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INFLUENCE OF INDUCED MOLTING ON HORMONAL STATUS OF AGED LAYING HENS

UTJECAJ IZAZVANOG MITARENJA NA HORMONSKI STATUS STARIH KOKOŠI NESILICA

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SUMMARY

Physiological mechanisms of reproduction, during induced molting, have not been investigated enough. From the investigations so far it is known that induction of molting with total fasting initiates drastic decrease of body weight, decrease of gonadotropic and sex hormones concentration in plasma and increase of the thyroid hormone concentration.

The aim of this investigation was to establish changes of the level of folicle-stimulating hormone (FSH), luteinizing hormone (LH) and progesterone in two genotypes of hens: ISA Brown, 88 weeks old and Hisex Brown, 91 weeks old in specific points of molting period. From the obtained results it can be seen that hormonal status of hens significantly changed because the FSH concentration in blood plasma during the preparing period for molting was 60.48 ng/ml, 62.36 ng/ml and 76.26 ng/ml for third, first and second group. After the period of stress it was significantly decreased: 23.38 ng/ml, 25.16 ng/ml and 44.59 ng/ml, and in the second egg laying cycle the concentration was stabilized and reached in 91.36 ng/ml in group 3 (Hisex Brown hens), 45.12 ng/ml in group 2 and 64.26 ng/ml in group 1 (ISA Brown hens).

Concentration of LH was also changed because during the preparing period it was: -16.88 ng/ml, 11.16 ng/ml and 23.85 ng/ml, in groups 1,2 and 3; during the stress period:-2.08 ng/ml, 13.96 ng/ml and 9.90 ng/ml in groups 1, 2 and 3. During the laying period it increased because in the time of maximum egg production it was 27.50 ng/ml, and 21.92 ng/ml, (first and second group)(ISA Brown), but the highest concentration was measured in hens from group 3, 37.35 ng/ml (Hisex Brown).

Progesterone in blood plasma during the preparing period in group 1 was 167.5 pg/ml, in group 2, 49.6 pg/ml (ISA Brown), but in group 3 it was 94.8 pg/ml (Hisex Brown). At the end of the stress period there was significant decrease and in group 1, it was 0, in group 2, 19.2 pg/ml, and group 3, 25.6 pg/ml. During maximum egg laying, the concentration of progesterone increased and it was: -135.3 pg/ml in group 3 (Hisex Brown), and 22.64 and 41.4 pg/ml in group 1 and 2 (ISA Brown).

Key words: artificial molting, hormonal status, laying hens.

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INTRODUCTION

Physiological mechanisms of reproduction, during artificial molting period, have not been sufficiently investigated. It is clear that induction of molting by fasting (feed restriction) initiates drastic decrease of body weight, gonadotropic and sex hormone concentration in plasma and increase of thyroid hormone concentration. In the period of about 14 days, large follicles of ovarium are resorbed and decreased, ovarium will contain only small follicles. Concentrations of T_3 and T_4 start to grow several days after decrease of LH and estradiol, and stay on this level 25 to 30 days after induced molting. High concentrations of thyroid hormone are required to support growing of new feathers. It is clear that natural and artificial molting has many common physiological changes (Etches R.J., 1979, 1996). Investigations suggest that laying hens began more sensible of increasing of photoperiod and feed restriction when they raise peak of laying. This sensibility is manifested with decreasing of egg production and beginning of molting on large part of flock.

The aim of this investigation was to establish changes of the level of follicle-stimulating hormone, luteinizing hormone and progesterone of hens of two genotypes during the induction of molting, and then during second egg laying cycle and during period of achieving the maximum egg laying.

MATERIAL AND METHOD

To get an answer to established tasks, there are few investigations with ISA Brown laying hens 88 weeks old and Hisex Brown 91 weeks old. Laying hens are accommodated in standard industrial poultry houses. Hens are separated in to 3 groups depending on genotype, lighting program and age: - group 1 hybrid ISA Brown 88 weeks old and lighting program A, group 2 hybrid ISA Brown 88 weeks old with lighting program B and group 3 hybrid Hisex Brown 91 weeks old and lighting program B. Plan of lighting program is given in Table 1.

Table 1. Plan for lighting of experimental laying hens
Tablica 1. Plan osvjetljenja za pokusne nesilice

Period	Group 1 lighting program A	Group 2 lighting program B	Group 3 lighting program B
Preparing period	24,00	24,00	24,00
Stress period (starvation)	9,00	7,00	7,00
1st week	9,00	7,00	7,00
2nd week	9,00	7,00	7,00
3rd week	9,00	7,00	7,00
4th week	10,00	700	7,00
5 th week	10,00	7,00	7,00
6 th week	11,00	8,00	8,00
7 th week	11,30	9,00	9,00
8 th week	12,30	10,00	10,00
9 th week	13,30	11,00	11,00
10 th week	14,00	12,30	12,30
11 th week	14,30	13,00	13,00
12 th week	15,00	14,00	14,00

Hormonal status was monitored with determination of hormones concentration in blood plasma: - FSH, LH and progesterone. For this purpose blood samples were taken from the same sort and marked laying hens directly from the heart (cardiaic puncture). Blood samples were centrifuged 15 minutes on 3500 rpm, not more than 1 hour after the cardiac puncture and the collected plasma was frozen at –20 ^{o}C in a freezer until analysis. Concentration of mentioned hormones was determined by radioimune (RIA) methods: - for determination of progesterone concentration

Progesterone extraction from plasma samples was done with hexane. Incubation of samples at –4 ^{o}C for 24 hours. Separation of binded complex antigen-antibody of free hormone was done with active charcoal. After centrifugation, supernatant was decanted and binded fraction was radio measure in period of 10 minutes.

Concentration of lutenizing hormone (LH) was determined by direct method without extraction of blood plasma. Separation of free and bound fraction was done by mediation of complex antigen-antibody separation with other antibody. After decantation of supernatant and complete removing of free fraction, samples were radio measured 1 minute separately on automatic counter. Hormone for marking NIDDK-LH-1-3 was marked with J^{125} according to modification hloramin-T method (Greenwood and Bryant, 1971) and clean up again with separate gel colon filtration Vitroget AoA-54.

Radioactivity was measured with spectrometer Beckman LS-8000. Gamma spectrometers RFT and NK-350 and automatic counter NZ-322 was used for reading the results of systems with the presence of hormone marked with J^{125}.

Blood samples were taken in the same time: at the end of egg laying, after preparing period, at the end of treatment (after 10 days starvation), during molting period (10^{th} day, 20^{th} day after treatment) and at maximum egg laying.

RESULTS AND DISCUSION

During the induction of molting and introduction in the second egg laying cycle of hens, we may notice changes in concentration of gonadotropic hormones (Table 2 and Figure 1, 2 and 3).

Table 2. Hormonal activity in hens in experimental period

Tablica 2. Hormonalna aktivnost u kokoši u pokusnom razdoblju

	Group 1				Group 2				Group 3			
	Age of hens, weeks	FSH ng/ml	LH ng/ml	Progesterone, pg/ml	Age of hens, weeks	FSH ng/ml	LH ng/ml	Progesterone, pg/ml	Age of hens, weeks	FSH ng/ml	LH ng/ml	Progesterone, pg/ml
1. First egg laying period												
– Last decade	88	51,98	30,04	157,8	88	34,32	9,38	153,6	91	47,14	6,92	112,6
2. Preparing period	89	62,36	16,88	167,5	89	76,26	11,16	49,60	92	60,48	23,85	94,80
3. Period of stress	91	25,16	2,08	0	91	44,95	13,96	19,20	94	23,38	9,90	25,60
4. Resting period												
– 10^{ti} day	92/2	60,41	5,71	5,84	92/2	44,98	13,62	0	95/2	51,30	16,00	0
– 20^{ti} day	94	61,62	12,64	11,12	94	45,01	11,64	5,60	97	72,40	22,71	77,18
5. Peak of laying	106	64,26	27,76	22,64	106	45,12	21,92	41,40	109	91,36	37,35	135,3

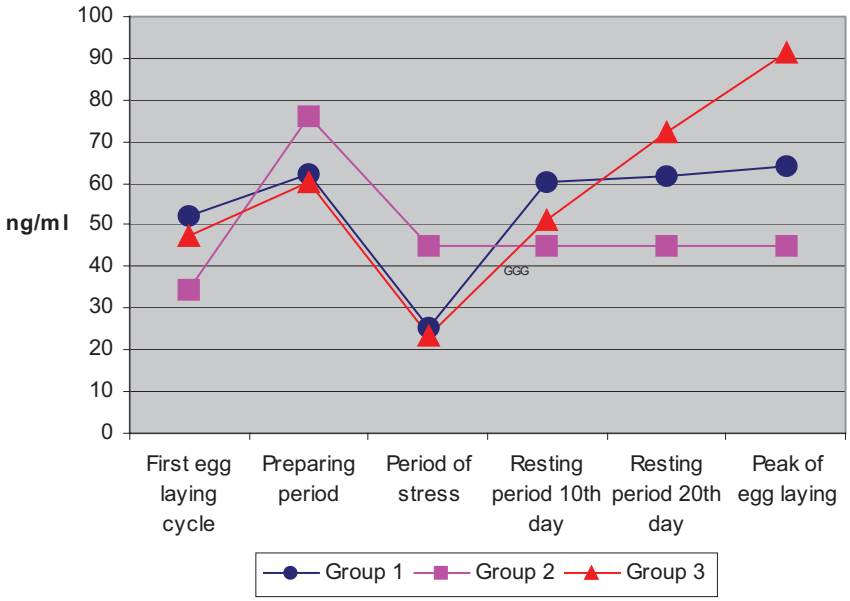

Figure 1. Hormonal status of FSH
Slika 1. Hormonalni status FSH

The most characteristic changes are notice in FSH concentration in blood plasma. At the end of the first egg laying cycle this concentration was from 34.32 ng/ml (group 2) to 51.98 ng/ml (group 1), and in group 3, FSH concentrations was in between 47.14 ng/ml.

In this period intensity of egg laying in three groups of hens was from 58.60% (group 3) to 62.60% (group 1) and 60.00% (group 2). This egg laying intensity was a result of very low intensity of the appearance of mature follicles, which occured under the direct influence of follicle-stimulating hormone.

During the preparing period for stress, hens when were treated with continuous lighting and ad libitum feed and water for 7 days, in all groups increased FSH concentration was noticed: 62.36 and 76.26 ng/ml in groups 1 and 2, and 60.48 ng/ml in group 3.

During the stress period, 10^{th} day, FSH concentration in plasma decreased in all groups to 23.38 (group 3), 25.16 (group 1) and 44.95 ng/ml (group 2) which was the result of reducing photoperiod and abstinence of feed. It is important to mention that the lowest concentration was observed in birds with the shortest photoperiod. In the following part of the procedure, resting period and increasing of egg laying, significant tendencies are increase of FSH concentration in all groups, a very small increase in groups 1 and 2, but in group 3 increase was linear starting in the stress period when FSH concentration was at the lowest level from 23.38 ng/ml to 91.36 ng/ml in the peak of egg laying.

In the luteinizing hormone content in blood plasma, in the end (last week) of the first egg laying cycle was observed oscillation, and in group 1 it was 30.04 ng/ml, in group 2, 9.38 ng/ml and in group 3, 6.92 ng/ml.

Collecting the blood samples was done in the period from 9 to 10 a.m. and always in the order from group 1 to group 3. On the sixth day of preparing period LH concentration was from 16.88 ng/ml in group 1; 11.16 ng/ml in group 2; and 23.85 ng/ml in group 3. There were significantly high oscillations which was the result of continuous light stimulation and significant changes in the time of LH secretion of hypophisis. In this preparing period for hens, dynamics of laying eggs in the morning hours and also ovulation changed and occured in the time of a whole day photoperiod. In the preparing period the morning laying eggs changed and occured proximally balanced during whole 24 hours lighting day.

During the 10 days period of stress blood sample were collected (9th day) when the concentration of LH was 2.08 ng/ml in group 1, 13.96 ng/ml in group 2 and 9.90 ng/ml in group 3. In this period the laying intensity was minimal. In some hens a low concentration of LH because was observed laying is not at the zero point level.

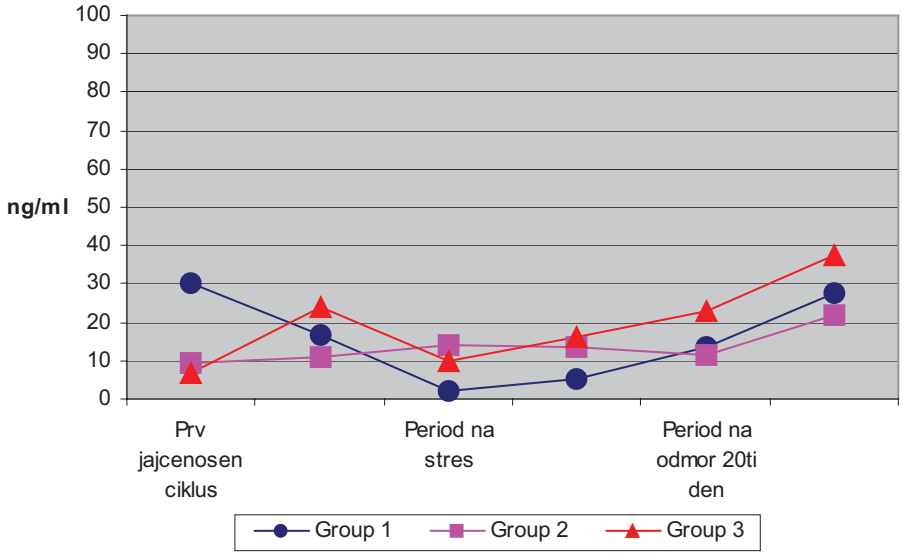

Figure 2. Hormonal status of LH
Slika 2. Hormonalni status LH

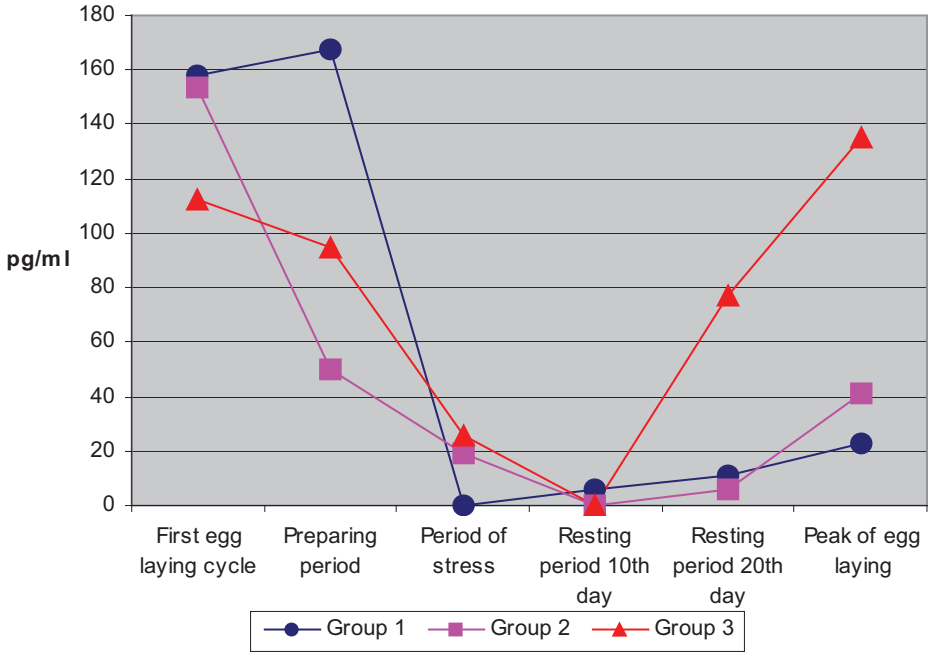

Figure 3. Hormonal status of progesterone
Slika 3. Hormonalni status progesterona

Similar cases are were observed in groups 1 and 2. After the period of ten days of stress the consumption of feed was constantly increasing as well as the photoperiod.

As a result of this in group 1 continuous increasing of LH concentration was observed from 5.26 ng/ml on the tenth day of resting period to 13.36 ng/ml on the twentieth day and 27.50 ng/ml in the peak of maximum egg laying. Similar tendencies were observed in group 2 where the LH content in 1 ml blood plasma at the peak of egg laying was 21.92 ng/ml and in group 3, where the highest average LH content in the plasma was - 37.35 ng/ml. In group 2 there was a little decrease of LH concentration on the 20-th day after the stress period which could be a result of timing of collecting samples. During the experiment, the progesterone content was recorded in blood plasma, which is ovary's product. Its function is not only to stimulate the oviduct for excretion of proteins, it also has an influence on hypothalamus excretion of realising hormone, the factor needed for excretion of luteinizing hormone from the hypothalamus. Its concentration was measured in picograms in milliliter, and in the last week from the first egg laying cycle it was from 112.6 pg/ml to 157.8 pg/ml in groups 3 and 1, and in group 2 it was 153.6 pg/ml. During the preparing period the concentration of progesterone in group 2 was 49.6 pg/ml in group 3, 94.8 pg/ml and in group 1 167.5 pg/ml. During the stress period was noticed drastically decreasing of its concentration. in group 1 it was at zero point, 19.2 in group 2 and 25.6 pg/ml in group 3. The concentration of progesterone was very low on 10^{th} day of the stressing period, it was not detected in groups 2 and 3, and in group 1 it was 5.84 pg/ml. This is natural for both periods because then the egg production and also the ovulation are at zero level. In the following period with the increasing of egg laying intensity increase of progesterone concentration in blood plasma was observed. The fastest increase of egg laying was observed in group 3 where progesterone concentration on the 20^{th} day after the stress increased to 77.18 pg/ml. In this group on the start of laying was 3.42% in the third week and 5.22% in the fourth week. In group 1 and 2 progesterone concentration on the 20^{th} day after stress was low, from 5.6 pg/ml (group 2) to 11.12 pg/ml (group 1). Intensity of egg laying of these groups in this period wat on the start 0.21% in group 2 and 1.47% in group 1.

In the peak of egg laying production, the concentration of progesterone in all groups increased and in group 3 was 135.3 pg/ml, in group 2, 41.4 pg/ml and in group 1, 22.46 pg/ml. The highest values was in group 3 which achieved the highest intensity of egg production of more than 80% and 50 grams of produced egg mass per day. Similar changes in hormonal status of molted hens with quantitative restriction of feed were mentioned by Decuypere E. and Verheyen G. (1986) and Etches R. J. (1979, 1996).

The above mentioned is graphically expresed in figure 3 where are clearly presented the mentioned tendencies of the decrease the progesterone concentration in blood plasma during the period from last week of first egg laying cycle and the period of stress, standing at low level in the resting period and increasing its concentration till achieving the maximum daily egg laying.

CONCLUSION

The significant changes were found in hormonal status of hens during the experimental period because the FSH concentration of blood plasma in the preparing period for molting was 60.48 ng/ml, 62.36 ng/ml and 76.26 ng/ml in third, first and second group. After the period of stress a significant decrease was found 23.38 ng/ml, 25.16 ng/ml and 44.59 ng/ml, and in the second egg laying cycle the concentration was stabilized and reaching:- 91.36 ng/ml in group 3 (Hisex Brown hens), to 45.12 ng/ml in second and 64.26 ng/ml in first group (ISA Brown hens).

Concentration of LH also changed and during the preparing period it was 16.88 ng/ml, 11.16 ng/ml and 23.85 ng/ml, in group 1,2 and 3, during the stress period it was 2.08 ng/ml, 13.96 ng/ml and 9.90 ng/ml in group 1, 2 and 3. In the following period it increased and in the time of maximum egg production was 27.50 ng/ml, 21.92 ng/ml, in first and second group (ISA Brown), but the highest concentration was measured in hens from group 3, 37.35 ng/ml (Hisex Brown).

Progesterone in blood plasma during the preparing period in group 1 was 167.5 pg/ml, group 2, 49.6 pg/ml (ISA Brown, but in group 3 it was 94.8 pg/ml (Hisex Brown). At the end of the stress period

there was a significant decrease and in group 1 it was 0, in group 2, 19.2 pg/ml, and in group 3, 25.6 pg/ml. During the maximum egg laying intensity, the concentration of progesterone increased and it was in group 3 (Hisex Brown) 135.3 pg/ml, and in group 1 and 2 22.64 and 41.4 pg/ml (ISA Brown), respectively.

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SAŽETAK

Fiziološki mehanizmi reprodukcije, u vrijeme umjetno izazvanog mitarenja, nisu dovoljno ispitani. Od dosadašnjih ispitivanja poznato je da izazvano mitarenje s potpunim ograničavanjem hrane uzrokuje nagli pad tjelesne mase, pad u koncentraciji gonadotropnih i spolnih hormona u plazmi i povećanje koncentracije tiroidnih hormona.

Cilj ovog rada je utvrditi promjene u koncentraciji folikulo-stimulirajućeg hormona (FSH), luteinizirajućeg hormona (LH) i progesterona (P) kod kokoši dva genotipa ISA Brown na uzrast od 88 tjedana i Hisex Brown na uzrast od 91. tjedna, u pojedinim točkama razdoblja mitarenja. Iz dobivenih rezultata može se vidjeti da se hormonalni status kokoši značajno mijenja, jer koncentracija FSH u krvnom serumu za vrijeme pripremnog razdoblja za mitarenje iznosi 60,48ng/ml, 62,36 ng/ml i 76,26 ng/ml u trećoj, prvoj i drugoj skupini. Nakon stresnog razdoblja ona se smanjuje i iznosi 23,38 ng/ml, 25,16 ng/ml i 44,95 ng/ml, dok se u tijeku drugog ciklusa nesenja stabilizira i dostiže 91,36 ng/ml u skupini 3 (nesilice Hisex Brown), 45,12 ng/ml u skupini 2 i 64,26 ng/ml u skupini 1 (nesilice ISA Brown).

Koncentracija luteinizirajućeg hormona isto tako se mijenja i za vrijeme pripremnog razdoblja iznosi: - 16,88 ng/ml, 11,16 ng/ml i 23,85 ng/ml u skupinama 1, 2 i 3; za vrijeme razdoblja stresa: -2,08 ng/ml, 13,96 ng/ml I 9,90 ng/ml u skupinama 1, 2 i 3. Za vrijeme drugog ciklusa nesenja koncentracija se povećava i iznosi 27,50 ng/ml i 21,92 ng/ml (prva i druga skupina) (ISA Brown), dok je najviša koncentracija izmjerena kod nesilica iz skupine 3, 37,35 ng/ml (Hisex Brown).

Progesteron u krvnom serumu za vrijeme pripremnog razdoblja u skupini 1 je 167,5 pg/ml, skupini 2 je 49,6 pg/ml (ISA Brown), dok u skupini 3 iznosi 94,8 pg/ml (Hisex Brown). Na kraju stresnog razdoblja značajno se smanjuje i u skupini 1 iznosi 0, u skupini 2, 19,2 pg/ml, a u skupini 3, 25,6 pg/ml. Za vrijeme maksimalne nosivosti koncentracija progesterone se povećava i iznosi: - 135,3 pg/ml u skupini 3 (Hisex Brown) i 22,64 odnosno 41,4 pg/ml u skupinama 1 i 2 (ISA Brown).

Ključne riječi: umjetno izazvano mitarenje, hormonalni status, kokoši nesilice.