Effect of supplemental dietary pepper capsaicinoids on some immunological indices in pheasants and laying hens

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SUMMARY
The effect of the supplemental dietary pepper capsaicinoids on some blood indicators of the innate and adaptive defense system (complement activity, Ig Y, IL-1\textbeta) in common pheasants and laying hens was investigated. Both species - pheasants and hens were allocated into control and two experimental groups respectively. The pheasant from two experimental groups received 0.5 and 1.0 ml per fowl dietary pepper capsaicinoids supplementation in two consecutive days, 4 times at intervals of 28 days. The fowls White Plymouth Rock mini from two experimental groups were treated 6 times in two consecutive days over the experimental period with pepper capsaicinoids at intervals of 28 days with 3ml per fowl pepper capsaicinoids per treatment supplied in the diet (I\textsuperscript{st} group) and water (II\textsuperscript{nd} group) respectively. The complement activity, Ig-Y and IL-1\textbeta were investigated at 1 hour and on the 7\textsuperscript{th} day after treatment.

Supplemental dietary pepper capsaicinoids increased complement activity in the pheasants from 2.0 to 2.2 times at 1 hour after treatment and these high levels kept in the next 7 days, while in the hens was not significantly variation at 1 h after the last pepper capsaicinoids treatment in comparison with control group. The complement activity almost doubled (1.85 times) on the 7\textsuperscript{th} day following the last treatment in I\textsuperscript{st} group and increased 4.4 times in II\textsuperscript{nd} group.

There was a clear-cut species-specific effect of supplemental dietary pepper capsaicinoids on IL-1\textbeta levels, manifested by an increase in pheasants until 4.2 times and a decrease in hens until 8.9 times on the 7\textsuperscript{th} day following the last treatment. Similar species-specific effects were observed on Ig Y levels too. Plasma immunoglobulin levels were inversely related to those of IL-1\textbeta.

Keywords: capsaicin, Ovocap, pheasant, hen, complement, Ig-Y, IL-1\textbeta
INTRODUCTION

The steady decline of the world’s biological diversity as a result of climate changes and human economic activity necessitates proper development and restoration of the fauna, particularly the shooting species as pheasants. In many countries, including Bulgaria, pheasants have been grown in farms and later resettled and set free. This practice is often accompanied with significant loss of birds. Nowadays, a number of investigators strive for finding ways to increase survival rate of the free-ranging pheasants. A possible way to improve pheasant’s vitality and adaptability is to add natural products to diet. Furthermore, the ever increasing rate of morbidity and drug resistance require the use of alternative natural products as immune system promoters.

Capsaicin affects various physiological functions which include intestinal peristalsis (Vazques-Olivencia et al., 1992; Hellgren et al., 2000), gastroprotection (Szoscanyi and Bartho, 2001), temperature regulation (Nomoto et al., 2004), modulation of the energy metabolism (Kawada et al., 1986), immune status (Lundblad et al., 1987; Sestini et al., 1990; Yu et al., 1998) and blood neutrophils (Zhukova and Makarova, 2002). Capsaicin has also been found to exert protective effect against Salmonella enteritidis infection in laying hens (Vicente et al., 2007). Previously we investigated the possible use of the supplemental dietary pepper capsaicinoids to enhance health status and production. The literature supports positive effect of Ovocap on the egg production, egg fertility, hatching and viable of the hens, common pheasants, ducks (Kitanov et al. 2004 a;b) and on the development and the function of some internal organs and endocrine glands (Kitanov et al. 2003).

The purpose of this study was to determine the effect of the supplemental dietary Ovocap on some immunological parameters (complement activity, immunoglobulin-Y, interleukin-IL-1β) in shooting pheasants and hens.

MATERIAL AND METHODS

The supplemental dietary Ovocap is a Bulgarian natural friendly product patented by Kitanov (1998) containing hot pepper alkaloids. All components of the feed supplement pepper capsaicinoids are natural. The active ingredients of the dietary supplement are capsaicinoids - capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin-I, homocapsaicin-II, homodihydrocapsaicin-I, homodihydrocapsaicin-II, called in short CAP. These amides were obtained by extraction of the fruit of Capsicum annuum var. Annuum.

The supplemental dietary Ovocap is manufactured in granulated form and its latest version Ovocap-6, which is in liquid form is not thoroughly tested yet.
The current study was conducted in the Institute of Animal Science – Kostinbrod with two poultry species - common pheasant (*Phasianus colchicus mongolicus* I. F. Brandt) and hens – parent stocks of White Plymouth rock-mini.

*Experiment with pheasants*

Pheasants were farmed in cages. At 6 months old they were moved at the experimental unit of Institute of animal science – Kostinbrod. All pheasants were labeled by the use of ring-shaped tags and then were blocked by weight and allocated into three analogous groups as follows: control group and two experimental groups (n=16 in each group). Pheasants were fed with a standard diet formulated to meet their nutritious requirements. They were allowed to adapt to the new environment for 22 days and then were treated with pepper capsaicinoids.

The experiment started when pheasants were 7 months old and ended at the beginning of the reproductive age. Pheasants were given supplemental dietary pepper capsaicinoids four times over the experimental period at intervals of 28 days. Each treatment was carried out in two consecutive days. First and second experimental groups received 0.5 and 1.0 ml pepper capsaicinoids per bird respectively per treatment.

*Experiment with hens*

A trial with White Plymouth Rock – mini, kept in cages, was bred at experimental unit in the Institute of Animal Science – Kostinbrod. All fowls (495 hens) were allocated into a control group and two experimental groups equally (3 cages with 164 hens per cage). The birds were treated 6 times over the experimental period with the feed supplemental pepper capsaicinoids at intervals of 28 days between the treatments. First and second experimental group received 3ml pepper capsaicinoids per bird per treatment supplied in two consecutive days in the feed and water respectively.

Hens were fed on a standard compound diet. Pepper capsaicinoids was added to a certain amount of feed in I experimental group while in II group it was added to the water according to the application scheme.

Venous blood samples were taken from10 birds/group 1 hour after the last pepper capsaicinoids treatment and 7 days later. All blood samples were collected by wing vein puncture using a sterilized syringe. Blood samples were labeled and fractioned. Some of the samples were used for direct measurement of complement and the remaining samples were allowed to clot and serum was stored at -20°C until assayed for immunoglobulin-Y (Ig-Y) and interleukin-1β (IL-1β).
Complement activity was measured by the method of Barta and Barta as modified by Grislova et al., (1978). Serum Ig-Y and IL-1β were determined using commercial ELISA kits.

Mode of Ovocap application in growing common pheasants and hens

The supplemental dietary pepper capsaicinoids is manufactured in liquid form. Before its addition to the pheasants feed it was diluted in small amount of drinking water and gradually mixed with the feed until the resulting mixture acquired homogenous, slightly moisten texture. Dietary pepper capsaicinoids supplementation was placed in the feeders 1h before the scheduled feeding. The other portion of the daily feed allowance was given as scheduled. The same procedure of supplementation was followed in hens except for the II group. An attempt was made to simplify and assess practical applicability of the process of supplementation by offering supplemental pepper capsaicinoids in the drinking water of II group. The hens were provided with the liquid supplement one hour before the scheduled time. During this period the water supply was suspended to ensure complete and uniform intake of the envisaged pepper capsaicinoids dose.

Statistical analyses

The data were subjected to one-way analysis of variance (ANOVA) and, in case of significant differences between control and experiental groups, a Duncan’s multiple-range test was applied (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of pepper capsaicinoids on complement activity

Both pepper capsaicinoids doses increased complement activity in either of the two experimental groups in pheasants (Fig 1). The rate of increment at 1 h after the last pepper capsaicinoids treatment was 2.2 times in I\textsuperscript{st} experimental group (P<0.01) and 2.0 times in II\textsuperscript{nd} experimental group (P<0.05) relative to the control value. The enhanced complement activity remained high at the 7 d after the last pepper capsaicinoids treatment in II\textsuperscript{nd} experimental group and increased further in I\textsuperscript{st} experimental group. There was no dose related difference in pepper capsaicinoids’s stimulatory effect on complement activity, although complement activity at 7 day after the last treatment tended to increase in I\textsuperscript{st} group (P<0.05) and decline in II\textsuperscript{nd} group (P<0.05).

Complement activity in hens (Fig 2) was not significantly influenced at 1 h after the last pepper capsaicinoids treatment as compared to that in control group. However, the activity almost doubled at 7 day following the last treatment in I\textsuperscript{st} group and increased 4.4 times in II\textsuperscript{nd} group relative to that in
control group (P<0.01). It is worth to note that pepper capsaicinoids had a clear-cut stimulatory effect on complement activity at 7 day following the last treatment when administered in the water (II group) rather than in the feed. This finding coincides with our earlier results showing higher effectiveness of pepper capsaicinoids on egg production when provided to the drinking water (Kitanov et al., 2003; Kitanov, 2004; Kitanov et al., 2004a, b).

**Fig. 1.** Complement activity in Ovocap-treated pheasants

**Fig. 2.** Complement activity in Ovocap-treated laying hens
The mechanisms that control complement protein synthesis are incompletely understood. Recent evidence suggests that cytokines are involved in the regulation of hepatic synthesis of circulating complement components. Interleukin-1β when combined with IL-6 exerts potent stimulatory effect on the secretion of component C₃, the major opsonic protein at the complement system (Andrews et al., 2003). Therefore, pepper capsaicinoids -induced increase of plasma complement activity could be due to its modulatory effect on IL-1β level in pheasants (Fig 5) and hens (Fig 6). Furthermore a large body of evidence has now accumulated that indicates that antibody-antigen (immune) complex and complement can stimulate each other, thus promoting the host defense. It has been demonstrated that C₁q, an integral part of the first component of complement (C₁), triggers the activation process when it binds onto antibody within this complex. Complement was also shown to lower the threshold for the activation of the B cell when it meets an antigen (Walport, 2001). Our data do not allow more detailed interpretation about complement-cytokine interaction. However, they are consistent with the view of Connor et al. (2005) that neuroendocrine-immune signaling is accomplished by various unknown until now signaling pathways, which are specific for mental, physical and antigenic stimuli, applied separately or in combination.

![Fig. 3. Serum immunoglobulin Y level in Ovocap-treated pheasants](image)

**Effect of pepper capsaicinoids on serum immunoglobulin Y level**

Serum Ig-Y in experimental groups declined both at 1h and at 7 d following the last pepper capsaicinoids treatment of pheasants (Fig 3). However the
magnitude of decline in II group did not achieve level of significance at 7 d after the last treatment. The most pronounced inhibitory effect of pepper capsaicinoids on serum Ig-Y level was registered at 1h after the last treatment of I group when the level of Ig-Y was 2.9 times lower than that in the control group. The higher pepper capsaicinoids dose tended to have less suppressive effect than the lower dose. There was an inverse relationship between plasma Ig-Y and IL-1β (Fig 5) at 7d following the last treatment.

Fig. 4. Serum immunoglobulin-Y level in Ovocap-treated laying hens

Fig. 5. Serum IL-1β in Ovocap-treated pheasants
Plasma Ig-Y levels in the laying hens increased by 50.79% and 20.63% at 1h following the last treatment and by 39.6% and 42.86% at 7d following the last treatment in I and II experimental group respectively (Fig 4). However, Ig-Y elevation achieved level of significance only at 1h after the last treatment in I experimental group. The observed increase of serum Ig-Y was registered against the background of sharply reduced IL-1β (fig. 6) in either of the experimental groups. The inverse relationship between Ig-Y and IL-1β in hens coincided with that found in pheasants and gives further support of the notion that the proinflammatory cytokine IL-1β modulates Ig-Y production.

Capsaicin has been reported to increase survival rate of salmonella infected mice (Park et al., 1998; Tellez et al., 1993). Consequently, the increased serum Ig-Y in experimental hens suggests that the reported protective effect of capsaicin against salmonella infection could be due to its stimulatory effect on Ig-Y production. However this view is not in agreement with the pepper capsaicinoids -induced reduction of IG-Y in pheasants. The opposite effect of pepper capsaicinoids on Ig-Y in hens and pheasants could be explained with a possible modulating effect of pepper capsaicinoids on IL-1β synthesis which on its turn could influence Ig-Y production, thus generating a pulsatile pattern of Ig-Y production. Besides, serum level of avian Ig-Y is higher than the other immunoglobulins and its protective effect against infections is well documented.

Blood Ig-Y was reported to be selectively incorporated into ovarian follicles at a higher rate than that of the other immunoglobulins (Kitaguchi et al, 2008). Further work of Carlander et al., 2003) suggested that the newly hatched chickens with limited amounts of Ig-Y from the hen may be more
susceptible to infections. These findings, as well as the higher Ig-Y level in pepper capsaicinoids treated hens are consistent with our recent data (in press) indicating higher vitality and survival rate in chickens hatched from eggs of pepper capsaicinoids -treated hens.

**Effect of pepper capsaicinoids on plasma IL-1β in pheasants and hens**

Supplemental dietary pepper capsaicinoids didn’t have any significant effect on serum IL-1β levels at 1h after the last treatment in both pheasant groups despite the twofold increase in I experimental group relative to control group (Fig 5). The lack of significance in I experimental group was due to sizable individual differences which are inherent feature of the hunting pheasant. Serum IL-1β increased significantly in either of the groups at 7d following the last treatment achieving values that were 4.2 and 3.5 times higher in I and II group respectively than the control value. These results suggest that pepper capsaicinoids may exert either fluctuating or delayed stimulatory effect on plasma IL-1β level.

It has been reported that capsaicin sensitive primary sensory neurons may play an important modulating role in the production of cytokines, including IL-1β (Park et al., 1998). These data are in agreement with the increased IL-1β levels in either of the experimental groups. A growing body of evidence now suggests that IL-1β stimulates adrenocorticotropin and glucocorticoids secretion as well as many other indices of hypothalamic-pituitary-adrenal axis activation. Most of the various IL-1β effects on stress hormones release are presented in the comprehensive review of Turnbul and Rivier (1999). Long term administration of IL-1β to rats has been found to increase corticotrophin-releasing hormone, adrenocorticotropin and adrenal weight (Turnbul and Rivier, 1999). In our earlier investigations with hunting pheasants, kept under identical experimental design (Kitanov et al., 2003), we found slight but significant adrenal weight enhancement which supports our assumption about possible involvement of IL-1β in hypothalamic-pituitary-adrenal axis activation. It is well known that adrenal weight can be increased only after prolonged stimulation, accompanied with increased secretion of glucocorticoids.

In our previous work we have established sustainable effect of pepper capsaicinoids on the investigated performance traits throughout a 20 weeks long experimental period, despite the fact that pepper capsaicinoids treatment was conducted at intervals of 28 days, which suggests that pepper capsaicinoids may have exerted prolonged aftereffect. This assumption is consistent with the reported delayed (at least 4 d ) and long lasting (at least 3 weeks) effect of single IL-1β administration on hypothalamic-pituitary-adrenal axis, particularly on arginine vasopressin stores in corticotrophin-releasing
hormone terminals in the external zone of the medium eminence (Schmidt et al., 1995) and on corticotrophin-releasing hormone secretion.

IL-1β has been found to be the most potent cytokine at stimulating adrenocorticotropic (ACTH) secretion (Turnbul and Rivier, 1999). It is hardly accidental that the potent stimulatory effect of IL-1β on ACTH secretion coincides with the reported capsaicin-induced augmentation of pituitary gland sensitivity to corticotrophin-releasing hormone (Watanabe et al., 1994). One of the proposed conceptions states that inflammatory cytokines, including IL-1β, signal central nervous system which in turn activates hypothalamic-pituitary-adrenal axis in an attempt to prevent immune response from overshooting. Therefore, the increase of plasma IL-1β in our case could be viewed as a message sent by immune system to central nervous system in response to pepper capsaicinoids s challenge.

Serum IL-1β levels in hens, unlike those in pheasants, declined significantly in either of the experimental groups both at 1d and at 7d following the last pepper capsaicinoids treatment in comparison with the level in the control group. The most pronounced decline of IL-1β levels was observed at 7d after the last treatment when IL-1β levels were 6.3 times lower in I experimental group and 8.9 times lower in II group relative to the control value. Besides, our results demonstrate higher inhibitory effectiveness of pepper capsaicinoids on IL-1β levels when supplemented to the water rather than to the feed. The inhibitory effect of Ovocap on IL-1β level was between 25% and 29% higher when administered in the water.

The observed discrepancy between IL-1β levels in pheasants and hens could be explained either by the reported differential modulating effect of corticotrophin releasing hormone on IL-1β system (stimulatory or inhibitory) depending on the state of activation of the monocytes (Pereda et al.,1995) or alternatively, by capsaicin-induced fluctuation in the negative feedback exerted by the hypothalamic-pituitary-adrenal axis on immune system. In our earlier report we have indicated that Ovocap exerts dose related differential effect on muscle color in pheasants (Tyufekchiev et al., 2005) which is consistent with the above proposed assumption. Our view is further supported by the well-known fact that meat color is directly related to the applied stress load. However, we should bear in mind that the bidirectional communication between immune and neuroendocrine systems is far more complicated and includes many mediators. Furthermore, lymphocytes and monocytes produce a number of cytokines and hormones which act at or above the level of the hypothalamus.

At first glance our hypothesis is not consistent with our previous work showing significant decline of plasma corticosteron level at 7d following the last pepper capsaicinoids treatment in hens kept under the same experimental
design (Kitanov et al., 2004). However, this does not necessarily mean that adrenal gland function has been permanently lowered throughout the experimental period. On the contrary, pepper capsaicinoids treated hens were found to have higher laying capacity which inevitably requires elevation of the metabolic rate and subsequent increase of glucose demand. This is why high producing animals, as a rule, have increased basal levels of glucocorticoids.

A large number of studies have confirmed the original findings that the administration of capsaicin to rats stimulates adrenal secretion, body fat mobilization as well as oxygen consumption and free fatty acids production (Ohnuki et al., 2001; Ohnuki et al., 2001; Oh and Ohta., 2003). Consequently, we are prone to believe that the lowered corticosteron level in pepper capsaicinoids treated laying hens, registered in our previous experiment, (Kitanov et al., 2004) was due to a possible fluctuation of corticosteron level which is compatible with the proposed hypothesis. Moreover, it is well documented that plasma corticosteron level is a dynamic and sensitive indicator of adrenal gland function. Corticosteron concentration in plasma increases within minutes in response to stress stimuli and subsides shortly after their abolishment. Consequently, when plasma corticosteron is measured only once or twice, over a prolonged period of time, as it is in our case, it gives information for the temporal activity of adrenal glands, but does not allow objective assessment of adrenal activity over the whole period.

Further investigations on the diurnal pattern and time course of the studied indices are required. They could give us better insight about pepper capsaicinoids -induced effect on the immunologic processes.

CONCLUSIONS

The supplemental dietary pepper capsaicinoids caused species-specific modulating effect on complement activity, serum Ig-Y and IL-1β level indicating that pepper capsaicinoids is implicated in the regulation of innate and adaptive immune systems.

REFERENCES


Connor, T. J.; Brewer, C.; Kelly, J. P.; Harkin, A., 2005: Acute stress suppresses pro-inflammatory cytokines TNF-α and IL-1β independent of a
catecholamine-driven increase in IL-10 production. Journal of Neuroimmunology 159, (1-2), 119-128


Kitanov, I., 1998: Feed supplement for animals and birds- Bulgarian patent - № 61634. Patent bulletin 2, 24-25. (Bg)

Kitanov, I., 2004: Stimulation of the laying ability of pheasants by means of Ovocap. Agricultural Science 5, 63-66


Szoscanyi, J; Bartho, L., 2001: Capsaicin-sensitive afferents and their role in gastroprotection: an update. Journal of Physiology 95, 181-188


