the insignificant conversion of tryptophan to niacin or serotonin. The results of the present experiment indicate that dietary supplementation with vitamins B_3+B_6 are able to improve the negative consequences of the applied stress stimuli on body weight. Plasma glucose level (Figure 2) declined in both groups of chickens at the end of stressor treatment (17 h) but the difference was significant ($P<0.05$) in control group only relative to the baseline level. There were no significant differences between the 2 groups by 17 h of stressor treatment. The observed dynamics is similar to that obtained in

stressed chickens supplemented with tryptophan in our previous study. The only difference is the lack of significance. The observed trend of higher glucose level by 17 h in experimental group in comparison with control group (Figure 2) coincides with similar trend in plasma corticosterone level (Figure 5) and suggest possible involvement of supplemental B_3+B_6 in gluconeogenesis. Futhermore, this suggestion is supported by the fact that pyridoxal -5- phosphate is coenzyme for reactions used to generate glucose from amino acids a process known as gluconeogenesis (Leklem, 1999; Mackey et al., 2006). However, this interpretation does not explain the similar effect of tryptophan on plasma glucose level in our previous ex-
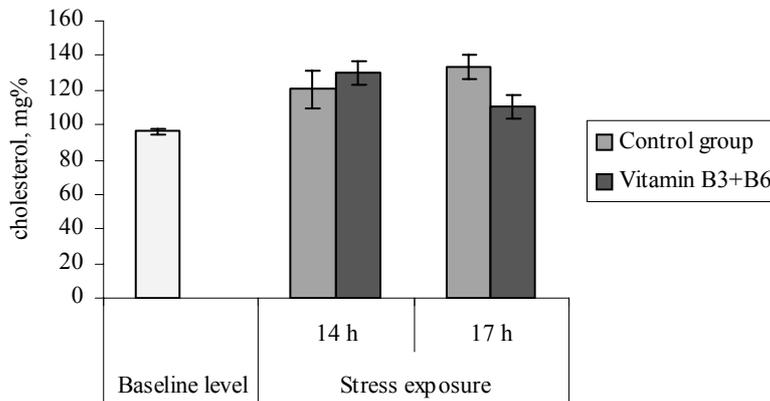

Fig.3. Effect of supplemental Vitamins B₃+B₆ on plasma cholesterol levels in chickens under stress
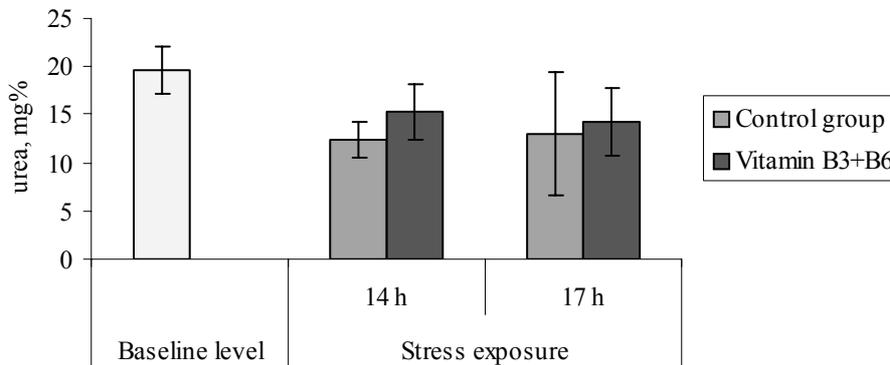

Fig. 4. Effect of supplemental Vitamin B₃+B₆ on plasma urea levels in chickens under stress

periment (Moneva et al., 2008). Therefore the similar pattern of plasma glucose level in both experiments could be due to the fact that tryptophan is precursor of melatonin, serotonin and niacin and that vitamin B_6 stimulate the conversion of tryptophan to niacin and serotonin. Basu and Mann (1997) reported that simultaneous administration of vitamins B_3+B_6 did not alter the hypolipidemic action of niacin. These results suggest that if dietary vitamin B_3 is sufficient to meet the metabolic needs, and then vitamin B6 has no additive effect.

Plasma cholesterol level (Figure3) declined in

Table 1

Relative weight of some lymphoid organs in chickens under stress

Lymphoid organs	Relative weights, g/kg BW	
	Control group	Experimental (Vit, B_3+B_6) group
Adrenal glands	0.067 ± 0.008 a*	0.108 ± 0.013a*
Bursa of fabricius	0.698 ± 0.059	0.792 ± 0.058
Spleen	2.112 ± 0.165 b*	1.628 ± 0.121 b*
Liver	30.134 ± 2.2415***	20.39 ± 0.72 ***

* P<0.05

***P<0.001

chickens supplemented with vitamins B_3+B_6 at the end of stressor treatment compared to that in control group (P<0.05). It is interesting that cholesterol dynamics, like that of glucose is similar to that observed in tryp-

tophan supplemented chickens under identical experimental design (Moneva et al, 2008). These data confirm once again that vitamins B_3+B_6 or tryptophan have a common mechanism of action involving one or more mediators. The most probable common mediator appears to be niacin, since tryptophan is a precursor of niacin and vitamin B_6 stimulates niacin synthesis from tryptophan. Besides, pharmacological doses of nicotinic acid have been found to reduce serum cholesterol (Knopp, 1999). However, bearing in mind that tryptophan conversion to niacin is only 45:1 we can not exclude serotonin as a possible common mediator. There are data showing close relation between cholesterol level of the cell membrane and serotonin binding and signaling via 5-hydroxitriptomine receptors (Sjцgren et al., 2006; Pucadyil and Chattopadhyay, 2007). These finding support our suggestion about the possible mediatory effect of serotonin in the B_3+B_6 induced cholesterol reduction (Leklem, 1999; Mackey et al, 2006; Suguro et al., 2006).

Plasma urea levels (Figure 4) were significantly lower (P<0.05) by 14h in control chickens as compared to basal urea level. Plasma urea levels in experimental chickens tended to be lower both by 14 h and 17 h. This reduction of urea level could be explained with the lack of dietary protein during the stressor treatment since protein degradation is the main source of plasma urea. The observed trend to higher urea level in experimental chickens, particularly by 14 h could be attributed to the stimulatory effect of PLP
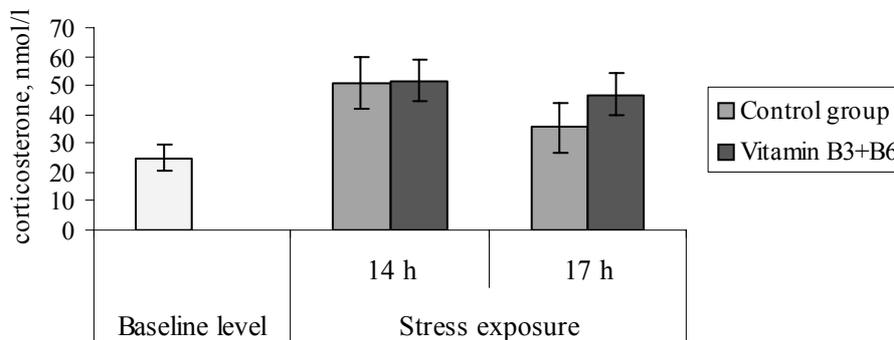

Fig.5. Effect of supplemental Vitamins B_3+B_6 on plasma corticosterone levels in chickens under stress
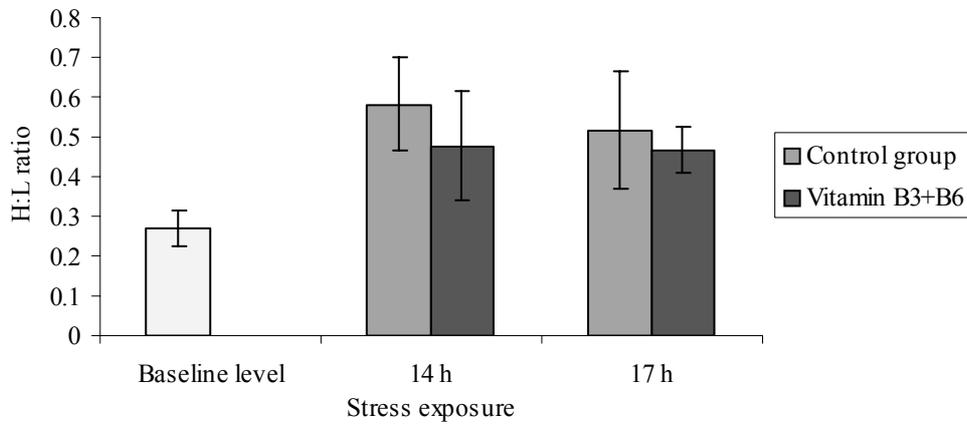
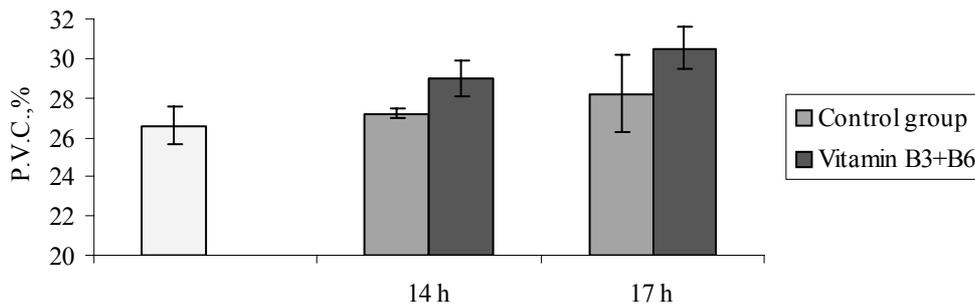**Fig.6. Effect of supplemental Vitamins B$_3$+B$_6$ on heterophil-to-lymphocyte ratio in chickens under stress**

Fig.7. Effect of supplemental Vitamins B$_3$+B$_6$ on P.V.C.levels in chickens' blood under stress
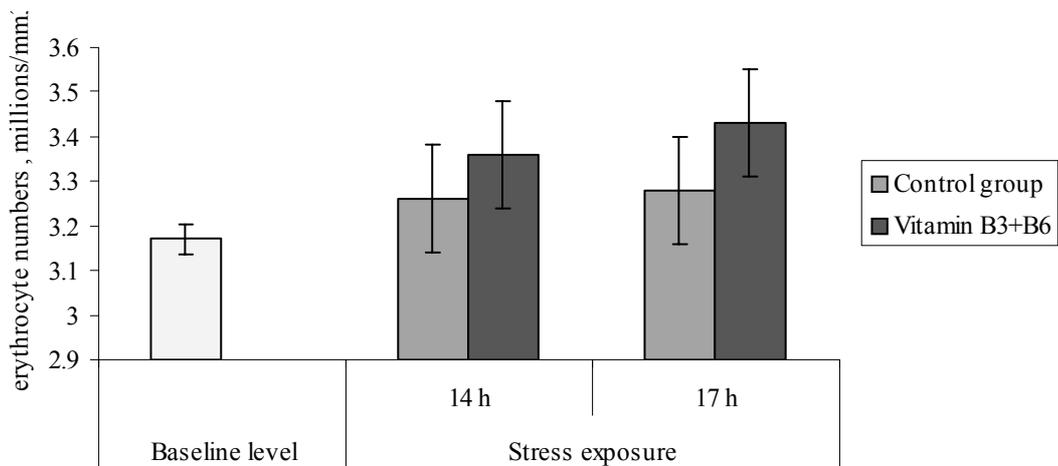

Fig. 8. Effect of supplemental Vitamins B$_3$+B$_6$ on blood erythrocyte numbers in chickens under stress

on gluconeogenesis (Leklem, 1999).

Vitamins B_3+B_6 increased significantly (P<0.05) the weight of adrenal glands (Table 1) which is in agreement with the increased plasma corticosterone level (Figure 5) and was possibly due to vitamin B6 stimulated serotonin synthesis, which in turn has a definit effect on hypothalamic-pituitary-adrenal axis activity (Gassano and D'mello, 2001). It is well known that higher corticosterone level has negative effect on growth and weight gain in chickens (Puvadolpirod and Thaxton, 2000a). However the increased adrenal weight accompanied with a trend towards higher corticosterone level in experimental group are not consistent with the lower rate of body weight decline in experimental chicken as compared to that of control group by 17 h. At first glance this data seem controversial, but they are probably not because vitamin B_6, tryptophan and their combination participate in biosynthesis and secretion of melatonin and melatonin is found to block glucocorticoid receptors (Tavartkiladze et al., 2006).

The same refers to liver and bursa of Fabricius rela-
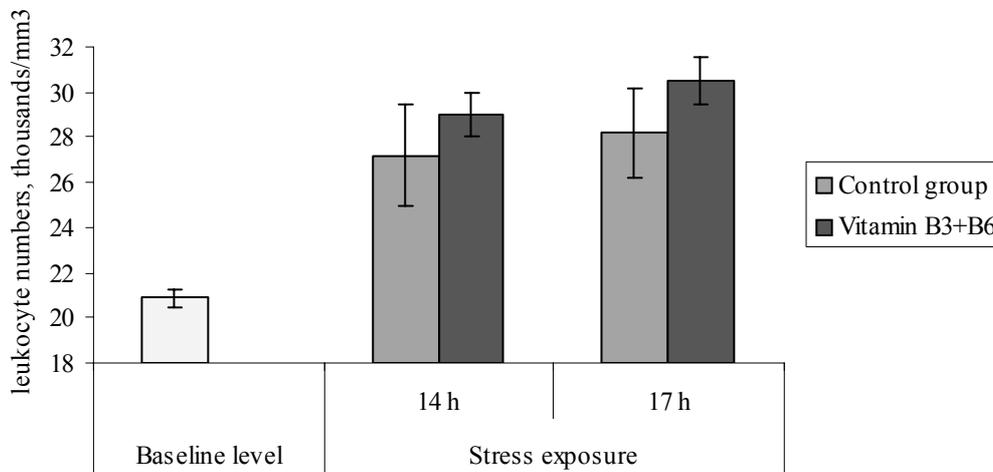

Fig. 9. Effect of supplemental Vitamins B_3+B_6 on blood leukocyte numbers in chickens under stress
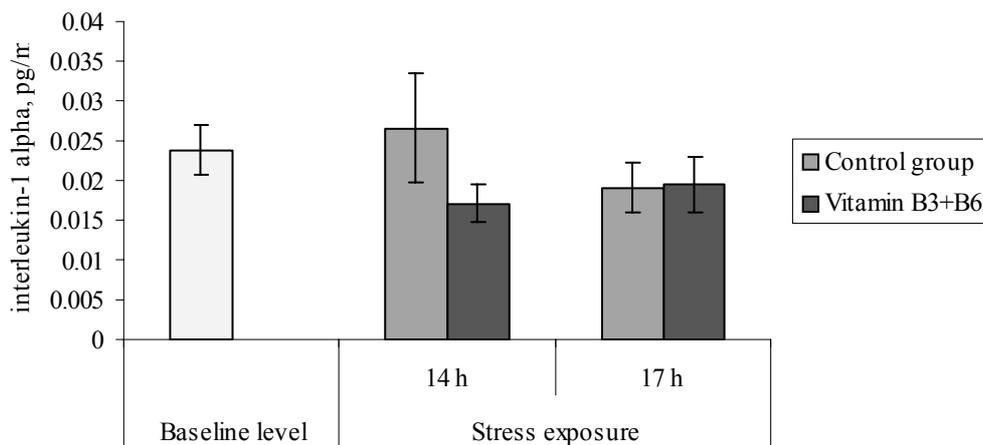

Fig.10. Effect of supplemental Vitamins B_3+B_6 on plasma Interleukin-1α levels in chickens under stress

tive weights (Table 1) which were decreased (P<0.001) or unchanged respectively in experimental birds relative to control birds at the end of stressor treatment despite of the documented opposite effect of stress on these organs (Puvadolpirod and Thaxton, 2000a). The same result was established in our previous experiment with tryptophan supplemented chickens under identical conditions. These data give further support of our assumption that tryptophan and vitamins B_3+B_6 have common mediator.

The decline (P<0.05) of spleen relative weight (Table 1) in experimental group is the only exception in the proposed scheme of vitamin B_6 metabolic involvement since vitamin B_6 did not prevent corticosterone reducing effect on spleen weight which was probably due to vitamin B_6 specific implication in immune cells production and red cell formation and function (Grimble, 1997; Leklem, 1999).

Plasma corticosterone levels (Figure 5) doubled by 14 h in either groups (P<0.05) and declined by 17 h in control (P>0.05) and experimental birds (P>0.05) as compared to those measured in 14 h. However, plasma corticosterone level in experimental birds remain significantly higher (P<0.05) relative to baseline level. The observed enhancement of plasma corticosterone by 14 h is consistent with the reported increase of plasma corticosterone in fasted or immobilized chickens (Beuving and Vonder, 1978; Harvey and Klandorf, 1983). The relatively lower rate of corticosterone elevation by 14 h could be due to the bird experience to the previous feed withdrawal (Zulkifli et al., 1995). Besides, low plasma corticosterone level has been found in long term- fasting birds which is consistent with the lower basal metabolic rate in this physiological state (Webster, 2003). Our data demonstrate that plasma corticosterone level can not be used as stress indicator in fasting chickens since the increase of plasma corticosterone was negligible compared to that reported after adrenocorticotropin injection (Puvadolpirod and Thaxton, 2000a) despite of the extreme stressor load measured by the body decline.

Heterophil–to-lymphocyte ratio (H:L) increased in both groups of chickens subjected to stress compared to the baseline ratio (Figure 6). However, the rate of H:L ratio elevation in either group by 17 h (about two times) is less than that reported by Mumma et al. (2006) in adrenocorticotropin treated laying hens (5-6 fold), and coincides with the similar rate of plasma corticosterone elevation. Heterophil- to- lymphocyte ratio in both groups remained higher by 17 h as compared to its baseline value. There were no significant differences among the groups at that time. These data give further support to our view that repetitive fasting related with gradual decline of the fasting induced emotional stress in each successive episode of fasting does not cause sharp increase in plasma corticosterone corresponding to severity of the applied stress.

Hematocrit (Figure 7) increased significantly (P<0.01) in the B_3+B_6 supplemented chickens in comparison with the baseline values, while in the control chickens they remained unchanged by 14 and tended to be higher by 17 h. These results demonstrate that supplemental B_3+B_6 have a definitive effect on hematocrit level which probably stems from the increased (P<0.05) erythrocyte numbers (Figure 8) rather than to the stimulatory effect of vitamin vitamin B_6 on heme synthesis (Leklem, 1999). This suggestion is further supported by the reported involvement of vitamin B_6 in erythrocyte production (Whithney et al., 2002). We have found similar trend of hematocrit values in our earlier experiment with tryptophan supplemented chickens under identical experimental design. Therefore, we can not exclude possible mediatory effect of niacin which is known to increase NAD concentration in erythrocytes (Fu et al., 1989).

The lack of significant changes in hematocrit values and erythrocyte numbers in control birds indicate that fasting has no effect on these indices. Other studies have reported controversial data on hematocrit and erythrocyte under diverse stress conditions (Puvadolpitod and Thaxton, 2000b; Olanrewaju et al, 2006) suggesting that hematocrit level is modulated by the specificity of the applied stress stimuli.

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 vitamin B_3+B_6 supplemented chickens. These results are simi-

lar to those obtained in our previous study with tryptophan supplemented chickens and are consistent with the results reported by Puvadolpiod and Thaxton (2000a) in chickens under normal feeding. It seems that vitamins B_3+B_6 supplementation has no preventive effect on the stress induced leukocyte numbers elevation and that fasting has specific stimulatory effect on leukocyte numbers. The observed effect of fasting on leukocyte numbers could be due to redistribution of leukocyte since more than 60% of the total immunoglobulins and more than 10^6 lymphocytes/g tissues are contained in the intestine (Salminen et al., 1998). Gut-associated lymphoid tissue contains the largest pool of immunocompetent cells in the body (Bourliox et al., 2003). Therefore the lack of food consumption would reduce the activity of the complex mucosal immune system which makes it possible to tolerate the massive load of dietary antigens. Ultimately this could lead to change in the systemic leukocyte redistribution.

Interleukin- 1α response to stress declined significantly ($P<0.05$) in vitamins B_3+B_6 supplemented chickens by 14h following the start of stressor treatment (Figure 10), while in the control chickens remained it unchanged by 14h and tended to decline by 17h ($P>0.05$) relative to the baseline levels. It is known that vitamin B_6 stimulates lymphocyte proliferation and cytokines production (Talbott et al., 1987; Meydani et al., 1991). However in our experiment we found significantly higher plasma corticosterone leves and adrenal weight in the vitamins B_3+B_6 supplemented chickens, and glucocorticoids are known to inhibit lymphocyte activity and the secretion of cytokines (Elenkov, 2008). Therefore, the observed interleukin-1 б decline in our case probably stems from the opposite effect of B_6 on adrenal and immune function.

Conclusion

Supplemental vitamins B_3+B_6 reduced the negative effect of fasting on most of the used stress indices but had specific stress synergistic effect on the relative weight of adrenal glands and spleen and on leukocyte numbers.

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