

Effects of Tamoxifen Dose and Nutrition Scheme during Growth on Stimulation of the Reproductive System in Cornish Breed Cocks

Mirosław LISOWSKI and Marek BEDNARCZYK

Accepted January 25, 2005

LISOWSKI M., BEDNARCZYK M. 2005. Effects of tamoxifen dose and nutrition scheme during growth on stimulation of the reproductive system in cornish breed cocks. *Folia biol. (Kraków)* 53: 1-6.

The effects of tamoxifen (TAM) antiestrogen dose as well as protein and energy content in feed on stimulation of the reproductive system development in meat type cocks were studied. The experiment was conducted on males of the Dominant White Cornish chicken breed. The optimum TAM dose for stimulation of reproductive system development was at the level of 5 mg per kg body weight. The effects of TAM administration on the following traits were investigated: body weight, breast muscle thickness, age at sexual maturity, egg fertilization and hatchability. No effect of TAM on breast muscle thickness was found. It was also noted that semen obtained from 11 to 12 week old males of the Dominant White Cornish breed could be used successfully in the insemination of females.

Key words: Meat type cocks, sexual maturity, antiestrogens, tamoxifen.

Mirosław LISOWSKI, Department of Animal Reproduction Biotechnology, Immuno- and Cytogenetics, National Research Institute of Animal Production, Zakrzewo n. Poznań, 62-069 Palędzie, Poland.

E-mail: mlisowski@tlen.pl

Marek BEDNARCZYK, Department of Animal Biotechnology, University of Technology and Agriculture, Mazowiecka 28, 85-225 Bydgoszcz, Poland.

E-mail: bednarczyk@atr.bydgoszcz.pl.

It is known that the main physiological functions of the reproductive organs of male chicken (testes activity and semen production) are affected by many factors: age, photoperiod, season, nutrition, diurnal rhythm, management system (cage vs. floor), inheritance, health status (disease), individual variability and other factors.

It is crucial for males to reach a minimum body weight typical for a given breed, strain or type of performance (DUNN & SHARP 1992), however, no clear relationships resulting from the composition of diet have been observed (ZHANG *et al.* 1999). On the other hand, photoperiod (daylight length) has the greatest effect on sexual maturity in domestic birds. (SAUVEUR & REVIERS, 1988). Numerous studies have confirmed a substantial effect of the lighting programme during rearing on the trait in question. REVIERS (1975) reported that extension of the lighting programme at 8 weeks of age from 8 to 16 hours per day resulted in an acceleration of sexual maturity in meat type cocks. It was reached between 15 and 18 weeks of age, i.e 6 to 8 weeks earlier in comparison with the control group. On the other hand, the application of an extended lighting programme at younger (4wk) or

older (14-20 wk) birds had no effect on the commencement of sexual maturity in meat type cocks. Similarly, constant lighting for 16 h stimulated faster testes growth in comparison with a control under an 8 h lighting programme per day. Intensity and wave length are among other light-triggered factors influencing sexual maturity in birds (OSOL *et al.* 1984).

Recently, studies have been conducted on various antiestrogen compounds used for the control of physiological processes. Clomiphen (CLM) and tamoxifen (TAM), selective estrogens receptor modulators-SERMs, have been used in poultry most frequently. Reports have been published on the effects of these preparations on birds at various ages, for example during the first days of embryo development (ROZENBOIM *et al.* 1990b) or even at 42 to 90 days of age (ROSENSTRAUCH *et al.* 1986). The application of CLM and TAM at various frequencies has been examined, e.g. from every second or third day (ZEMAN *et al.* 1989) until eight weeks (ROSENSTRAUCH *et al.* 1986); Various administration techniques were also studied: *in ovo* (ROZENBOIM *et al.* 1990b), *per os* in gelatine capsules (BEDNARCZYK & DOBALOVA 1990), intra-

muscular injection (LEITNER *et al.* 1996). as well as the use of different preparation doses, e.g. 0.5; 1.0; 5.0; 10.0 or even 25 mg per kg of body weight.

Most frequently light laying type birds were used in studies on acceleration of sexual maturity in males (ROZENBOIM *et al.* 1990b). These birds are characterized by a high level of reproductive traits and have never been selected for negatively correlated meat traits (PINGEL 1990).

An earlier study (LISOWSKI *et al.* 2003) on the influence of TAM on testosterone level in blood and testes tissue development has shown that stimulation of the reproductive system in heavy meat type males of the dominant white Cornish breed selected for high body weight and growth rate is possible. More advanced testes tissue development as a result of a dosage effect of TAM in this experiment wasn't equivalent to earlier sexual maturity and production of better quality semen allowing for successful insemination and embryo development.

The purpose of this study was to evaluate the effect of TAM dose on rearing and sexual maturity in meat type cocks. The effects of the nutrition regimen (feed protein level) on the studied traits were also examined.

Material and Methods

Experiments were carried out on 105 cocks of the Dominant White Cornish breed, strain CE-2, from the Institute of Animal Husbandry, Poultry Research Division, Zakrzewo n. Poznań, Poland. The design of the experiment is presented in Table 1.

Birds were kept in cages under a 24 h continuous lighting programme of 15 lux intensity. In the experiment (see Table 1) two isoenergy diets of different protein content were used. The A and the control (TAM 0) groups were given feed of 19.1% protein content during their first 6 weeks of age and 16.6% protein content from the 7th week on-

Table 1

Design of the experiment

Group		Control (TAM 0)	TAM 1			TAM 5		TAM 10	
Tamoxifen dose (mg/kg body weight)		0	1			5		10	
		Bird's nutrition scheme							
		A	A	B	A	B	A	B	
Rearing period up to 6 weeks of age	ME (MJ/kg)	12.13	12.13	12.13	12.13	12.13	12.13	12.13	
	C. Protein (%)	19.1	19.1	21.0	19.1	21.0	19.1	21.0	

Table 2

Significance of differences among groups in body weight of males

Group	Weeks																															
	6				7				8				9				10				11				12							
	3	4	5	6	7	2	3	4	5	6	7	3	4	5	6	7	3	4	5	6	7	3	4	5	6	7	4	5	6	7	6	7
1	*	*	**	*	*	*	**	*	*	*	**	*	**	**	*	**	*	**	*	**	**	*	**	*	**	*	**	*	**	*	**	*
2			*	—			**	**	**	**	**	**	**	**	*	**	*	*	*	*	**	*	*	*	*	*	*	*	*	*	*	
3	—	*		—			—	*			—	*	—																			
4		—		—			—	—			—	—	—																			
5			—	**			—	**			—	**			—	**			—	**	**	*	—	*	—	*	—	*	—	*	—	

1-group TAM 1A, 2-group TAM 1B, 3-group TAM 5A, 4-group TAM 5B, 5-group TAM 10A, 6-group TAM 10B, 7- TAM 0 (control group).

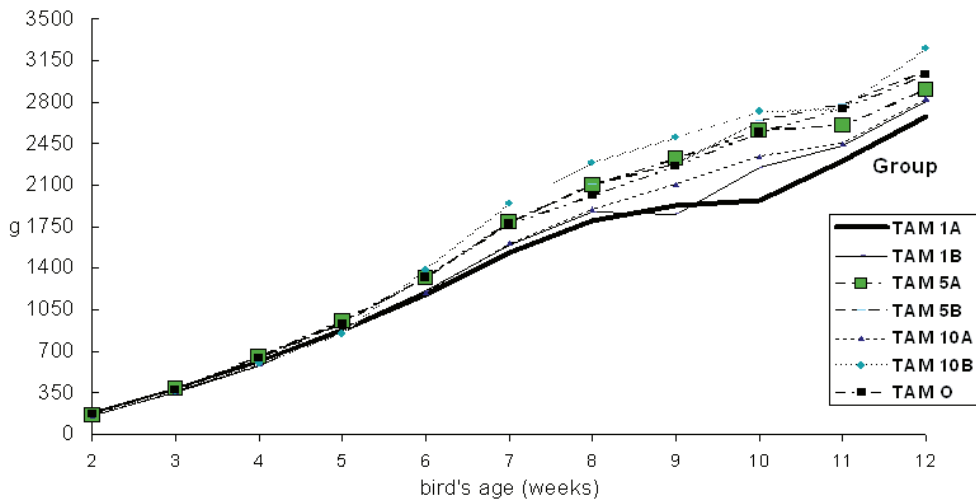


Fig. 1. Body weight of males treated with different dose of tamoxifen.

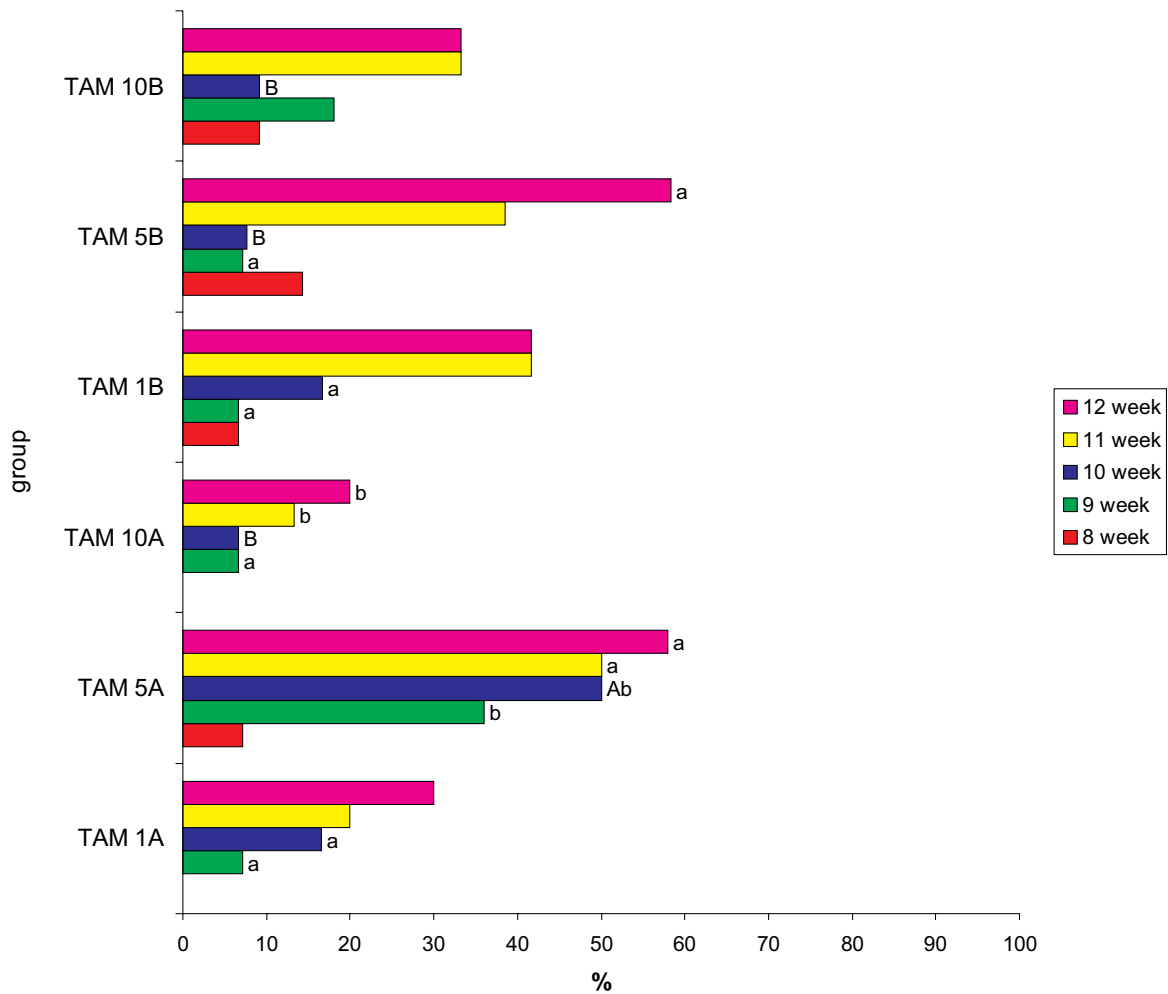


Fig. 2. Percentage of males producing semen at various age. Note: means signed by the different letters in columns differ significantly: a – $P \leq 0.05$; A – $P \leq 0.01$.

Table 3

Reproduction results of males treated with different dose of tamoxifen. Note: means signed by the different letters in columns differ significantly: a – $P \leq 0.05$; A – $P \leq 0.01$. (within a weeks)

Group	Number of eggs set	Fertility (%)	Percentage of chicks:	
			In ratio to eggs set	In ratio to fertile eggs
11 week				
TAM 1 (TAM 1A + TAM 1B)	18	16.6 Aa	11.1 Aa	66.6
TAM 5A	15	53.3 b	46.6 b	87.5
TAM 5B	16	43.8	31.3	71.4
TAM 10 (TAM 10A + TAM 10B)	22	77.2 B	63.6 B	82.3
12 week				
TAM 1 (TAM 1A + TAM 1B)	42	54.8 a	50.0 a	91.3
TAM 5A	42	76.2 b	76.2 Ab	100.0 a
TAM 5B	38	60.5	47.4 Ba	78.3 b
TAM 10 (TAM 10A + TAM 10B)	43	74.4 b	74.4 b	100.0 a

wards. Group B was fed a diet of 21% protein content for the first 6 weeks of age and 19.1% protein from the 7th week onwards. Tamoxifen (SERVA) was administered to birds *per os* in the form of dihydrochloride containing around 65% pure substance. Doses of tamoxifen of 1, 5 and 10 mg/kg body weight were used.

Tamoxifen doses were administrated every 48 h from two up to 12 weeks of age and dose size was estimated once a week on the basis of current body weight and the anticipated body weight at the end of the given week. All cocks were assessed taking into account the following traits: body weight (determined once a week), and breast muscle thickness by using a needle probe (BOCHNO *et al.* 1988) at 42 days of age.

From 8 weeks onwards the cocks were massaged abdominal massage to get the birds accustomed to semen secretion. In this way an individual assessment of sexual maturity of each cock (the lack or presence of semen on a zero-one scale) could be estimated. Semen quality was also evaluated *in vivo* after insemination of 36 hens from pure strain CE-2. The percent of egg fertilization, hatchability from the egg set and from the eggs fertilized were calculated.

The data pertaining to body weight and breast muscle thickness were subjected to two-factorial analysis of variance with interaction. The significance of differences among groups of birds was verified by using Student's *t*-test (OKTABA 1980). The significance of differences among percentage values concerning the number of cocks which se-

creted semen, egg fertilization, hatchability of the egg set and fertilization was assessed by the use of the χ^2 test (LUNGHIN & LANDY 1976).

Results and Discussion

Cock body weight and differences among experimental groups are presented in Figure 1 and Table 2. Between the second and fifth week of age no statistically significant effect of TAM dose (mg/kg b.w.) on cock body weight was noted in TAM 1 (groups 1A and 1B); TAM 5 (groups 5A and 5B); and TAM 10 (groups 10A and 10B). Over this growing period the birds gained from 156 to 892 g (group TAM 1B) or from 158 to 955 g (group TAM 5A).

Beginning from the sixth week of age, however, statistically higher body weight (b.w.) was observed in birds fed on a diet containing higher doses of TAM (5 and 10 mg/kg b.w.) in comparison with birds given the lowest TAM dose (1 mg/kg b.w.) which resulted in body weight differences in birds from TAM groups 5 and 10. For example at 12 weeks of age their body weight ranged from 2,831 to 3,250 g, whereas those from TAM group 1 from 2,684 to 2,812 g on average. In this study no significant interaction between the two experimental factors (TAM, protein content) on cock body weight was observed. It was also found that none of the examined experimental factors significantly influenced breast muscle thickness (data are not given).

In meat type birds, the body weight is the principal selection criterion since the level of this feature in a breeding stock determines its formation in commercial stock (broilers) significantly. The differentiated effect of TAM on bird body weight has been reported in the literature since some authors have not observed any significant effect of antiestrogens on this trait (LEITNER *et al.* 1996). However, others noted either positive (ZEMAN *et al.* 1989; ROSENSTRAUCH *et al.* 1986) or negative effects (ROZENBOIM *et al.* 1990a) on bird body weight.

It seems that these different experimental findings may be caused by SERMs properties which have antagonistic effects on estrogens in certain tissues or imitate those hormones in others (PATTERSON 1981). ROBINZON *et al.* (1984) demonstrated that in males of light laying strains, injection of synthetic estrogens resulted in four times higher blood testosterone content and substantial obesity at 270 days of age. The injection of testosterone in the same breed of males reduced their obesity (SNAPIR *et al.* 1983) whereas in females, similarly as in meat type chicken of both sexes, testosterone had no effect on obesity level (ROBINZON *et al.* 1987). IRISAWA *et al.* (1990) conducted a study on mice and found that body weight reduction under the effect of SERMs was dependent on the age of the treated males and decreased with animal age.

Figure 2 presents percentage data of cocks that produced semen at various ages. The first semen was obtained from birds at eight weeks of age. The highest percentage of cocks producing semen over the period from the 8th until the 12th week of age was noted in group 5A. The birds of the control group – TAM 0 are not shown in Figure 2 since none had produced semen. At the 12th week of age, sixty percent of cocks produced semen. ROZENBOIM *et al.* (1990b) reported that seventy percent of White Leghorn males produced semen during their 10th week of age. This difference was probably caused by the age of sexual maturity, i. e. 20 week in light birds (White Leghorn) and about 24 weeks in heavy birds (Dominant White Cornish).

In Table 3 the hatchability of eggs laid by hens inseminated with semen from 11 and 12 week old cocks is presented. Because of the results obtained earlier (% of cocks that delivered semen), and the relatively low quantity of collected semen, the experimental groups TAM 1A and TAM 1B as well as TAM 10A and TAM 10B are presented together. The highest fertilization (77.2%) was noted in eggs from hens inseminated with semen produced by 11 week old cocks of the TAM 10 group, whereas the lowest (16.6%) was from the TAM 1 group. Hatchability from egg set was found highest (63.6%) in group TAM 10 while

lowest (11.1%) in group TAM 1. On the other hand, the highest hatchability from the fertilized eggs was observed in group 5A (87.5%) and the lowest in group TAM 1 (66.6%). If semen collected from cocks at 12 weeks of age was used in insemination, the best fertility was noted in group TAM 5A (76.2%) and the poorest in group TAM 1 (54.8%). The following hatchability levels from the fertilized eggs were found: 100% in groups TAM 5A and TAM 10; 91.3% in group TAM 1 and 78.3% in group TAM 5B.

Results of this study concerning fertilization and hatchability confirm the previous study in turkey and Muskovy Ducks (ROZENBOIM *et al.* 1990a) where the optimum dose of tamoxifen for the acceleration of sexual maturity in poultry males was 5 mg per kg body weight. The comparison of groups in which males obtained the same dose of tamoxifen but in different feed showed the beneficial influence of the reduction of protein and energy content on sexual maturity. The positive influence of protein reduction in the diet on semen production by males of meat type has been described by ZHANG *et al.* (1999).

The experimental findings of this study indicate that it is possible to obtain and successfully use the semen of 11 and 12 week old White Dominant Cornish cocks in insemination. The optimum dose of tamoxifen for stimulation of the reproductive organs in these birds from the 2nd to 11th week of age was found to be 5 mg per kg body weight. This indicates a possibility for the development of a breeding programme in broiler breeders where the best males could be used in reproduction as early as a few weeks, just after meatiness performance evaluation. In this way the length of time between subsequent generations is decreased and breeding effect is increased.

References

- BEDNARCZYK M., DOBALOVA M. 1990. Influence of antiestrogens – tamoxifen on some sex traits in cocks. Symp. PTZ, Szczecin: 5. (In Polish).
- BOCHNO R., LEWCZUK A., JANISZEWSKA M., MAZANOWSKI A., WAWRO K. 1988. Use multiple regression equations for estimation of muscle and FAT weight of ducks. Acta Acad. Agricult. Tech. Olst. 31: 197-203.
- DUNN I. C., SHARP P. J. 1992. The effect of photoperiodic history on egg laying in dwarf broiler hens. Poultry Sci. 71: 2090-2098.
- IRISAWA S., IGUCHI T., TAKASUGI N. 1990. Critical period induction by tamoxifen of genital organ abnormalities in male mice. Zool. Sci. 7: 541-545.
- LAUGHLIN K. F., LUNDY H. 1976. The influence of sample size on the choice of method and interpretation of incubation experiments. Brit. Poultry Sci. 17: 53-57.
- LEITNER G., LANDSMAN T., BLUM O., ZALTSMANN N., HELLER E. D. 1996. Effects of gonadal steroids and their antagonists on the humoral immune response of immuneselected broiler chicks. Poultry Sci. 75: 1373-1382.

- LISOWSKI M., BEDNARCZYK M., KŁOSOWSKA D., ELMINOWSKA-WENDA G., MAĆKOWIAK P., NOGOWSKI L., NOWAK K. 2003. The influence of tamoxifen on testosterone concentration in blood and on development of cocks testis tissue. *Med. Wet.* **58**: 344-347.
- OKTABA W. (1980). *Statistical Methods in Experimentation*. PWN, Warszawa. (In Polish).
- OSOL G., FOSS C., CARREW L. B. 1984. Effects of selected wavelengths of light reproductive development in cockerels. *Gen. Comp. Endocrinol.* **55**: 227-232.
- PATTERSON J. S. 1981. Clinical aspects and development of antiestrogen therapy: A review of the endocrine effects of tamoxifen in animals and men. *J. Endocrinol.* **89**: 67-75.
- PINGEL H. 1990. *Poultry Breeding and Genetics*. R. D. Crawford ed., Elsevier, Amsterdam, The Netherlands. Pp. 691-704, d 771-780.
- REVIERS M. 1975. Le développement testiculaire du coq. *Ann. Biol. Anim. Biochem. Biophys.* **15**: 633-641.
- ROBINZON B., NOV Z., SAYAG N., SNAPIR N. 1984. Early hormonal treatments and adiposity and sexual activity of the mature cock. Abstracts of the Congress of Comparative Physiology and Biochemistry, Liege, p. D31.
- ROBINZON B., ROZENBOIM I., SAYAG N., GVARVAHU G., WAXLER J., SNAPIR N. 1987. Testosterone and adiposity in chicken: The effect of breed and sex. *Pharmacol. Biochem. Behav.* **19**: 617-624.
- ROSENSTRAUCH A., DEGEN A., BEDRAK E., FRIEDLANDER M. 1986. Improvement of fertility in Cornish roosters by the use of clomiphene citrate. Proc. 7th Europ. Poultry Conf.: 1025-1028.
- ROZENBOIM I., MELTZER A., ROBINZON B., GAHALY S., AMON E., SNAPIR N. 1990a. The effect of tamoxifen on sexual puberty of turkey toms and muscovy drakes. *Poultry Sci.* **69**: 176-178.
- ROZENBOIM I., ROBINZON B., AMON E., SNAPIR N. 1990b. The effect of tamoxifen on semen fertilization capacity in White Leghorn male chicks. *Poultry Sci.* **69**: 1220-1222.
- SAUVEUR B., REVIERS M. 1988. *Reproduction des Volailles et Production d'Oeufs*. INRA, Paris.
- SNAPIR N., ROBINZON B., SHALITA B. 1983. The involvement of gonads and gonadal steroids in the regulation of food intake, body weight and adiposity in the White Leghorn cock. *Pharmacol. Biochem. Behav.* **19**: 617-624.
- ZEMAN M., KOSUTZKY J., UHRIN V. 1989. Effect of antiestrogen clomiphene citrate on sexual development and plasma testosterone levels in cocks. *Vedeck Prace Vh.* **24**: 64-74.
- ZHANG X., BENY W. D., MCDANIEL G. R., ROLAND A., LIU P., CALVERT C., WILHITE R. 1999. Body weight and semen production of broiler breeder males as influenced by crude protein broiler hens. *Poultry Sci.* **78**: 190-196.