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Hormonal and metabolic changes at the late oestrous phase after stimulation of ovulation rate by lupin grain in sheep

Zmiany hormonalne i metaboliczne w ostatniej fazie rui u owiec po podwyższeniu OR wywołanym podawaniem nasion łubinu

Key words: FSH, 17β-oestradiol, progesterone, ovulation, sheep, lupin feeding
Słowa kluczowe: FSH, 17β-oestradiol, progesteron, owulacja, owce, łubin

INTRODUCTION

The role of FSH (follicle-stimulating hormone) and steroid hormones in the ovulation rate responses to lupin grain is not well understood. Furthermore, the increase in OR (ovulation rate) in ewes after high protein and high energy feed (7, 9, 12, 13, 15, 16, 19, 20) suggests that some specific metabolic or nutritional signals evoke gonadal response (7). The type and site of their action on the reproductive axis remains unclear, although the ovary and pituitary were pointed out as the places of metabolic influences of these compounds. Inhibition of glycolysis with 2-deoxyglucose mimics the tissue starvation induced by hypoglycaemia or low insulin and leads to suppression of follicle growth and deletions in the LH surge from pituitary (7, 21).

This paper aims at the following two objectives: 1) to check whether increased ovulation rate in response to short-term feeding with lupine grain during the oestrous cycle results from changes in gonadotropin (FSH) and steroid hormones (17 β-oestradiol – E_2, progesterone – P_4, testosterone – T and androstenedione – Ad) secretion; 2) to extrapolate glucose and protein level to OR in sheep kept under different nutritional conditions. The other important point which needs consideration is that the increase in OR stimulated with lupine grain appears as a result of action of single or multidirectional influences.

MATERIALS AND METHODS

Experimental design. Ninety-six female Polish Lowland sheep, 3–5 years of age, were used. Their mean body weight was 62.05 ± 3.55 kg. The experiments were carried out in summer throughout the breeding season during three years. The 1st day of the experiment was that on which meadow hay feeding commenced. From the beginning to the end of the experiment each ewe was given 1.5 kg of meadow hay (6% protein on a dry matter basis, gross energy – 5.2 MJ/kg of dry matter). On the 1st day of the experiment all the ewes were given first injections of 250 μg prostaglandin $F_2\alpha$ analogue (Cloprostenol, Oestrophan, Leciva – Czech Republic) to synchronise the oestrous cycle. They were weighted and divided into two groups: control (C, n = 48) and experimental (E, n = 48). The groups were similar with regard to mean age (C – 4 years, E – 4 years), mean body weight (C – 61.50 ± 3.20 kg, E – 62.60 ± 3.90) and mean fertility in the previous years (C – 1.29, E – 1.27). On the 12th day of experiment the ewes were given the 2nd dose of prostaglandin $F_2\alpha$. After 48–72 hours (on the 14th and 15th day of the experiment), blood samples from the jugular vein were collected to check the efficiency of the oestrous synchronisation by analysis of 17β-oestradiol (E_2) and progesterone (P_4) concentration.

The results of hormonal analysis (mean E_2 concentration – 26.28 ± 1.66 pg/ml, mean P_4 concentration – 0.68 ± 0.11 ng/ml), enabled to recognise the 15th day of the experiment as the 1st day of the oestrous cycle. Since the 2nd to 13th day of the oestrous cycle (the 16th–27th days of the experiment) the ewes received a supplement in the form of ground lupin (*Lupinus angustifolius*) grain. The ewes were given this high-protein (35% protein on a dry matter basis) and high-energy (gross energy – 20.1 MJ/kg of dry matter) supplement at increasing doses: on the 2nd day of the cycle – 150 g, the 3rd day – 200 g, the 4th day – 400 g, the 5th day – 600 g and from the 6th to 13th day – 750 g of lupin grain per ewe. Since the 14th day of the oestrous cycle (the 28th day of the experiment) all ewes were fed only hay. On the 17th day (the 31st day of the experiment) 5 ml blood samples for hormones, glucose and protein analysis were collected every 15 min for 12 h starting at 10.00 h. Blood was centrifuged (20 min. at 4°C and 1000 g) and plasma was stored at 20°C. The ovulation rate in all ewes was determined by laparoscopy of ovaries on the 37th day of the experiment (the 6th day of another oestrous cycle).

Determination of hormones. Steroid hormones (oestradiol, testosterone, and progesterone) were extracted from plasma with dichloromethane and analysed by HPLC method (Beckman, Gold System, USA). The analysis employed a reversed phase, 250 × 4 mm I.D., 5 μm analytical column (LiChrospher 100, Merck, Germany). The mobile phase, consisting of 0.25% ortophosphoric acid and acetonitrile, was pumped at 1.0 ml/min (125 SM, Beckman, USA). The separation was performed in a gradient of acetonitrile (40–100% in 20 min.). UV detection (DAD 168, Beckman, USA) was at 220 nm. As an internal standard dehydrocholic acid was used (11). Plasma concentrations of FSH were determined by immunoradiometric assay (FSH [^{125}I] IRMA, Spectria, Orion Diagnostica, Finland). Intra- and interassay coefficients of variation were 9 and 11%.

Plasma protein and glucose determination. The total protein plasma level was determined by the modified Lowry method (14). Total glucose plasma level was determined by enzymatic method using diagnostic KIT (Liquick Cor – Glucose 120, Cormay, Poland).

Statistical analysis. Statistical analysis of the obtained results was performed using Excel 97. Differences in the ovulation rate and concentrations of hormones, amino acids, proteins and glucose were compared by the Student's t test.

RESULTS

OVULATION RATE

The ovulation rate of the ewes supplemented with lupin grain was significantly ($P \leq 0.05$) higher (1.687 ± 0.463) than that of the control fed ewes (C) (1.291 ± 0.454). In the experimental group (E) (n = 48) double ovulation was found in the thirty-three ewes, whereas in the control group (n = 48) in the fourteen. The rest of the sheep in each group have single ovulation in the examined oestrous cycle.

PLASMA FSH

The mean plasma FSH level was increased in the experimental group (143.27 ± 24.25 ng/ml) in comparison to the control (129.45 ± 18.09 ng/ml) on the 17th day of the oestrous cycle (Fig. 1). There were also found the higher mean plasma FSH concentrations in the ewes with double ovulation (C – 133.56 ± ± 21.45 ng/ml, E – 147.85 ± 32.14 ng/ml) than in the ewes with single one (C – 125.34 ± 20.17 ng/ml, E – 138.69 ± 20.86 ng/ml) (Fig.1). These differences

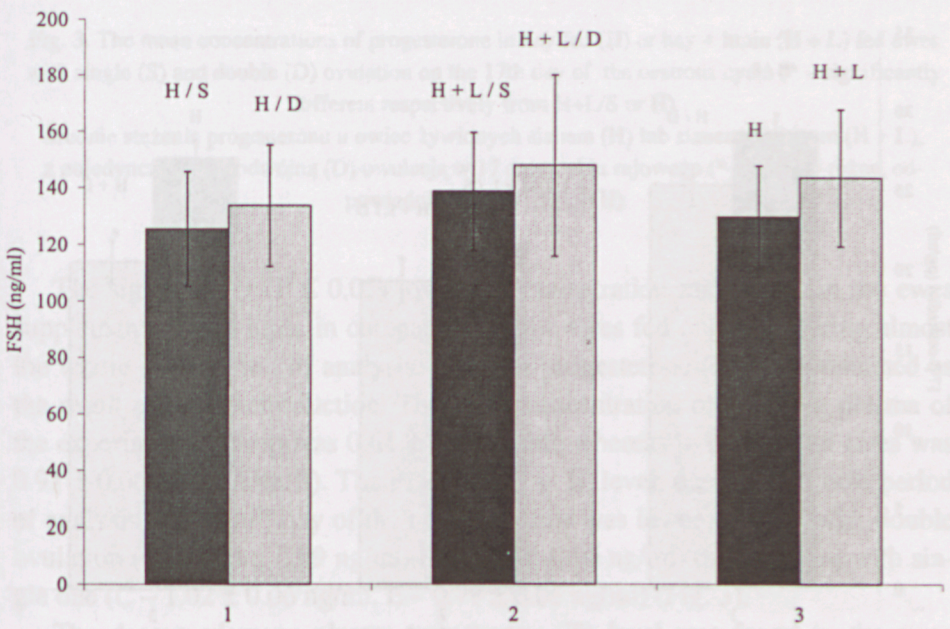

Fig. 1. The mean concentrations of FSH in hay fed (H) or hay + lupin (H + L) fed ewes with single (S) and double (D) ovulation on the 17th day of the oestrous cycle
Średnie stężenia FSH u owiec żywionych sianem (H) lub sianem i łubinem (H + L), z pojedynczą (S) i podwójną (D) owulacją w 17 dniu cyklu rujowego

were maintained during the whole period of analysis both in the experimental and control sheep. The FSH pulse frequency was similar both in the control and lupin fed ewes. There were observed three poorly marked pulses per 12 h. The mean pulse amplitude was higher in the ewes fed lupin + hay (16.61 ± 7.35 ng/ml) in comparison to the sheep fed only hay (10.18 ± 2.98 ng/ml). The nadir and the maximum were 122.00 and 140.08 ng/ml for ewes fed hay whereas for ewes fed hay + lupin – 132.82 and 154.95 ng/ml.

PLASMA 17β-OESTRADIOL, PROGESTERONE AND TESTOSTERONE

The plasma 17β-oestradiol (E_2) level significantly ($P \leq 0.05$) decreased after lupin grain feeding (E) in comparison to control (C) ewes (Fig. 2). The mean plasma E_2 concentration in the experimental group was 20.51 ± 1.35 pg/ml, whereas in the control one 26.99 ± 1.14 pg/ml. Furthermore, the E_2 level during the whole period of analysis on the 17th day of the oestrous cycle was lower in sheep with double ovulation (C – 25.32 ± 1.31 pg/ml, E – 19.32 ± 1.43 pg/ml) than in these with single one (C – 28.67 ± 1.15 pg/ml, E – 21.69 ± 1.57 pg/ml) (Fig. 2).

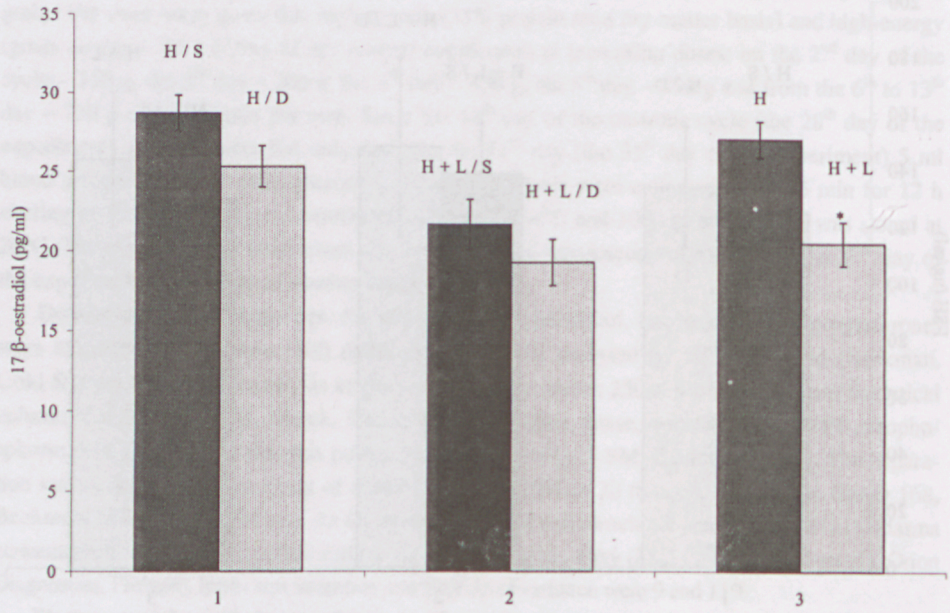

Fig. 2. The mean concentrations of 17β-oestradiol in hay fed (H) or hay + lupin (H + L) fed ewes with single (S) and double (D) ovulation on the 17th day of the oestrous cycle (* – significantly different from H)

Średnie stężenia 17β-estradiolu u owiec żywionych sianem (H) lub sianem i łubinem (H + L), z pojedynczą (S) i podwójną (D) owulacją w 17 dniu cyklu rujowego (* – istotnie różne od H)

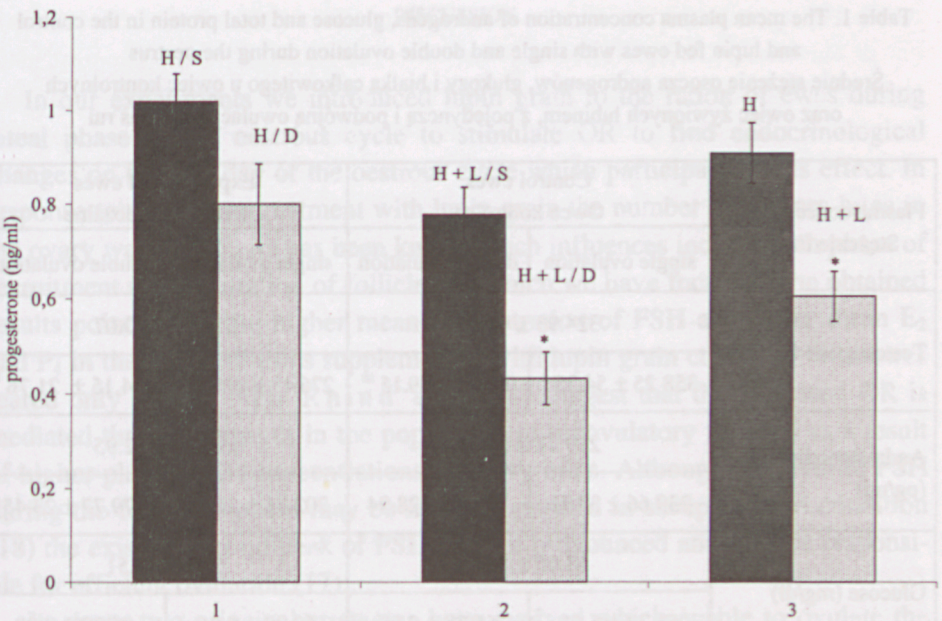

Fig. 3. The mean concentrations of progesterone in hay fed (H) or hay + lupin (H + L) fed ewes with single (S) and double (D) ovulation on the 17th day of the oestrous cycle (* – significantly different respectively from H+L/S or H)

Średnie stężenia progesteronu u owiec żywionych sianem (H) lub sianem i łubinem (H + L), z pojedynczą (S) i podwójną (D) owulacją w 17 dniu cyklu rujowego (* – istotnie różne, odpowiednio od H + L/S lub H)

The significantly ($P \leq 0.05$) lower E_2 concentration maintained in the ewes supplemented with lupin in comparison to the ewes fed only hay during almost the whole 12-h period of analysis. Also the progesterone (P_4) level declined as the result of lupin introduction. The mean concentration of P_4 in the plasma of the experimental sheep was 0.61 ± 0.05 ng/ml, whereas in the control ewes was 0.91 ± 0.06 ng/ml (Fig. 3). The P_4 level just as E_2 level, during the whole period of analysis on the 17^{th} day of the oestrous cycle was lower in sheep with double ovulation (C – 0.80 ± 0.09 ng/ml, E – 0.43 ± 0.06 ng/ml) than in these with single one (C – 1.02 ± 0.06 ng/ml, E – 0.78 ± 0.06 ng/ml) (Fig. 3).

The decreased mean plasma testosterone (T) level was found in the ewes following lupin grain feeding (250.29 ± 30.07 pg/ml) in comparison to control (324.93 ± 48.05 pg/ml). The concentration of T both in the experimental and control group was lower in the case of double ovulation (C – 291.62 ± 39.15 pg/ml, E – 224.15 ± 21.76 pg/ml) than in the case of single one (C – 358.25 ± 54.35 pg/ml, E – 276.43 ± 19.98 pg/ml) (Table 1).

Table 1. The mean plasma concentration of androgens, glucose and total protein in the control and lupin fed ewes with single and double ovulation during the oestrus
Średnie stężenie osocza androgenów, glukozy i białka całkowitego u owiec kontrolnych oraz owiec żywionych łubinem, z pojedynczą i podwójną owulacją podczas rui

Plasma concentration Stężenie osocza	Control ewes Owce kontrolne		Experimental ewes Owce doświadczalne	
	single ovulation	double ovulation	single ovulation	double ovulation
Testosterone (pg/ml)	324.93 ± 48.05		250.29 ± 30.07	
	358.25 ± 54.35 [a]	291.62 ± 39.15 [ab]	276.43 ± 19.98 [ab]	224.15 ± 21.76 [b]
Androstenedione (pg/ml)	219.77 ± 35.33		191.27 ± 22.95	
	239.66 ± 37.72	199.87 ± 28.94	202.76 ± 21.24	179.77 ± 23.45
Glucose (mg/dl)	63.05 ± 5.55		65.10 ± 8.51	
	63.72 ± 5.51	65.78 ± 8.88	60.99 ± 5.96	65.79 ± 8.98
Total protein (g/l)	62.34 ± 13.12		82.32 ± 14.63	
	56.62 ± 12.51	68.05 ± 11.71	79.12 ± 15.99	85.51 ± 12.99

a, b – values signed with other letters are significantly ($P \leq 0.05$) different.
a, b – wartości oznaczone innymi literami są istotnie ($P \leq 0.05$) różne.

PLASMA GLUCOSE

The plasma level of glucose increased after lupin feeding and achieved 65.10 ± 8.51 mg/dl in the experimental group whereas in the control – 63.05 ± 5.55 mg/dl. It was also higher in the ewes with double ovulation than with single one (Table 1).

PLASMA PROTEIN LEVEL

The total plasma protein level was higher on the 17th day of the oestrous cycle in the ewes supplemented with lupin grain (82.32 ± 14.63 g/l) than in the ewes fed only hay (62.34 ± 13.12 g/l). The concentrations of plasma protein in the double ovulated ewes (group C – 68.05 ± 11.71 g/l, group E – 85.51 ± 12.99 g/l) exceed these in single ovulated ewes (group C – 56.62 ± 12.51 g/l, group E – 79.12 ± 15.99) both in the control and experimental group (Table 1).

DISCUSSION

In our experiments we introduced lupin grain to the ration of ewes during luteal phase of the oestrous cycle to stimulate OR to find endocrinological changes on the 17th day of the oestrous cycle which participate in this effect. In response to short-term treatment with lupin grain the number of corpora lutea in the ovary was higher. As has been known, such influences include both phase of recruitment and maturation of follicles on which we have focused. The obtained results point out to the higher mean concentration of FSH and lower mean E_2 and P_4 in the group of ewes supplemented with lupin grain compared with ewes reared only on hay. Also Rhind et al. (18) suggest that the increased OR is mediated through changes in the population of preovulatory follicles as a result of higher plasma FSH concentrations in heavy ewes. Although the level of FSH during the follicular phase may be more suppressed in sheep in good condition (18) the existing second peak of FSH is more pronounced and may be responsible for efficient ovulation (17).

In sheep two principal strategies have evolved which enable to ovulate the greater number of follicles. Firstly, by reducing the sensitivity of the hypothalamic-pituitary unit (HP) to the feedback effects of ovarian hormones (inhibin, and/or oestradiol) which suppress FSH. Secondly, by the mechanism associated with the FecB gene comprising "precocious" development of a large number of antral follicles (2). The product of this gene is a factor which enhances follicular development by a reduction of the circulating FSH level in the late follicular phase (3, 8), as well as affecting gonadotrophic function. But in both of these statements there were not taken into account the two phases of FSH secretion during the late follicular stage of the oestrous cycle. The importance of the follicular recruit phase has been known from the first work by Wallace et al. (21). From his paper it is known that there are some separate peaks of FSH during the initiation of follicular growth. In ewes in good conditions provoked by lupine supplementation higher concentrations of FSH during initial phase of oestrous cycle (as has been stated in another paper) which are not suppressed by E_2 and inhibin may determine the ovulation rate.

We found that the concentration of E_2 declines in sheep with double ovulation with more distinct suppression in the group supplemented with lupine grain. As has been established earlier (6) oestradiol (E_2) exerts atretogenic influence on folliculogenesis although the locus of this action has not been accurately determined. Some results show that E_2 enhances androgen production (10) and in this way augments a detrimental influence on growing follicles. Decrease in E_2 and androgens concentration observed in our experiments in the ewes with double

ovulation supports the importance of these steroids in determination of the number of selected follicles. Taken together with the finding that the suppressed E_2 stimulus during the follicular period is permissive for the growth of additional follicles, it is reasonable to suggest that exposition of ewes to nutritional signals, derived from lupin, induces first of all changes in FSH secretion followed by modification of steroidogenesis in growing follicles. However, as was postulated by many authors (5, 18) there is a causal relationship between ovulation rate (OR) and the numbers of E_2-secreting dominant follicles (18). According to Rhind et al. (18) in ewes with a single dominant follicle total E_2 secretion may be lower than in ewes with more than one dominant follicle. It may result in lower LH and higher FSH levels. This contrasts with the view of Boukhliq et al. (4) who have stated that although heavy ewes may produce more E_2 but its clearance rate is greater so that overall the amount feedback on FSH secretion is less than in light ewes.

Food ingredients, like glucose, may influence OR by stimulation of the greater responsiveness to FSH. In prolific sheep like Romanov the greater FSH receptor mRNA levels both in small and large follicles has been observed (1) given the greater responsiveness to FSH especially in ewes with good condition. Thus, it can be concluded that short-term (12 day) lupin grain feeding exerts stimulatory influence on FSH secretion and suppressive one on secretion of E_2, P_4 and androgens from ovary which together with elevation of protein precursors and glucose lead to augmentation of ovulation rate in ewes.

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SUMMARY

Apart from well-recognized data that energy level is the significant dietary component with respect to the determination of ovulation rate (OR) there are also reports about the increased OR under the influence of protein-rich supplements like *Lupinus angustifolius* grain. In the experiment 96 female Polish Lowland sheep, synchronized with analogue of $PGF_{2\alpha}$, were used. A half of them were fed hay and additionally supplemented with increasing portions (from 150 to 750 g

/ewe/day) of lupin grain during 12 days starting on the second day of the oestrous cycle. The rest of ewes received only hay (control group). On the 6th day of another oestrous cycle the ovulation rate was determined by laparoscopy of ovaries. The ovulation rate of the ewes supplemented with lupin grain was by 30.67% higher than that of the control fed ewes. There was a trend towards a higher mean concentration of FSH and lower mean E_2 and P_4 in the group of experimental ewes compared with those reared only on hay. It is concluded that the elevated level of FSH during oestrous phase accentuates ovulatory response to lupin grain in ewes. Larger follicles in ewes supplemented with lupin grain, may suggest that the effect of body condition on the number of follicles was mediated at least in part through increased circulatory FSH levels.

STRESZCZENIE

Istotnym czynnikiem żywieniowym, który determinuje liczbę owulacji (OR) u owiec, jest wartość energetyczna paszy. Istnieją jednak również doniesienia o wzroście OR pod wpływem dodatku wysokobiałkowego w postaci nasion łubinu. W przeprowadzonym doświadczeniu wykorzystano 96 zsynchronizowanych maciorek rasy PON, z których połowa, stanowiąca grupę eksperymentalną, oprócz siana przez 12 dni, począwszy od 2 dnia cyklu rujowego, otrzymywała suplement żywieniowy w postaci śruty z nasion łubinu wąskolistnego. Owce otrzymywały dodatek we wzrastających dawkach – od 150 do 750 g łubinu/dobę/owcę. Pozostałe maciorki stanowiące grupę kontrolną otrzymywały wyłącznie siano. 6 dnia kolejnego cyklu rujowego wykonano badanie laparoskopowe jajników w celu określenia OR. Liczba owulacji u owiec otrzymujących łubin była o 30,67% wyższa niż u kontrolnych.

W wyniku badań wykazano wyższe średnie stężenia FSH oraz niższe 17β-estradiolu i progesteronu w grupie owiec doświadczalnych w porównaniu do żywionych wyłącznie sianem. Otrzymane wyniki wskazują, że jednym z czynników prowadzących do wzrostu liczby owulacji w efekcie podawania łubinu u owiec jest podwyższony poziom FSH w czasie rui. Zwiększona liczba dużych pęcherzyków jajnikowych u owiec otrzymujących suplement sugeruje, że wpływ kondycji owiec na liczbę owulujących pęcherzyków jest przynajmniej częściowo spowodowany przez wzrost poziomu krążącego FSH.