Immune cells distribution and some biochemical parameters in Ovocap-treated laying hens

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Abstract

The effects of the Bulgarian, biologically active product, Ovocap, which contains capsaicin, were studied in 3 groups of laying hens: I[st]- control, II[nd] and III[rd] – experimental. Ovocap was added in the concentrate mix (II group) or in the water (III group) at intervals of 28 days except for the last Ovocap treatment (21 day from the previous treatment). Each treatment was carried out in two consecutive days. In the present study we examined the immediate effect and after – effects of Ovocap on adrenal function, leukocyte distribution and some metabolic parameters. Plasma corticosterone levels declined (P<0.1) 7 days after the last Ovocap treatment (on day 133) regardless of the mode of its application. There was an increase (P>0.05) in the percentage of lymphocytes and a decline (P>0.05) in neutrophils percentage, registered 1 hour after the last treatment and 7 days later. Ovocap evoked an increase (P>0.05) in hematocrit value and a decline (P<0.05) in cholesterol concentration, when it was added to the concentrate mix only. Plasma urea level declined (P<0.01) 1 hour after the last treatment in III[rd] group of hens, whereas 7 days later (day 140) urea levels declined in both experimental groups. Plasma indol levels tended to be lower in both experimental groups on day 140. Our data suggest that Ovocap stimulates oxygen consumption and has an inhibitory effect on adrenal function 7 days following Ovocap. Besides it has selective modulatory effect on leukocyte subpopulations.

Keywords*: blood cells , Ovocap, cholesterol, urea, corticosterone, hens*

Introduction

Ovocap is a Bulgarian product, containing Capsaicin. It has been shown to stimulate egg production (*Kitanov et al., 2002*) and hatchability in hens (in press). Capsaicin has been reported to be implicated in plenty of physiological functions, including gastroprotection (*Szoscanyi and Bartho, 2001*), temperature regulation (*Nomoto et al.,2004*), modulation of energy metabolism (*Kawada et al., 1986*) and regulation of the immune status (*Yu et al., 1998*). Most of the above mentioned effects of capsaicin have been observed in experimental designs involving single or

repetitive daily injections of capsaicin. It has been found, that the phase effect of capsaicin on neutrophils activity is closely related with the functional activity of sensory neuropeptides (*Zhukova and Makarova, 2002*). The same authors have reported, that the return of the neuropeptides to their baseline level takes place on day 21 following capsaicin. The objective of this study was to determine plasma cholesterol, urea, indol, corticosterone and leukocyte subpopulations distribution in laying hens treated with capsaicin at intervals of 28 days for 20 weeks.

Material and methods

Eighteen laying hens were allocated into 3 groups as follows: Ist group –control, II nd and IIIrd – experimental. Experimental hens were treated 5 times for 20 weeks at a dose of 1.5 ml per hen. Each Ovocap treatment was conducted at intervals of 29 days except for the last treatment that was carried out on day 21 following the previous treatment. The two experimental groups differed from one another by the way of Ovocap application – in the concentration mix (IInd group) or in the water (III rd). The foreseen Ovocap dose for each treatment was given in two consecutive days. Blood samples were taken from the ulnar vein one hour after the last Ovocap treatment (d 133) and 7 days later (d 140). Plasma corticosterone levels were determined by the RIA methods of Persengiev and Konakchieva (199). Plasma urea was assayed as described by Rerat et al. (1976). Plasma cholesterol and indol were determined by the methods of Watson (1960) and Balahovskii (Chilov, 1959), respectively. Peripheral blood leukocytes were counted microscopically in smears (Giemsa-Romanovsky stain). Data were analyzed by the Student t-test.

Results and discussion

Plasma corticosterone levels tended to decline in the IInd group of hens whereas in the IIIrd group of hens they remained unchanged on day 133 (Fig.1). These data indicate that capsaicin had no effect on adrenal function 1 hour following the treatment. It has been established that a single dose of capsaicin stimulates sympathoadrenal activity in mice (*Masuda et al., 2003*), and pigs (*Alving et al.,1991*). However, repeated capsaicin injection do not influence catecholamines and cortisol secretion (*Alving et al., 1991*).

Furthermore according to *Watanabe et al. (1988)* the increase of catecholamine secretion by capsaicin is mainly through activation of the central nervous system. The author did not find any capsaicin – induced enhancement of catecholamine secretion from the adrenal glands. The lack of corticosterone enhancement on day 133 is consistent with the above mentioned facts, concerning the effect of capsaicin on adrenal glands activity during repeated treatment.

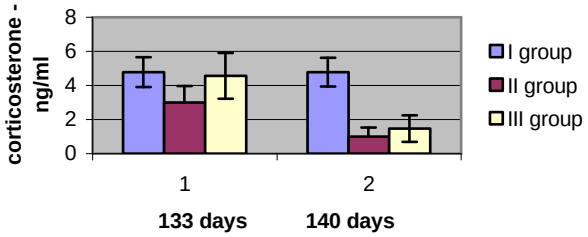

Fig. 1. Plasma corticosterone concentration in Ovocap treated laying hens

Plasma corticosterone levels, determined 7 days following the last capsaicin treatment (d 140) declined significantly both in IInd (P<0.01) and IIIrd group (P<0.05) of hens (Fig.1). These data suggest that capsaicin itself does not have a direct effect on adrenal function, because of the transient depolarization of primary afferent c-fibers, related with an increased release of substance P (SP) from capsaicin sensitive nerve endings (*Lambrecht, 2001*). The initial short –term depolarization of afferent fibers and the increase of SP is followed by a long-term attenuation of the synthesis and concentration of SP in the primary afferent fibers (*Harmar et al.,1986*). According to *Zhukova and Makarova (2002)* the return of neuropeptides concentration to their baseline levels lasts around 21 days.

Table 1. *Peripheral blood leucocytes distribution in Ovocap treated laying hens-133d and 140 d*

White blood cells	133 d			140 d	
	I group	II group	III group	II group	III group
Basophiles	1.1 ± 0.21	1.3 ± 0.18	1.2 ± 0.25	1.4 ± 0.17	1.2 ± 0.13
Eosinophils	1.2 ± 0.12	1.1 ± 0.17	1.3 ± 0.15	1.2 ± 0.26	1.3 ±0.32
Neutrophils	24.2 ± 8.4	15.0 ± 7.2	17.1 ± 5.8	11.4 ± 0.54	84.7 ± 0.54
Lymphocytes	69.0 ± 8.1	76.6 ± 7.6	74.5 ± 5.9	81.0 ± 0.54	84.0 ±0.35
Monocytes	4.7 ± 0.41	5.3 ± 0.41	5.8 ± 0.74	5,0 ± 0.23	5.3 ±0.41

Therefore we could not expect any effect of SP, originating from capsaicin sensitive nerve endings, on d 7 after the last Ovocap treatment. We hypothesize that the observed corticosterone decline 7 days after capsaicin treatment was due to SP, produced by lymphocytes. Substance P is known to have an inhibitory effect on corticotropin–releasing factor (*Larsen et al., 1993*). Further support for this hypothesis stems from the observed percentage change in lymphocyte numbers (Table 1) showing an increase of lymphocyte numbers (P>0.05) at 1st hour and 7 days following the last treatment with Ovocap. Besides , human T lymphocytes have been shown to produce endogenous SP under baseline – conditions (*Lai et al., 1998*). Therefore the increase in lymphocytes percentage could contribute for enhancement of the endogenous substance P production, the more so as SP

stimulates both T and B lymphocytes proliferation (*Payan et al., 1983; Scicchitano et al., 1988; Madden and Felten, 1995*).
Furthermore, most lymphocytes producing SP also express its receptor (*Lai et al., 1998*).

Table 2.. *Hematocrit, erythrocytes and leucocytes in Ovocap treated laying hens- 140 d*

Group	Hematocrit %	Erythrocytes millions/mm3	Leucocytes thousands/mm3
I group (control)	31.47 ± 1.48	31.47 ± 1.48	21060 ± 383
II group	35.00 ± 1.06	3.48 ± 0.10	21000 ± 300
III group	30.80 ± 1.68	3.56 ± 0.10	20708 ± 430

Our assumption that SP plays a key role in Ovocap – induced changes is consistent with the higher egg productivity , egg hatchability and the better food utilization in Ovocap treated hens (in press) since SP has been found to stimulate ovulation by influencing LH and FSH surges (*Potargowicz and Traczyk, 1999; Kerdelhue et al., 2000*). Furthermore capsaicin modifies gastrin secretion (*Nojima et al., 2000*) and stimulates appetite. Neonatal capsaicin is implicated in thymocyte proliferation and its effect on thymocyte function is dependent on vaniloid – mediated regulation of SP (*Santoni et al., 2004*). Hematocrit value tended to decline in second group of hens (P>0.05) and remained unchanged in IIIrd group (Table 2). These data show that capsaicin supplemented to the concentrate mix unlike that added to water had a pronounced effect on red cells volume since the number of erythrocytes in both experimental groups was similar to that of the control group. The increased erythrocytes volume is related with higher hemoglobin level (*Dessypris, 1991*). Therefore oxygen-binding capacity in IInd group of hens is higher, compared to Ist and IIIrd group of hens. The higher oxygen binding capacity of erythrocytes in IInd group of hens is consistent with the result of *Masuda et. al. (2003)* who have reported higher oxygen consumption in capsiate treated mice. The lack of hematocrit increase in IIIrd group could be due to the faster water passage through the gastrointestinal tract that could influence the rate of capsaicin absorption.
Blood percentage of neutrophils declined (P>0.05) both at 1 h. and 7 days following capsaicin (Table 1). The decreased neutrophil percentages against the background of unchanged total number of leukocytes could be explained with decreased influx of neutrophils from the marrow or increased egress of neutrophils from the circulation. It is known that glucocorticoids have contrariwise effect on leukocyte subpopulations distribution (*Dhabhar et.al.,1995*). Therefore Ovocap – induced decline in corticosterone level could have an effect on the observed changes in leukocyte subpopulations. This assumption however is not consistent with the unchanged corticosterone level found 1 h. following capsaicin (133 d) in both

experimental groups. Taken together our findings suggests that Ovocap has a certain effect on both leukocyte subpopulations distribution and hematocrit value.
Plasma cholesterol level declined on day 133 ($P<0.05$) and day 140 ($P>0.05$) in IInd group of hens (Fig. 2). The very fact that the decline of cholesterol coincides with the enhancement of hematocrit in the group in which Ovocap was provided with the concentrate mix suggest that the observed cholesterol decline is closely related with the metabolic rate. This assumption is consistent with the observed lower cholesterol level in high oxygen consuming chicks (*Sutton et al., 1985*).

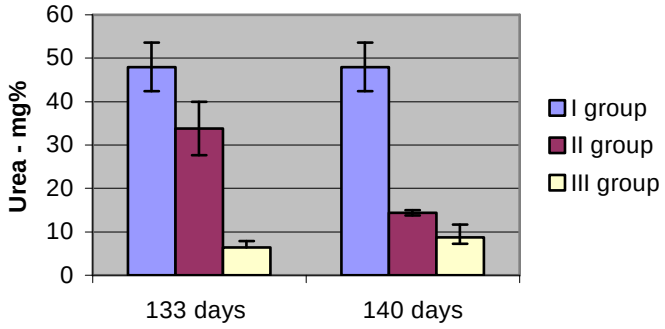

Fig 2. Plasma cholesterol concentration in Ovocap treated laying hens

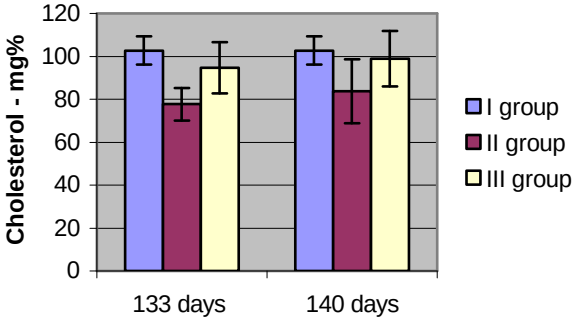

Fig. 3. Plasma urea concentration in Ovocap treated laying hens

In addition oxygenated cholesterol has been found to be 10 to 100 times more effective, than cholesterol in inhibiting the activity of the rate – limiting enzyme in cholesterol biosynthesis (*Bell et. al., 1976*).
Plasma urea levels determined 1 h. following Ovocap were lower in IInd ($P>0.05$) and IIIrd ($P<0.001$) groups on d 133. Seven days later (d 150) they were significantly lower in both experimental groups ($p<0.001$) compared with the corresponding values in the control group (Fig.3). The lower plasma urea suggests more efficient

utilization of protein, since high urea concentration reflects losses of nitrogen (`). These data indicate that Ovocap reduces plasma urea level regardless of the way of supplementation – in the forage or in the water.

There were no significant changes in plasma indol levels at 1 h. following Ovocap (Fig. 4). However plasma indol levels tended to decline in both experimental groups on day 7 following the last treatment (d 140) indicating less undigested protein in the distal part of the large intestine.

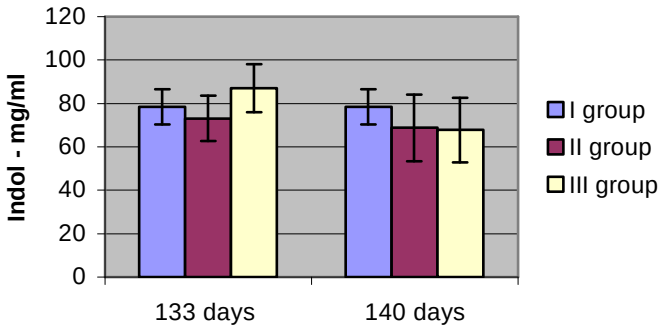

Fig. 4. Plasma indol concentration in Ovocap treated laying hens

Conclusions

The results from this study indicate that Ovocap reduces plasma corticosterone level 7 days after the treatment, regardless of the mode of its addition (in the water or in the concentrate mix). Ovocap increased lymphocytes percentage and decreased neutrophils percentage 1 h. after the last treatment and 7 days later.

It caused an augmentation of hematocrit value and reduction of cholesterol level, when added in the concentrate mix. Ovocap reduced plasma urea level 1 h. after the treatment in III group of hens. Seven days after the last treatment plasma urea levels declined in both experimental groups. Plasma indol levels tended to be lower in both experimental groups 7 days following Ovocap.

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