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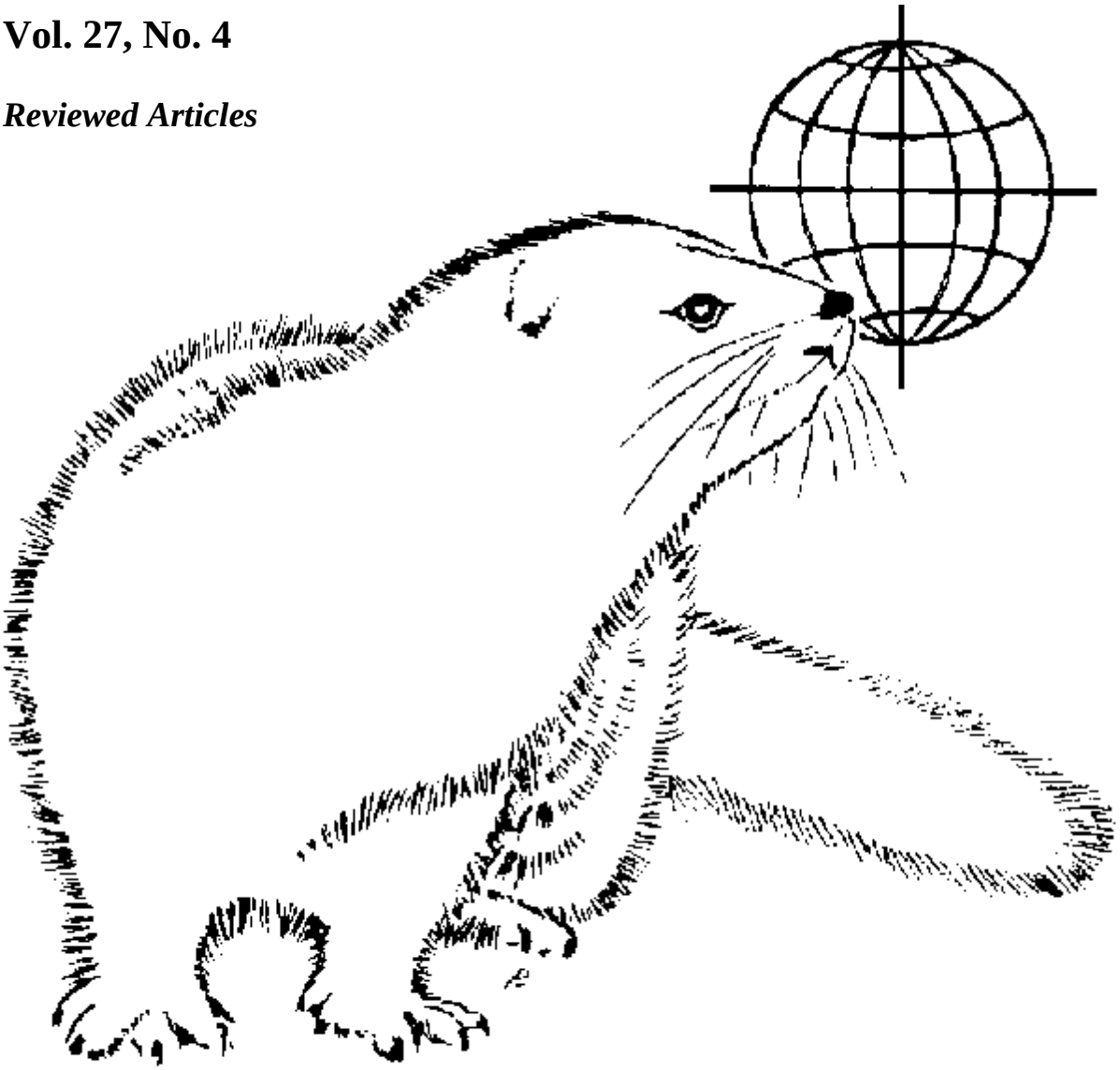
Reviewed Articles



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SCIENTIFUR
ISSN 0105-2403
Vol. 27, No. 4
Reviewed Scientific Articles

1. **Contents** **61**

2. **Notes** **63**

3. **A proved technique for preimplantation embryos recovery in *Myocastor* Coypus (Coypu).** *A.E. Felipe, M.T. Teruel, J.A. Cabodevila, S.S. Callejas* **65**

4. **Apparent ileal digestibility of fat and fatty acids in polar foxes.** *K. Burlikowska, R. Szymeczko, J. Błaszyk* **71**

5. **Why certain silver fox genotypes develop red hairs in their coat.** *D.I. Våge, E.B. Stavdal1, J.Beheim, H. Klungland* **79**

6. **Management problems and tools for strictly synchronised animal production systems exemplified by mink production.** *S.H. Møller, J.T. Sørensen* **85**

Notes from the Group of Editors

This version of Scientifur, which is the fourth issue of volume 27, contains three reviewed articles. More reviewed articles are on their way, but the reviewing procedure takes some time. We do, however, hope to be able to publish a considerably larger number of reviewed articles in the next volume of Scientifur.

As always, we invite our readers to submit proceedings from congresses and seminars with relation to fur animal production. We also invite you to submit short communications, abstracts and letters on fur animal production, and in particular we ask you to send us articles for reviewing.

On behalf of the
Group of Editors

Birthe Damgaard

A proved technique for preimplantation embryos recovery in *Myocastor Coypus* (Coypu)

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Abstract
The aim of this work was to determine the applicability and viability of collection techniques for preimplantation embryos of *Myocastor coypus*. Sexually mature animals were used. A colpocytological follow-up was made to the females and once estrus was determined they were mated. Copulation was confirmed by the presence of spermatozoids in the colpocytological sample 1 hour after copulation. Females were sacrificed from day 2 to day 8 after copulation. To apply the collection technique the reproductive tract was sectored into oviducts and hemiuteri. As it was impossible to use the standard washing procedure, a 30G needle was inserted until the ampullary-isthmic junction performing the washing towards the uterotubal junction. Embryos were washed down from the uterus inversely, introducing the needle at the cervical extreme of the hemiuteri. The use of washing techniques adapted to the anatomy of the *Myocastor coypus* allowed us to obtain embryos.

Keywords: embryo collection, Myocastor coypus, preimplantation embryos

Introduction

The *Myocastor coypus* (coypu) is a neo-tropical mammal exploited as a zootechnical species for a century (García Mata, 1985). There are works that consider different aspects of the species comprising studies on its relationship with the environment and reproductive behaviour (Brown, 1975; Willner et al., 1979), on morphology of the reproductive apparatus (Afasaniev et al., 1970; Felipe et al., 1998; Felipe et al., 1999), and on its physiology (Bura et al., 1985; Cotea et al., 1986; Jakubicka et al., 1989; Bura, 1992 a and b; Iudica & Alberio, 1995).

Considering that the coypu is a species of easy breeding in captivity its possible to do research similar to that perform with other laboratory mammals and domestic animals. Among the procedures applied to the improvement in animal production those destined to the achievement of a better reproductive efficiency are outstanding (Hafez, 1993). This requires a basic knowledge of aspects related to the reproductive function, such as the anatomicohistological components, the physiology, and the processes that occur during the development. For the last two decades the use of biotechnology has been of great importance in the advancement of agricultural production. One main beneficiary of the advancements has been animal breeding, mainly with the use of new technologies in animal reproduction (Jalkanen, 1993; Mapletoft, 1995). Nevertheless, their application and success, particularly of embryotechnologies, depend partly on having knowledge of preimplantation development and of the characteristics of the reproductive cycle of the species (Montes et al., 1983; Heyman, 1988; Seidel, 1991).

Actually, there is no systematised information on the biology of the coypu's development, except general references when mentioning other hystricomorph rodents (Roberts & Perry, 1974). Therefore, it is necessary to amplify the knowledge of aspects related to the reproductive physiology and to the embryonic development of the *Myocastor coypus* and for that, techniques adjusted to that species are required. Thus, the purpose of this work was to determine the applicability and viability of flushing techniques for the collection of preimplantation embryos of *Myocastor coypus*.

Materials and methods

Animals

One male and twelve virgin and sexually mature females (Groenland mutation) destined to zafra were used. The male was 8 month-old and weighed 5.8 kg. The age of the females was of 8.7 ± 1.1 months (means ± SEM.) with a weight of 4.6 ± 0.6 kg. (means ± SEM.). Groups of four females were kept in 15-m2 partially roofed corrals with concrete floor in an experimental place. They received 300g/day of balanced food and water was administered ad-libitum with a nipple-type trough at both sides of the corrals.

Mating program

The mating program implied a daily colpocytological follow-up by the routine technique (Felipe et al., 2001). Copulation and ejaculation were determined by behaviour observation and by spermatozoids detection in the vaginal cavity one hour after mating respectively. The confirmation of mating by colpocytology allowed us to assume the day of its occurrence as time 0 of gestation and to establish the moment for the collection of the embryos in post-coital days.

Exteriorisation of the reproductive apparatus

The technique of extraction of oviducts and uteri was designed considering the protocols by Barros & Yanagimachi (1972) for hamsters and by Barros (1992) for mice and guinea pigs. The complete reproductive apparatus was extracted by medial laparotomy through white line. Once the abdominopelvic cavity was approached, the cut of the pubic symphysis and the separation of the vagina sectioning the surrounding tissues and pulling the bladder upwards were performed. Holding the vagina with forceps, the cervical area of the uterus was lifted and each horn was separated cutting the mesometrium at its uterine insertion to guarantee a complete remotion of the adipose tissue. The cut was made until the uterotubal junction. Next, holding the mesovarium with blunt-tip forceps, the adipose tissue surrounding the oviducts and the ovary was partially cut, carefully separating them from the perirenal tissue. The reproductive apparatus thus extracted was put into a 10-cm diameter Petri dish with physiological saline solution at 37°C and the apparatus was sectored. To that end, the vagina was separated under stereomicroscope by a transverse cut at the caudal point of the cervix. Then, the cervix was separated by a cut at cephalic level of the vaginal fornix. After, the uterine horns and body (hemiuteri) were divided by a perpendicular cut of the intercornual ligament and of their area of external fusion. Subsequently, the oviduct-ovary segments were separated from each hemiuterus by sectioning at 5 mm at the caudal point of the uterotubal junction. The segments thus obtained were put in Petri dishes with physiological saline solution at 37°C.

Embryo recovery

The flushing of organs for embryos collection was made at 48-h intervals from day 2 until day 8 post-coitus.

Flushing of the ovarian surface

Due to the possibility that the newly ovulated oocytes were in the ovarian surface because of the adherence of the cumulus, the oviduct-ovary segments were put in capsules and the ovaries were sprayed three times with washing liquid, keeping the fimbrias folded. Ovaries were not separated from the oviducts due to the closeness of their union and to avoid the dragging of the oocytes by the scalpel during the handling. The ovaries were analysed with a stereomicroscope for identifying and counting of ovulation sites and corpora lutea.

Flushing of the oviducts

The flushing of the oviducts was made under stereoscopic microscope. Physiological saline solution at 37° was perfused in the lumen of each segment using 1-ml syringes with blunt-tip 30G needles.

The procedures used in other species (Selwood, 1980; Ratky & Brussow, 1995; Palma, 2001) was not applicable for oviducts of *Myocastor coypus*. When the needle was introduced through the

infundibulum until the middle part of the ampulla (about 5 mm) and this was holded with forceps, the liquid injected re-flowed after expanding the segment (Figure 1a). Due to those difficulties, the washing procedure was modified. The infundibulum was located under the microscope and the fimbrias were unfolded and displaced with blunt-tip forceps to clear the opening of the ampulla. A 30G needle was introduced once the ampulla was found until the ampullary-isthmic junction, approximately at 1 cm from the infundibular opening. Once this was achieved, the curvature present in the same junction was gently straightened and holding the needle with forceps the washing liquid was gently injected three times, without resistance (Figure 1b).

Flushing of the hemiuteri

The needle was introduced into each hemiuterus through the cephalic extreme of the horns, in the area of section of the uterotubal junction, finding resistance to the injection of the washing solution. Considering this difficulty the washing was performed in each hemiuterus (right and left) in the opposite direction to that initially followed. A 30G needle was introduced about 5 mm through the cervical extreme of each segment and, holding the needle and the cervical wall with forceps, physiological saline solution at 37° was injected three times, observing its way out without difficulty through the cranial sector (Figure 1c).

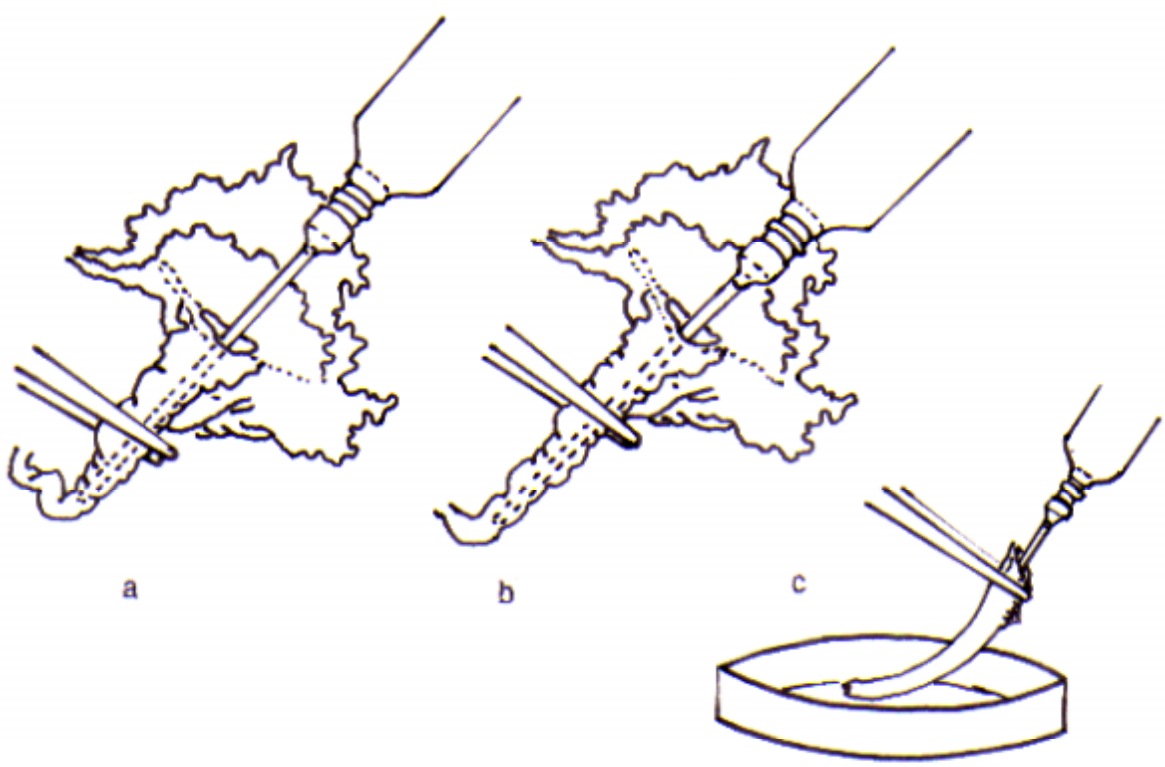

Figure 1. Flushing techniques: a) insertion of the washing needle into the ampulla; b) insertion of the washing needle slightly exceeding the ampullary-isthmic junction; c) flushing of a hemiuterus towards caudocephalic direction

Complementary flushings
In addition to the three flushings indicated, two other complementary flushings were made to the oviduct and hemiuterus segments, to guarantee their complete washing.

Search of embryos
The search for embryos in the washing solution was made in dishes divided into squares, square by square three times per washing to guarantee a complete observation of the dish area. A stereoscopic microscope with two optical magnifications (10x and 25x), was used. The final magnification used was 10x. The morphological evaluation of the embryos was made according to Palma (2001).

Statistical analysis
Analysis of variance was used to compare mean values of the number of embryos collected at each side of the reproductive tract. The results were expressed by means ± standard error.

Results
With the technique used, seventy one embryos at different stages of development were collected. The totality of them was considered morphologically normal. The stages of development included zygotes, first stages of segmentation, non-compacted morulae, compacted morulae, and blastocysts (Table 1). The number of embryos collected was greater than the copora lutea counted (71 recovered embryos / 55 corpora lutea counted).

Embryos at the zygote to non-compacted morulae stages were collected from the oviducts and the uterotubal junctions. After performing the flushing of uteri, non-compacted morulae, compacted morulae and blastocysts at different degrees of development were obtained (Table 1).

Table 1. Results of the embryo recovery in females of *Myocastor coypus* from day 2 to 8 post-coitus.

Code female	Embryos collected (n) and development stage	Nº of oocytes and embryos		Nº of days from copulation	Collection site	
		Right side	Left side		Oviduct	Uterus
1/1073/2	(6) zygotes	3	3	2	X	
2/1052/2	(5) zygotes	3	2	2	X	
1323/6	(7) zygotes	3	4	2	X	
1/sp/4	(9) 2 cells	6	3	4	X	
4/v/4	(6) 2 cells	1	5	4	X	
1/1212/4	(1) zygote + (7) 2 to 3 cells	5	3	4	X	
4/1244/5	(4) 7 to 9 cells + (1) NCM	3	2	6	X	
1/1074/1	(2) 8 and 9 cells +(5) NCM	4	3	6	X	
2/1101/1	(1) NCM	1		6		X
1297/6	(5) blastocysts	3	2	8		X
4/1048/1	(4) blastocysts	2	2	8		X
9/1111/3	(3) CM + (5) blastocysts	4	4	8		X

References: NCM, non - compacted morulae; CM, compacted morulae.

The means of embryos collected per female was of 5.9 ± 2.5, with a range of 1 to 9. The mean number of embryos collected from the right and left sides was of 3.2 ± 1.5 and 2.8 ± 1.3 respectively without significant differences (P>0.5).

Discussion
One method to improve reproductive efficiency is the embryo transfer that requires technical procedures to guarantee the collection of the specimens. The results of this work show that the collection of preimplantation embryos for a hystricomorph rodent like *Myocastor coypus* can be made following their location pattern in the maternal reproductive tract and that is similar to other species, that is from oviducts at the first development stages or else from the uterus at more advanced stages.

Collection methods based on reproductive routes alone and surgical and non-surgical procedures in living animals have been used for embryos of different species (Hunter et al., 1969; Brackett et al., 1971; Hendrickx, 1971; Eddy et al., 1975; Hsu, 1979; Selwood, 1980; Enders & Schlafke, 1981; Selwood, 1982; Ratky & Brussow, 1995; Pereira et

al., 1998). Though the non-surgical way is preferred considering its advantages, however in species like the *Myocastor coypus* where nowadays the first attempts are being made in the collection, the use of reproductive routes alone is chosen, taking into account that preliminary studies on its reproductive histomorphology are currently being made (Felipe et al., 1998).

The techniques of embryos collection have been applied in carnivorous fur animal species too. Lindeberg et al. (2002) highlight that the domestic ferret (*Mustela putorius furo*) was the first species of carnivorous in which the embryo transfer technology was successfully applied. Other references to studies in carnivores are the works of Valtonen et al. (1985) and Farstad et al. (1995), who describe the fertilization and the early embryonic development in the blue fox (*Alopex lagopus*). Maksimovskii et al. (1994) analyzed the preimplantational development of *Mustela erminea* and *Mustela mink*, making a characterization using light microscopy. The surgical transfer of embryos has also been developed in the silver fox (*Vulpes vulpes*) (Jalkanen and Lindeberg, 1998).

The collection technique employed in this work allowed us not only to successfully perform the collection of embryos from the oviduct and from the uterus, but also to start establishing the development table corresponding to preimplantation embryos of *Myocastor coypus* (Felipe et al., 2002).

Future works will accurately define in which specific segment of the maternal reproductive tract are the embryos at their different development stages. In his turn, it will be necessary to develop surgical collection methods in animals in vivo and to try the development of non-surgical techniques.

Acknowledgements

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References

Afasaniev, V.A., Pereldik, N.S., Kladovscikov, V.F., Kuznetov, G.A. & Hronopulo, N.P., 1970. Cresterea animalelar de blana, Ed. Ceres Press, Bucuresti.

Barros, C., 1992. Biología del desarrollo de mamíferos y criopreservación de embriones. C. Barros (ed.), Santiago, Chile, pp. 6 - 9 and 19 - 21.

Barros, C. & Yanagimachi, R., 1972. Polispermy-preventing mechanisms in the golden-hamster egg. Journal of Experimental Zoology, 180, 251 - 266.

Brackett, B.G., Killen, D.E. & Pearce, M.D., 1971. Cleavage of rabbit ova inseminated in vitro after removal of follicular cells and zonae pellucidae. Fertility and Sterility, 22, 816 - 828.

Brown, L.N., 1975. Ecological relationship and breeding biology of the nutria (*Myocastor coypus*) in the Tampa Florida Area. Journal of Mammalogy, 56, 928 - 930.

Bura, M., 1992 a. Studiu asupra indicelui de fecunditate la nutrie (*Myocastor coypus Molina*). Lucrari Stiin Zootechnie, 26, 141 - 145.

Bura, M., 1992 b. Studiu asupra ratei conceptiei la femele primipare si lutipare de nutrie (*Myocastor coypus Molina*). Lucrari Stiin Zootechnie, 26, 147 - 151.

Bura, M., Gluhovschi, N. & Radulescu, V., 1985. Incercári de combatere a infecundit tii determinate de anestru prelungit la femelele de nutria. Revista Cresterea Animalelor, 1, 52 - 55.

Cotea, C., Cotofan, V. & Hritcu, V., 1986. Histology and histochemistry of accesory sex glands of the male coypu, *Myocastor coypus*. Seminarul tehnologia, Animal Ferma, 10, 527 - 530.

Eddy, C.A., García, R.G., Kraemer, D.C. & Pauerstein, C.J., 1975. Detailed time course of ovum transport in the rhesus monkey (*Macaca mulatta*). Biology of Reproduction, 13, 363 - 369.

Enders, A.C. & Schlafke, S., 1981. Differentiation of the blastocyst of the Rhesus monkey. American Journal of Anatomy, 162, 1 - 21.

Farstad, W., Hyttel, P., Grondahl, C., Mondain-Monval, M. & Smith, A.J., 1995. Fertilization and early embryonic development in the blue fox (*Alopex lagopus*). Molecular Reproduction and Development, 36, 331 - 337.

Felipe, A., Cabodevila, J. & Callejas, S., 1998. Anatomicohistological characteristics of female genital tubular organs in the south american nutria (*Myocastor coypus*). Anatomia, Histologia, Embryologia, 27, 245 - 250.

Felipe, A., Cabodevila, J. & Callejas, S., 1999. Anatomicohistological characteristics of the ovary of the coypu (*Myocastor coypus*). Anatomia, Histologia, Embryologia, 28, 89 - 95.

Felipe, A., Teruel, M., Cabodevila, J. & Callejas, S., 2001. Controlled mating in *Myocastor coypus* (coypo). Scientifur, 25, 99 -103.

Felipe, A., Teruel, M., Cabodevila, J. & Callejas, S., 2002. Preimplantational timetable of embryonal development of *Myocastor coypus* (coypu). Reproduction, Nutrition and Development, 42, 15 - 24.

García Mata, R., 1985. Producción en cautividad de pelíferos. In: Ministerio de Agricultura (ed.), Primeras Jornadas de Producción Nutriera y otros pelíferos, Santa Fe, 1985, 20 - 41.

Hafez, E.S.E., 1993. Foliculogénesis, maduración del óvulo y ovulación. In: Hafez, E.S.E. (ed.), Reproducción e inseminación artificial en animales, (México, Interamericana), 142 - 181.

Hendrickx, A.G., 1971. Embryology of the baboon. University of Chicago Press Chicago, Chicago, USA.

Heyman, Y., 1988. Moment de la transplantation et succes de la gestation chez les mammiferes. Reproduction, Nutrition and Development, 28, 1773 - 1780.

Hsu,Y.CH., 1979. In vitro development of individually cultured whole mouse embryos from blastocyst to early somite stage. Developmental Biology, 68, 453 - 461.

Hunter, R.R.F., Hunt, D.M. & Chang, M.C., 1969. Temporal and cytological aspects of fertilization and early development in the guinea pig, *Cavia porcellus*. Anatomical Record, 165, 411- 429.

Iudica, C.E. & Alberio, R.H., 1995. Preliminary study of the sexual cycle of the South-American nutria (*Myocastor coypus*) by the method of exfoliative colpocytology. Scientifur, 19, 33 - 42.

Jakubicka, I., Barta, M., Nitray, J. & Szeleszezukova, O., 1989. Fluctuations in the progesterone concentration of the peripheral blood of nutria during pregnancy. Veterinary Medicine, 34, 251- 256.

Jalkanen, L., 1993. Prospects of embryotechnology in fox research. Scientifur, 17, 287 - 292.

Jalkanen, L. & Lindeberg, H., 1998. Successful embryo transfer in the silver fox (*Vulpes vulpes*). Animal Reproduction Science, 54, 139 - 147.

Lindeberg, H., Amstislavsky, S., Jarvinen, M., Aalto, J. & Valtonen, M. 2002. Surgical transfer of in vivo produced farmed European polecat (*Mustela putorius*) embryos. Theriogenology, 57, 2167 - 2177.

Maksimovskii, L.F., Amstislavskii, S.I., Golubitsa, A.N., Zhelezova, A.I., Ternovskaia, I.G. & Ternovskii, D.V., 1994. The preimpantation embryonic development of 2 species of mammals from the family *Mustelidae* (*Mustela erminea* and *Mustela vison*). Ontogenez, 25, 45 - 51.

Mapletoft, R.J., 1995. The use of embryo transfer technology in the 21st century. In: C.H. Del Campo, M. Patiño and M. Del Campo, (eds), Relaciones embriomaternas y biotecnologías reproductivas (Univ. Austral de Chile Press, Valdivia), 80 - 90.

Montes, G.S., Luque, E.H., Alonso, C.R. & Ponce, B.V., 1983. Obtención y transferencia de embriones en animales de interés zootécnico. Gaceta Veterinaria de Buenos Aires, 361, 453 - 462.

Palma, G.A. 2001. Biotecnología de la reproducción. Ed. Instituto Nacional de Tecnología Agropecuaria, R. Argentina.

Pereira, R.J., Sohnrey, B. & Holtz, W., 1998. Nonsurgical embryo collection in goats treated with prostaglandin F2alpha and oxytocin. Journal of Animal Sciences, 76, 360 - 363.

Ratky, J. & Brussow, K. , 1995. Successful embryo recovery from swine by a minimal invasive technique. Acta Veterinaria Hungara, 43, 371 - 375.

Roberts, C.M. & Perry, J.S., 1974. Hystricomorph embryology. Symposium of Zoological Society of London, 34, 333 - 360.

Seidel, G.E., 1991. Embryo transfer: the next 100 years. Theriogenology, 35, 171 -180.

Selwood, L.A., 1980. Timetable of embryonic development of the dasyurid marsupial *Antechinus stuartii* (Macleay). Australian Journal of Zoology, 28, 649 - 668.

Selwood, L.A., 1982. The management of breeding colonies of the dasyurid marsupial *Antechinus stuartii* (Macleay) to obtain embryonic material and pouch young. In: D. Evans (ed.), The management of australian mammals in captivity, (Melbourne, Zoological Board of Victoria), 31 - 37.

Valtonen, M., King, W.A., Gustavsson, I. & Makinen, A., 1985. Embryonic development in the blue fox. Nordvegian Veterinary Medicine, 37, 243 - 248.

Willner, G.R., Chapman, J.A. & Pursley, D., 1979. Reproduction, physiological responses, food habits, and abundance of nutria on Maryland marshes. Wildlife Monograph, Maryland, 65.

Apparent ileal digestibility of fat and fatty acids in polar foxes

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Abstract
The purpose of this study was to evaluate apparent digestibility of fat and fatty acids in "end to end" ileorectal anastomosed four blue foxes fed diets used in the nutrition of parent stock of polar foxes during the winter furring period. The experimental diets (A and B) were composed of different animal offals and cereals. In diet A the main source of fat were poultry offals (55%), while diet B contained mainly fat from flounder (34.5%) and pork offals (32.1%). The content of fat in the evaluated diets was 23.3%. The higher ($P<0.05$) digestibility of fat (98.2%), TFA (98.2%), SFA (97.6%) and MUFA (98.9%) were observed in B diet containing fish and pork offals. Digestibility coefficient of PUFA (C18:2 and C18:3) did not statistically differ between the tested diets.

Introduction
In the nutrition of carnivorous fur bearing animals, fat constitutes the cheapest and the most efficient source of energy (NRC, 1982; Hansen, 1992). Diets for those animals contain fish, poultry, pork, beef and utilisation fats (mixture of various fats) and plant fats, mainly soybean and sunflower fats (Pölönen et al., 2000).

The nutritive value of feed depends not only on the content of nutrients but also their digestibility and availability. So far mostly a fecal method of measuring apparent digestibility of respective nutrients in carnivorous fur bearing animals feeds, including fat and fatty acids, has been traditionally used. Apparent fat digestibility defined for the entire gastrointestinal tract, based on the analysis of the composition of feces excreted, does not cover endogenous fatty acids secreted from the small intestine and their production by microflora in the large intestine (Ajuyah et al., 1996). Besides biohydrogenation of unsaturated fatty acids recorded in colon and ceacum can overestimate the digestibility of respective fatty acids. The accuracy of the method determining total fat digestibility in piglets can be also questioned due to a negative apparent fat digestibility in the lower gut of these animals (Endres et al., 1988).

A more precise method applied to define apparent digestibility of nutrients, including fat and fatty acids, is therefore the ileal method, according to which digestibility is calculated as remainder left after subtraction of the content of the nutrient in feed from that in digesta passing through the final segment of the small intestine. In dogs with cannulas inserted into the final section of small intestine there were estimated also an effect of supplementing various animal offals, various kinds of fibre and plant protein on apparent ileal digestibility of fat (Muir et al., 1996; Murray et al., 1997; Cole et al., 1999; Bednar et al., 2000; Clapper et al., 2001; Hill et al., 2001). However no reports have been noted in the literature available on determining apparent ileal digestibility of fat and fatty acids in mink and foxes. The purpose of the present study is, therefore, to compare apparent ileal digestibility of fat and fatty acids in polar foxes fed on diets used in the foxes nutrition during the winter furring period.

Material and methods
The experiment included 4, from the same litter, one-year-old male polar foxes of a similar body weight (7.91±0.31kg). In those animals 'end-to-end' ileorectal anastomosis was made surgically following the method developed and used in foxes by Szymeczko (2001). Foxes were premedicated with tranquilizer (0.08ml/kg) and atropine (0.03 mg/kg). General anaesthesia was induced with ketamine (10mg/kg) and foxes were placed on back side for the surgical procedure. The abdominal

cavity was opened and the segment of large intestine for resection was located. It was taken out beyond the abdominal cavity and in the mesentery anastomosed to it – successive blood vessels were ligated. Then the segment of the large intestine marked was resected and free sections of ileum and rectum were anastomosed with two continuous sutures with the 'end-to-end' technique. Having completed the anastomosis, the ends of the free mesentery and body layers were sutured, the area around the wound was washed and disinfected. Over five days following the operation, antibiotic and tranquilizers, antihaemorrhage and anti-swelling medications were administered to the foxes with dosages recommended by the producers. Over the first two days following the operation, liquids were supplemented, administering subcutaneous physiological saline solution and glucose. Over the following days the foxes were fed with liquid diet with an increasing concentration of nutrients. Starting from the tenth day, the animals were given an entire feed ration. After the veterinary examination in the third week after the operation, the foxes were considered clinically healthy. They were housed separately in metabolic cages, in an environmentally controlled room at 18°C and a 14-10 light-dark cycle. The use and handing of animals for this experiment were approved by the Local Ethical Committee in Bydgoszcz.

The digestibility experiment evaluated diets (A and B) used in the feeding of parent stock of polar foxes over the winter furring period on two selected reproduction farms (Table 1). Diet A included 55% of poultry offals, while in diet B – mostly flounder offals (34.5%) and pork offals (32.1%) were most substantial. The level of calculated metabolic energy in diets A and B was 1523 and 1646 kcal/kg, respectively. The distribution of metabolic energy from protein, fat and carbohydrates in those diets was similar (diet A: 37, 40, 23; diet B: 38, 37, 25) and consistent with feeding recommendations for this animal species (Hansen, 1992). To estimate apparent digestibility of nutrients in the feeds 0.3% chromic oxide (Cr_2O_3) was used as an indicator (Szymeczko and Skrede, 1991;Hill et al., 1996).

Testing each diet was divided into two 4-day periods: adaptation and digesta collection period. Over the adaptation period the animals were being made accustomed to experimental diets, and during the digesta collection period digesta excreted from the small intestine was collected. Both diets were tested one after another. The animals were fed to approximate daily maintenance energy (ME) requirements (90kcal ME/kg body weight) once a day at 8:00 (NRC, 1982). Throughout the experimental periods foxes had free access to water and remained under a constant inspection of a veterinary doctor.

All experimental digesta was immediately collected after being excreted into plastic boxes with tightly fitting lids kept on ice trays which were stored at +4°C between successive collections. Having completed the collections of digesta, the containers had been stored at -25°C before being analysed. Experimental diets and digesta were then freeze-dried, having removed the hairs and ground. Chemical analyses of feed and freeze-dried digesta were carried out in the laboratory of the Agricultural Academy in Bydgoszcz using standard methods. Crude fat concentration was analysed using Soxlet method, according to application notes for Soxtec System HT6 apparatus. Prior to determining fat, diets and digesta samples were hydrolysed using Tecator Soxtec Hydrolizing Unit. Fatty acid methyl esters were prepared according to Polish Norm (PN-ISO 5509, 1996) using methanol-chloroform (1:2) mixture. The composition of fatty acids was analysed with Hewlett Packard gas chromatograph according to Polish Norm (PN-EN ISO 5508, 1996) at Meat and Fat Research Institute in Warszawa.. The analysis of fat and fatty acids were made in duplicate.

The results obtained were statistically verified with Student's t-test for dependent samples using STATISTICA software (Stanisz 1998). The level of significance was set at $P<0.05$.

Results and discussion

Diets contained similar concentrations of dry matter and fibre. Crude protein and ash concentrations were higher in B diet (Table 1). The content of fat in diets A and B was 23.3%. The content of fatty acids (TFA) in fox diets slightly exceeded 22% (Table 2). Fatty acids present in dietary fat in minor amounts (≤0,5%) are omitted. Diet B contained higher content of saturated acids (SFA), with the highest content of palmitic acid (C16:0), which accounted for 66% of SFA. In diet A its content was over 74% of SFA. Stearic acid (C18:0), similarly to all the saturated acids, is characteristic of mammal-origin fat products. In A diet the level of this acid was

1.4% and it was lower then its content in B diet (2.2%) composed of the higher percentage of animal by-products. Monounsaturated fatty acids (MUFA) were accounted for over 50% of those fatty acids-marked in the diets tested. A considerable per cent share in MUFA was accounted for by oleic acid (C18:1 C9), which content in the diets exceeded 70%. Diet A, based on poultry offals, appeared richer in polyunsaturated fatty acids (PUFA), represented by linoleic acid (C18:2) and linolenic acid (C18:3). One shall stress that these acids are essential fatty acids for foxes (NRC, 1982).

Table 1. Composition of winter furring diets used in nutrition of experimental polar foxes (%).

Ingredient	Diet	
	A	B
Flounder offals	15.00	34.50
Poultry offals	55.00	3.80
Pork offals	2.00	32.10
Meat meal 55%	-	2.60
Beef offals	-	3.10
Protein-fat concentrate	-	2.60
Lard	-	2.60
Soybean oil	-	0.40
Extruded cereals	15.00	18.00
Vitamin-mineral mix.(1) (Polfamix)	-	0.20
Vitamin-mineral mix.(2) (Ewomix)	0.05	
Iron preparation (Taiga Fur)(3)	0.10	0.10
Water	14.85	
Proximate comp:		
Dry matter	97.64	98.61
Protein	31.97	36.34
Fat	23.28	23.32
Fiber	1.63	1.64
Ash	10.72	12.98

1) Concentration per 1g of mixture: vit. A 3500 IU; D_3 500IU; E 28mg; K_3 0.2mg; B_1 1.5 mg; B_2 2.8mg; B_6 2.8mg; B_{12} 0.02 Mg;H 0.2mg; folic acid 0.2mg; PP 10mg; Ca-panthotenate 7mg; methonine 200mg; choline chloride 50mg; Fe17mg; Zn 2mg; Cu 1mg; Mn 1mg; Co 1mg; J 0.1mg; Se 0.6mg

2) Concentration per 1g of mixture: vit. A 7200j.m; D_3 720j.m; E 82mg; $B_1$30.8mg; $B_2$12mg; H 0.1mg; B_6 6mg; B_{12} 0.04mg; PP 20.4mg; folic acid 0.6mg; Ca-panthotenate 8mg; choline chloride 50mg; Mg 66.8mg; Mn 6mg; Zn 8.6mg; Cu 1mg; Fe 9.2mg; J 0.3mg

3) Concentration per 1ml of preparation: ferrous glukonate205mg; ferrous sulfate 97.5mg; cupric sulfate 5.9mg; cobalt sulfate 1.76mg

Table 2. Content of fat and fatty acids (% d.m.) in winter furring diets fed to experimental foxes.

Ingredient	Diet A	Diet B
Fat	23.28	23.32
14:0	0.29	0.36
16:0	5.76	5.51
16:1 C7	0.13	0.11
16:1 C9	2.07	1.38
18:0	1.45	2.21
18:1 TRANS	0.09	0.33
18:1 C9	8.72	8.98
18:1 C11	0.69	0.78
18:2	2.11	1.43
18:3	0.13	0.09
20:1	0.29	0.33
TFA(1)	22.19	22.18
SFA(2)	7.72	8.34
MUFA(3)	12.15	12.26
PUFA(4)	2.31	1.58

1) Total fatty acids
2) Saturated fatty acids
3) Monounsaturated fatty acids
4) Polyunsaturated fatty acids

The level of fat in digesta A (3.5%) determined in chemical analyses was significantly higher (P<0.05) than in digesta B (2.1%). The content of all the fatty acids analysed was also higher (P<0.05) in digesta after A diet (Table 3).

Table 3. Content of fat and fatty acids (%d.m.) in digesta of experimental foxes fed winter furring diets.

Ingredient	Diet				P - value
	A		B		
	$\bar{x}$	SD(5)	$\bar{x}$	SD	
Fat	3.51[a]	0.46	2.13[b]	0.65	0.003
14:0	0.06[a]	0.02	0.03[b]	0.01	0.011
16:0	1.23[a]	0.20	0.55[b]	0.16	0.000
16:1 C7	0.01[a]	0.00	0.01[b]	0.00	0.024
16:1 C9	0.11[a]	0.03	0.04[b]	0.01	0.002
18:0	0.41	0.06	0.35	0.09	0.132
18:1 TRANS	0.01[a]	0.01	0.04[b]	0.02	0.017
18:1 C9	0.78[a]	0.12	0.48[b]	0.15	0.047
18:1 C11	0.08[a]	0.01	0.05[b]	0.01	0.008
18:2	0.36	0.08	0.28	0.11	0.186
18:3	0.03	0.01	0.02	0.01	0.359
20:1	0.04[a]	0.01	0.02[b]	0.01	0.043
TFA(1)	3.33[a]	0.44	2.02[b]	0.62	0.003
SFA(2)	1.80[a]	0.28	0.99[b]	0.28	0.001
MUFA(3)	1.08[a]	0.16	0.68[b]	0.22	0.039
PUFA(4)	0.45	0.11	0.34	0.13	0.189

1) Total fatty acids
2) Saturated fatty acids
3) Monounsaturated fatty acids
4) Polyunsaturated fatty acids
5) Standard deviation
a, b – Means in the same row within the same component followed by different letters are significantly different (P<0.05)

Ileal digestibility of fat of the evaluated feeds was high and the digestibility coefficient was significantly higher (P<0.05) for B diet (98.2%), based on fat from fish and pork offals (Table 4). The experiments which included minks defined digestibility of various kinds of fat, e.g. soybean (95.5%), fish (94.4%) and poultry (91.7-94.2%) (Austreng et al. 1979; Mertin et al., 1999). Ileal digestibility of fat was measured in dogs (Muir et al., 1996; Murray et al., 1997; Cole et al., 1999; Bednar et al., 2000; Clapper et al., 2001) which, similarly to foxes, are adapted to high-fat-content diets. These animals have gastric stomach, relatively short large intestine in which some bacterial fermentation takes place. Digestibility data obtained for foxes and dogs are therefore very similar due to the functional similarity and similar anatomy of their digestive system (Ahlstrøm and Skrede, 1998). In studies on dogs with cannulas in the end of the small intestine, ileal digestibility of fat, depending on the diet composition, ranged from 88.3 to 96.1% (Muir et al., 1996; Murray et al., 1997; Cole et al., 1999; Bednar et al., 2000; Clapper et al., 2001). The values were, however, much lower than those of the digestibility coefficients obtained in the present study, especially for B diet. In the experiment reported by Hill et al. (2001) in dogs fed with diets of a considerably higher quantity of beef fat, however, there were obtained higher values of apparent ileal digestibility of fats (98.8-99.5%). In large intestine there was recorded a slight increase in its secretion (≈30mg/kg), which was reflected by a lower apparent ileal digestibility of fat measured over the entire gastrointestinal tract of those animals (Hill et al., 2001).

Table 4. Apparent ileal digestibility of fat and fatty acids (%) in experimental polar foxes fed winter furring diets.

Ingredient	Diet				P - value
	A		B		
	$\bar{x}$	SD[5]	$\bar{x}$	SD	
Fat	96.34[a]	0.89	98.16[b]	0.65	0.003
14:0	94.88[a]	1.41	98.13[b]	0.66	0.006
16:0	94.81[a]	1.33	97.97[b]	0.71	0.002
16:1 C7	98.41[a]	0.71	98.96[b]	0.47	0.044
16:1 C9	98.65[a]	0.47	99.37[b]	0.25	0.009
18:0	93.09[a]	1.70	96.80[b]	1.07	0.003
18:1 TRANS	96.33	2.35	97.38	1.18	0.256
18:1 C9	97.80[a]	0.61	98.92[b]	0.38	0.024
18:1 C11	97.01[a]	0.78	98.78[b]	0.42	0.007
18:2	95.83	1.29	96.11	1.70	0.716
18:3	95.41	1.70	95.48	1.89	0.944
20:1	96.33[a]	0.87	98.51[b]	0.50	0.014
TFA[1]	96.35[a]	0.89	98.17[b]	0.65	0.003
SFA[2]	94.33[a]	1.38	97.59[b]	0.82	0.002
MUFA[3]	97.83[a]	0.59	98.88[b]	0.40	0.020
PUFA[4]	95.27	1.61	95.70	1.80	0.627

1) Total fatty acids
2) Saturated fatty acids
3) Monounsaturated fatty acids
4) Polyunsaturated fatty acids
5) Standard deviation
a, b – Means in the same row within the same component followed by different letters are significantly different (P<0.05)

Higher (P<0.05) digestibility of saturated fatty acids was recorded for B diet (97.6%) than for A diet (94.3%). One shall stress that an increase in the length of carbon chain coincided with a decrease in digestibility of respective acids SFA, which confirms the reports of study on rats which showed that more saturated fats show a lower availability (Al-Othman, 2000). In pigs and broilers, saturated short- and medium-chain fatty acids, similarly to unsaturated acids, are better digested than saturated long-chain fatty acids (Reis de Souza et al., 1995; Dänicke et al., 1999). Digestibility of saturated fatty

acids, measured over the entire digestive tract, decreases with an increase in the chain length to C18, however any further increase in its length to C22 results in yet another increase in SFA digestibility (Austreng et al., 1979).

In the present experiment the digestibility of stearic acid (C18:0) was poorest of all SFA (Table 4), which coincides with the results obtained for soybean-oil-fed broilers. Higher levels of lipids in chicken feces, as compared with the amount determined in digesta, can reflect their synthesis by large intestine microorganisms (Ajuyah et al., 1996). A low digestibility of stearic acid (C18:0) in piglets can be also due to the activity of large intestine microorganisms which participate in biohydrogenation of oleic acid (C18:1 C9) and of linoleic acid (C18:2) and their transformation into stearic acid C18:0 (Reis de Souza et. al., 1995). As a result, the digestibility of stearic acid can be systematically underestimated, while the digestibility of unsaturated fatty acids of C18 group – overestimated. The traditional method of measuring the digestibility of fat and fatty acids is not, therefore, fully accurate when evaluating digestion processes of these nutrients (Reis de Souza et al., 1995; Mountzouris, 1999).

In the present study monounsaturated acids were better digested from B diet (98.9%) than from A diet (97.8%) ($P<0.05$). Oleic acid (C18:1 C9), constituting over 70% of all MUFA in the diets evaluated, was better absorbed from B diet (98,9%), than from A diet (97.8%) ($P<0.05$). In minks, apparent digestibility of oleic acid, determined in feces, depended on the source of fat; when soybean oil was administered, it reached 97.2%, for fish oils – it did not exceed 95% (Austreng et al., 1979). According to the authors of the present study, monounsaturated acids are better digested than their saturated counterparts, which is also confirmed in piglets (Reis de Souza et al., 1995). Digestibility coefficients of linoleic (C18:2) and linolenic (C18:3) acids, representing polyunsaturated acids PUFA, did not statistically differ between the tested diets (Table 4). In mink diets which included soybean oil and various fish fats, traditionally measured digestibility of linoleic acid was 96.6, 70.8 and 74.3%, respectively. Apparent digestibility of linolenic acid for the soybean-oil diet was 100% (Austreng et al., 1979).

Conclusions

Inclusion high level of poultry offals (55%) in diet tended to depress apparent fat and fatty acids digestibilities in the small intestine of polar foxes. For the diet composed of high level of flounder (34.5%) and pork offals (32.1%) significantly higher ($P<0.05$) ileal digestibilities of fat (98.2%), TFA (98.2%), SFA (97.6%) and MUFA (98.9%) were recorded. Taking into account microbial activity in the large intestine of carnivore animals, the estimation of nutrients digestibility by the ileal method seems to be more precise in polar foxes.

References

Al-Othman, A.A., 2000. Growth and lipid metabolism responses in rats fed different dietary fat sources. Int. J. Food Sci. Nutr. 51, 159-167.

Ahlstrøm, Ø. & Skrede. A., 1998. Comparative nutrient digestibility in dogs, blue foxes, mink and rats. J. Nutr. 128, 2676-2677.

Ajuyah, A.O., Balnave, D. & Annison, E. F., 1996. Determination of apparent and true dietary fatty acid digestibilities and metabolisable energy using ileal digesta and excreta from broiler chickens. Anim. Feed Sci. Tech. 62, 131-139.

Austreng, E., Skrede, A. & Eldegard, Å., 1979. Effect of dietary fat source on the digestibility of fat and fatty acids in rainbow trout and mink. Acta Agr. Scand. 29, 119-126.

Bednar, G. E., Murray, S. M., Patil, A. R., Flickinger, E. A., Merchen, N. R. & Fahey, G. C. Jr., 2000. Selected animal and plant protein sources affect nutrient digestibility and fecal characteristics of ileally cannulated dogs. Arch. Anim. Nutr. 53, 127-140.

Clapper, G. M., Grieshop, C.M., Merchen, N. R., Russett, J. C., Brent, J. L Jr. & Fahey, G. C. Jr., 2001. Ileal and total tract nutrient digestibilities and fecal characteristics of dogs as affected by soybean protein inclusion in dry, extruded diets. J. Anim. Sci. 79, 1523-1532.

Cole, J.T., Fahey, G.C. Jr., Merchen, N.R., Patil, A.R., Murray, S.M., Hussein, H.S. & Brent, J. L. Jr., 1999. Soybean hulls as a dietary fiber source for dogs. J. Anim. Sci. 77, 917-924.

Dänicke, S., Jeroch, H., Böttcher, W., Bedford, M. R. & Simon, O., 1999. Effects of dietary fat type, pentosan level, and xylanases on digestibility of fatty acids, liver lipids, and vitamin E in broilers. Fett/Lipid. 101, 90-100.

Endres, B., Aherne, F.X., Ozimek, L. & Spicer H., 1988. The effects of fat supplementation on ileal versus fecal fat digestibilities, performance and

body composition of weaned pigs. Can. J. Anim. Sci. 68, 225-231.

Hansen, N.E., 1992. Recent advances in the nutrition of fur animals. Norw. J. Agr. Sci., Suppl. 9, 221-231.

Hill, R. C., Burrows, C. F., Ellison, G. W. & Bauer, J. E., 1996. The use of chromic oxide as a marker for measuring small intestinal digestibility in cannulated dogs. J. Anim. Sci. 74, 1629-1634.

Hill, R. C., Burrows, C. F., Ellison, G.W. & Bauer, J. E., 2001. The effect of texturized vegetable protein from soy on nutrient digestibility compared to beef in cannulated dogs. J. Anim. Sci. 79, 2162-2171.

Mertin, D., Süvegová, K., Tocka, I., Fl'ak, P., Cerešnáková, Z., Podolanová, E. & Barabasz, B., 1999. Digestibility of nitrogen and fats from feed rations for mink at various proportions of poultry by-products. Scientifur 23, 25-28.

Mountzouris, K. C., Fegeros, K. & Papadopoulos, G., 1999. Utilization of fats based on the composition of sow milk fat in the diet of weanling pigs. Anim. Feed Sci. Tech. 77, 115-124.

Muir, H. E., Murray, S. M., Fahey, G. C. Jr., Merchen, N. R. & Reinhart, G. A., 1996. Nutrient digestion by ileal cannulated dogs as affected by dietary fibers with various fermentation characteristics. J. Anim. Sci. 74, 1641-1648.

Murray, S. M., Patil, A. R., Fahey, G. C. Jr., Merchen, N. R. & Hughes, D. M., 1997. Raw and rendered animal by-products as ingredients in dog diets. J. Anim. Sci. 75, 2497-2505.

NRC, 1982. Nutrient Requirements of Mink and Foxes. 2nd revisted Edition. National Research Council. National Academy Press, Washington, DC, pp.72.

Polish Standards PN-EN ISO 5508. December 1996. Animal and vegetable fats and oils – Analysis by gas chromatography of methyl esters of fatty acids.

Polish Standards PN-ISO 5509. December 1996. Animal and vegetable fats and oils – Preparation of methyl esters of fatty acids.

Pölönen, I., Käkelä, R., Miettinen, M. & Asikainen, J., 2000. Effects of different fat supplements on liver lipids and fatty acids and growth of mink. Procceedings of the VIIth International Scientific Congress in Fur Animal Production, Kastoria. Scientifur 4, 92-93.

Reis de Souza, T., Peiniau, J., Mounier, A. & Aumaitre, A., 1995. Effect of addition of tallow and lecithin in the diet of weanling piglets on the apparent total tract and ileal digestibility of fat and fatty acids. Anim. Feed Sci. Tech. 52, 77-91.

Stanisz, A., 1998. Przystępny kurs statystyki w oparciu o program Statistica PL na przykładach z medeycyny. StatSoft Polska Sp z o.o. Kraków, pp.362.

Szymeczko, R. & Skrede, A., 1991. Protein digestion on fistulated polar foxes. Scientifur 15, 3: 227-232.

Szymeczko, R., 2001. Ileal and total digestibility of amino acids in feeds used in mink and polar fox nutrition. J. Anim. Feed Sci. 10, Suppl.1, 211-222

Why certain silver fox genotypes develop red hairs in their coat

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Abstract
Two genetic variants of the red fox (Vulpes vulpes) do both express the silver fox phenotype. A genomic deletion in the *agouti* gene explains the black Standard Silver fox phenotype. A substitution (C125R) mutation causing a constitutively active melanocyte stimulating hormone receptor (MC1R), explains the Alaska Silver fox phenotype. The large amount of red pigment found in Cross foxes is distinct from the uniformly black pigment synthesis in other mammalians harbouring a constitutively active MC1R, like for example mice, sheep and cattle. It was therefore predicted that animals being homozygous for the constitutively active *MC1R* - mutation might also display some red hairs in the otherwise black coat, provided that a functional agouti protein is present. In the present study, using a novel genotype test, we showed that red hairs are exclusively found in Alaska Silver and Sub-Alaska Silver genotypes, confirming this hypothesis. The limited amount of red hairs found in these genotypes is considered as a quality failure, and should be avoided in commercial fur breeding.

Keywords: pigmentation, coat colour, genotype test, MC1R, Agouti

Introduction

Two naturally occurring mutations in the Red fox produce genotypes that are phenotypically very similar (Våge et al., 1997). These genotypes are referred to as Alaska Silver and Standard Silver. The Alaska Silver phenotype is caused by a mutation in the *extension* gene, encoding the melanocyte stimulating hormone receptor (MC1R). It has been demonstrated pharmacologically that this amino acid change from cysteine to arginine in position 125 (C125R) transforms the receptor into a constitutively active state (Våge et al., 1997; Lu et al., 1998). The Standard Silver fox phenotype is caused by a large deletion in the *agouti* gene, encoding an antagonist of MC1R. In the absence of agouti protein, the melanocyte stimulating hormone (α-MSH) binds to MC1R in a non-restricted manner, and the receptor is activated. This means that both the Alaska Silver and the Standard Silver mutations are causing an activated receptor, either by changing the structure of the receptor itself, or by removing the antagonist.

In mice (Robbins et al., 1993), cattle (Klungland et al., 1995) and sheep (Våge et al., 1999), dominant *MC1R* locus alleles encode mutant forms of the MC1R that have constitutive activity. In these species *MC1R* is epistatic to *agouti*, hence animals with dominant alleles at both loci remain darkly pigmented. In the fox, a non-epistatic interaction of *agouti* and *MC1R* is observed in the Cross foxes ($AAE^{+}E^{A}$ or $Aa\ E^{+}E^{A}$), as these animals produce a significant amount of red pigment around the flanks, midsection and neck (Våge et al., 1997). We therefore predicted that foxes being homozygous for the constitutively active MC1R also could display

some red hairs in the presence of a functional agouti protein. Visible red hairs in the black Silver fox coat are considered as a quality failure in commercial fur production, and are therefore unwanted. The availability of genetic tests for both *agouti* and *MC1R* have made it possible to identify the five different Silver fox genotypes.

In this study, 6 different matings were performed in order to obtain Silver fox offspring being homozygous for the E^A allele on different *agouti* backgrounds. To facilitate the agouti genotyping, a PCR-based test for the relatively large genomic *agouti* deletion was developed.

Materials and methods

Primers used

The following primers were used to amplify the fox agouti gene:

A1 5'-GTCTCCCTGTGCTTCCTCAC-3' (exon 2, forward)
A2 5'-CCGCCTCTTTTCTGCTGATC-3' (exon3, reverse)
A3 5'-ATATACTCTTTGTGTGCTGG-3' (intron 2, forward)
A4 5'-ATTCTAGATGTATCGGGAAG-3' (intron 2, forward)
A5 5'-M13TGTACATGATACTGAGTCCA-3' (intron 2, forward)
A6 5'-CTTCTTTTCCGCCTCTTTTCTGCTGATC-3' (exon3, reverse)
A7 5'-GAGAGACAGAAGGAGCAAAGCCAGATGT-3' (intron2, reverse)
A8 5'-GGTTGGCTGGATGAATCACAACTTGAGA-3' (intron2, reverse)
A9 5'-TGCCATCCAAGAAAGCAACA-3 (intron1, forward)
A10 5'-M13ATAGGGTCCTGTAATGTCCG-3 (intron 2, reverse)

Localisation of the 3' break point of the deletion

The second intron of the fox *agouti* gene was amplified by primers A1/A2, and the resulting 1418 bp fragment was sequenced by standard methods (AJ250364). As the Standard Silver fox deletion includes the entire second exon (Våge et al., 1997), it was assumed that the 3' breakpoint of the deletion was localised in the second intron. To narrow down the possible region of the breakpoint, downstream primers A3, A4 and A5 were designed at intron 2 positions 484, 707 and 984. These three primers were separately combined with the A2 reverse primer, and genomic DNA from both Standard Silver fox (*aa*) and Red fox (*AA*) were used as template. Only primer combination A5/A2 did amplify in both Standard Silver fox and Red fox, while the two other combinations only amplified in the Red fox. These results indicated that the break point of the deletion is located in the region between primers A4 and A5.

Construction of a Genome Walker Library

In order to identify the upstream region of the deletion, a "Genome Walker Library" was used according to the protocol (Clontech, Palo Alto, USA). Five different restriction enzymes were used for constructing the different libraries (*DraI*, *EcoRV*, *PvuII*, *ScaI* and *StuI*) from Standard Silver fox DNA (*aa*). The *agouti* fragment was amplified by a nested PCR-protocol, using gene-specific reverse primers A6, A7 and A8 combined with two primers complementary to the ligated adaptor. An amplified fragment of approximately 2 kb (ScaI digested) was selected for sequencing by conventional methods (AJ250101).

A PCR based method for detecting the Standard Silver fox deletion

Based on the available sequences, three different primers (A3, A9 and A10) were combined in one PCR reaction, in order to detect both genotypes of the *agouti* gene in the *Vulpes vulpes*. Primer A9 is located in intron 1 (upstream of the 5' break point) while A3 is located in intron 2 (upstream of the 3' break point). The A10 primer is a reverse primer located downstream of the 3' break point. If the Standard Silver fox deletion is present, only the combination A9/A10 (252 bp) will amplify, while if the wild type allele is present only A3/A10 (349 bp) will amplify. In heterozygous animals, both fragments will be amplified (Fig. 1A).

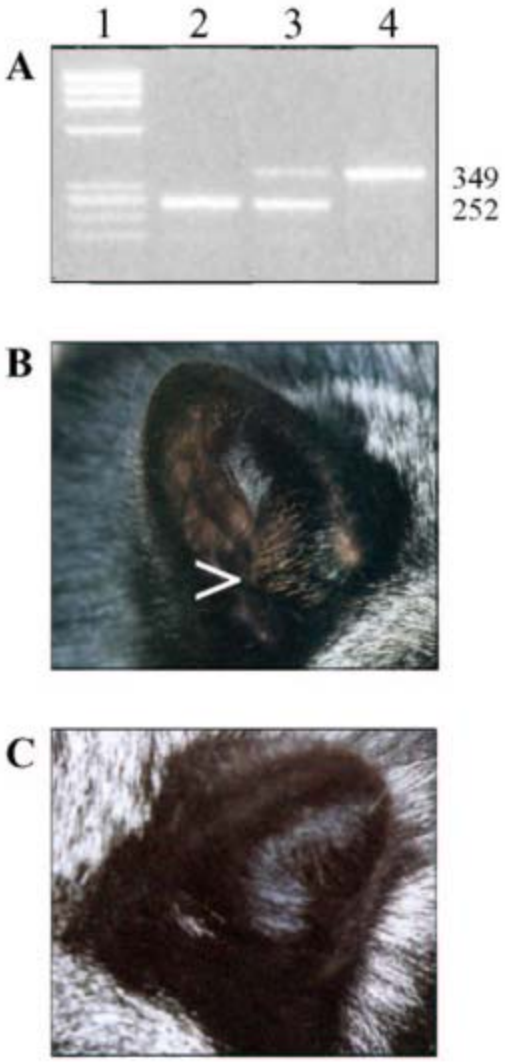

Figure 1. (**A**) PCR-based genotype test for the genomic deletion in *agouti* found in the Standard Silver fox. Lane 1: φX174 digested with *HaeIII.* Lane 2: An animal homozygous for the Standard Silver fox deletion (*aa*). Lane 3: An animal heterozygous for the Standard Silver fox deletion and the wild type *agouti* allele (*Aa*). Lane 4: An animal homozygous for the wild type *agouti* allele (*AA*). Molecular sizes are shown to the right. Genomic DNA is amplified with the primers A3, A9 and A10. Primer A9 is located in intron 1 (upstream of the 5' break point) while A3 is located in intron 2 (upstream of the 3' break point). The A10 primer is a reverse primer located downstream of the 3' break point. If the Standard Silver fox deletion is present, only the combination A9/A10 (252 bp) will amplify, while if the wild type allele is present only A3/A10 (349 bp) will amplify. In heterozygous animals, both fragments will be amplified. (**B**) The arrow indicates the presence of red hairs in the ear of a Sub-Alaska Silver fox (AaE^AE^A). (**C**) The ear of a Double Silver fox (aaE^AE^A), where no red hairs are present.

PCR conditions

The PCR reaction was carried out in 30 ul containing 50 ng genomic DNA, 30 pmol of primers A3 and A9, 60 pmol of primer A10, 200 uM dNTP, standard buffer conditions and 1.5 U Taq polymerase. DNA was denatured for 3 min at 94 ^{o}C, and PCR run for 30 cycles at 94 ^{o}C for 15 sec, 60 ^{o}C for 15 sec, and 72 ^{o}C for 40 sec. The amplified fragments were separated on an 1.5 % agarose gel (illustrated in Fig. 1A).

Detecting the Alaska Silver fox mutation

The PCR-based test described by Våge et al., (1997) was performed to identify the C125R Alaska Silver fox mutation.

Animals

Six litters were produced over two breeding seasons with the parental genotype combinations described in Table 1. Altogether 27 offspring had a Silver fox phenotype, while 4 were Cross foxes. Before the genotyping, all Silver fox offspring were scored for presence of red hairs in the coat and especially in the ears (Fig. 1B and 1C). All offspring were subsequently genotyped for *agouti* and *MC1R* alleles, and the results of the 27 Silver foxes are given in Table 2.

Table 1. Matings used to produce different Silver fox genotypes.

Litter no.	Maternal genotype	Paternal genotype
Litter 1:	Double Silver (aaE^AE^A)	Blended Cross (AaE^+E^A)
Litter 2:	Double Silver (aaE^AE^A)	Blended Cross (AaE^+E^A)
Litter 3:	Double Silver (aaE^AE^A)	Blended Cross (AaE^+E^A)
Litter 4:	Blended Cross (AaE^+E^A)	Double Silver (aaE^AE^A)
Litter 5:	Double Silver (aaE^AE^A)	Sub-Alaska Silver (AaE^AE^A)
Litter 6:	Sub-Alaska Silver (AaE^AE^A)	Sub-Alaska Silver (AaE^AE^A)

Table 2. Presence of red hairs in coat/ears of 27 Silver fox offspring from the matings given in Table 1.

Agouti	Extension	Type	Number	Presence of red hairs
aa	E^+E^+	Standard Silver	1	No
aa	E^+E^A	Sub-standard Silver	5	No
AA	E^AE^A	Alaska Silver	1	Yes
Aa	E^AE^A	Sub-Alaska Silver	8	Yes
aa	E^AE^A	Double Silver	12	No

Results and discussion

A Genome Walker Library was constructed from Standard Silver fox (*aa*) DNA to obtain sequence upstream of the genomic deletion. In addition, intron 2 was amplified from Red fox DNA (AA) and sequenced. By comparing these two sequences (AJ 250101 and AJ250364), the 3'-breakpoint of the deletion was located between position 723 and 724 in the intron 2 sequence. A PCR - test to differentiate between the two fox *agouti* alleles (A and a) was designed based on these sequences (Fig.1A).

Based on the genotyping results, we found that all animals expressing red hairs in the coat/ears are either of the Alaska Silver (AAE^AE^A) or Sub-Alaska Silver (AaE^AE^A) genotype (Table 2). In one case, an animal initially scored as containing red hairs in the ears, appeared to be of the Standard Silver fox genotype. Re-examination of this particular animal revealed a high number of white hairs in the ears of this animal, which might have been scored as diluted red in the initial scoring. We therefore conclude that truly red hairs in the coat or ears are merely a phenomena in Alaska Silver and Sub-Alaska Silver foxes (Fig. 1B). The fact that red hairs are only expressed in Silver foxes carrying functional agouti protein strongly suggests that *agouti* is responsible for the observed red hairs. Pheomelanic hairs are typically seen around the flanks and in the ears, a pattern comparable to the Cross foxes, but less pronounced.

The red colour of the Cross foxes could possibly be explained by an antagonising effect of the agouti protein on the E^+- variant of MC1R, that conquer the cAMP-stimulating effect of the E^A-variant in certain body regions. However, when red hairs are clearly expressed in animals homozygous for the constitutively active MC1R, this shows that agouti also antagonises the constitutively active receptor (E^A). The regional distribution of red hairs found in Cross foxes, and to a lesser extent in homozygous E^AE^A animals, most likely reflects the regional expression pattern of *agouti*. High concentrations of agouti protein in the individual hair follicle will increase the chance of conquering the cAMP-stimulating effect of the constitutively active MC1R.

Based on the present study we will conclude that the penetration of red hairs in the Alaska Silver and Sub-Alaska silver fox pelage is explained by the antagonising effect of the agouti protein present in these two genotypes. The rapid DNA tests developed for both the *agouti* deletion (present study) and the *MC1R* substitution mutation (Våge et al., 1997), provide a novel tool for identifying all the nine genotypes that can be realised by these two loci. It is thus possible to avoid combinations of parents giving offspring that will develop fur coat with reduced colour clarity caused by the production of red hairs. This strategy will be most efficient when applied on elite males that are used for artificial insemination.

References

Klungland, H., Våge, D.I., Raya, L.G., Adalsteinsson, S. & Lien, S., 1995. The role of melanocyte-stimulating hormone (MSH) receptor in bovine coat color determination. Mammalian Genome 6, 636-639.

Lu, D., Våge, D.I. & Cone, R.D., 1998. A ligand-mimetic model for the constitutive activation of the melanocortin-1 receptor. Molecular Endocrinology 12, 592-604.

Robbins, L.S., Nadeau, J.H., Johnson, K.R., Kelly, M.A., Roselli-Rehfuss, L., Baack, E., Mountjoy, K.G. & Cone, R.D., 1993. Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. Cell 72, 827-834.

Våge, D.I., Lu, D., Klungland, H., Lien, S., Adalsteinsson, S. & Cone, R.D., 1997. A non-epistatic interaction of agouti and extension in the fox, Vulpes vulpes. Nature Genetics 15, 311-315.

Våge, D.I., Klungland, H., Lu, D. & Cone, R.D., 1999. Molecular and pharmacological characterization of dominant black coat color in sheep. Mammalian Genome 10, 39-43.

Management problems and tools for strictly synchronised animal production systems exemplified by mink production

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Abstract
The characteristics of seasonally synchronous versus continuous animal production systems are revealed by analysing the interaction between the management and the production system. A strictly synchronous animal production is illustrated by the annual cycle of mink production. By regarding the mink production as a cybernetic system, the interaction between the management system and the production system is analysed. The systems description of the strictly synchronised mink production disclosed a time lag in the three steps of the feedback loop of management at the tactical level. Therefore the sequence of 1) Measurements of the production system's behaviour. 2) Comparison with a goal or a plan, and 3) Adjustment of controllable factors is often postponed until the relevant production period next year. In this situation, characteristic needs for management support are identified as tools to plan and prepare for the production period to come, to evaluate the effect of changes in management and to evaluate the need for adjustments from year to year. Systematic Operation Programmes, On-farm experiments and Evaluation of production results between years are presented as tools to meet the need for management in mink production. The generality of the needs for management support and of the tools presented are discussed in relation to other seasonally synchronous animal productions.

Key words: cybernetic system, decision support, Systematic Operation Programmes, On-farm experiments.

Introduction
The production of many domesticated farm animals varies with the seasons in the same way as that of their seasonally breeding wild ancestors. In traditional animal production systems, sheep, goats, horses, deer, mink, foxes, ferrets and trout are seasonal in their reproduction, while cattle, pigs and poultry are not. During the process of domestication, breeding and management have weakened the inherent seasonality. Intensive dairy, pig and poultry production have become continuous in the sense that the same production processes are repeated several times throughout the year, e.g. calves and piglets are born and eggs are laid year round. Under extensive outdoor production conditions dairy, pig and poultry production tend to be more seasonally synchronised rather than continuous (Thatcher, 1974; Mauget, 1982; Sharp, 1993). The key point is, that it is possible in practice to engage these animals in continuous production. In synchronous production systems, the different production periods (e.g. breeding, gestation, lactation, growth) are restricted in time to specific seasons and the different management procedures are performed in only one period each year, as all animals enter each period of the annual production

as a group. The number of animals in a given production period is high in a synchronised system, while in a continuous production system only a fraction of the animals is in the same production period at a given point in time. The number of animals affected by a change in management procedures also differs. In a synchronous system potentially all individuals are affected at the same time, while in a continuous system animals are affected over a period of time as they pass through the production period (Møller et al., 2003). In relation to management synchronisation has benefits as well as disadvantages.

The potential advantages of continuous animal production (and thus disadvantages of synchronised animal production systems) are:

- better utilisation of fixed resources like buildings and labour
- the ability to supply fresh products (like milk, meat and eggs) continuously to the market
- increased reproduction by shortening of the interval between successive breedings
- that only the fraction of animals in a given production period is at risk to a given period-specific hazard
- period-specific animal data can be sampled from a fraction of the animals in any season

Potential advantages of synchronised animal production systems (and thus disadvantages of a continuous production) are:

- no need to provide natural seasonal factors such as feed, light and heat when out of season (e.g. as pasture preserved as silage for cows, light programmes and heating for pigs and poultry).
- collection of a representative sample of animal data within a short time, i.e. in a given production period

The aim of this paper is to analyse the management in strictly seasonally synchronous animal production systems exemplified by mink production. The need for management information and decision support in mink production is identified and relevant management solutions are suggested.

Characterisation of the interaction between management and production

The farm as a cybernetic system

One way to describe the management in animal production is to look at the farm as a cybernetic system (Sørensen & Kristensen, 1992). In this context the farm is organised as a production system defined by the animals, buildings, machines, land and labour, and a management system defined by feedback of information performed by the farmer (Fig. 1). It is an open system, as it produces animal products (e.g. meat, milk, wool, pelt) and by-products (e.g. manure, wastewater) by use of controllable inputs (e.g. feed, water, fuel, medicine, labour) and uncontrollable inputs (e.g. climate, price relationships, restrictions from society, infectious diseases). By regulating the controllable factors the farmer strives to maintain the production in harmony with the overall purpose of farming (Sørensen & Kristensen, 1992). Adjustments in the controllable factors are needed, when uncontrollable factors induce deviations from the intended course of production. The interaction between the production system and the management system is illustrated in Fig. 1. Management is seen as a chronological series of:

1. Measurements of the production system's behaviour
2. Comparison with a goal or a plan
3. Adjustment of controllable factors

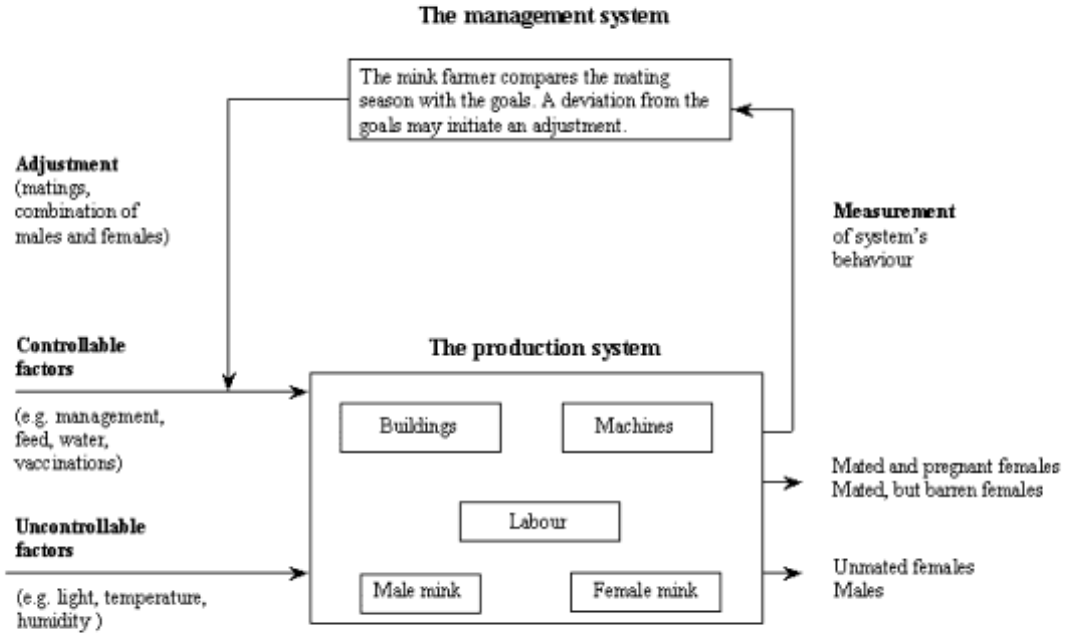

Figure 1. Mink production as a cybernetic system (modified from Sørensen & Kristensen, 1992).

The process of measurement, comparison and adjustment is a negative feedback, as it reduces the deviation between the goal and the measurement. Important aspects of this interpretation of management are the chronology of and the time lag between the measurement and the comparison and the adjustment in the feedback cycle. In the measurement step, information is gathered during a period of time. In the comparison step, it takes time to analyse deviations from the goal in order to determine whether adjustments are needed. In the adjustment step it may take time to implement the adjustments and subsequently for the adjustments to take effect. Another important aspect is that the feedback loop of measurement, comparison and adjustment is continuously repeated because uncontrollable factors continuously affect the production system. In the seasonally synchronous mink production, the possibility to complete the feedback loop depends on the time horizon.

Time horizons in farm management

A distinction between the strategic, tactical and operational time horizons is often found to be relevant in farm management (e.g. Huirne, 1990). Management at the strategic level has a long time horizon of several years, is closely related to the overall objective of the farm and has a major effect on the entire production for a long period of time. Adjustments are typically based on a long period of measurement and evaluation and usually with a long period of implementation.

Management at the tactical level has an intermediate time horizon of months to years, is related to the goals of the production and may have an effect on the entire production for a period of time. Adjustments are local and based on an intermediate period of measurements and evaluations. Implementation takes some time.

Management at the operational level has a short time horizon of hours to weeks, is related to production plans and usually has short-term effects. Adjustments are local and based on an accordingly short period of measurement and evaluations and are implemented immediately.

Seasonality and management horizons

As the time horizon at the strategic level is longer than one reproduction cycle or year, the management cycle does not differ between the continuous and the synchronous animal production at the strategic level, e.g. the building of a new shed or buying new breeding stock is independent of the type of production (synchronous or continuous).

Management at the tactical level (e.g. how many and which females to mate when in heat) may be influenced by the type of production. In a strictly synchronous production it is important to mate when the females are receptive, as they may not come into heat again until next year. In a continuous system the animal may be inseminated in the following oestrus cycle, and it may sometimes even be desirable to skip the first heat. In general, management at the tactical level differs between the continuous and synchronous production systems due to the time lag in the chronological sequence of measurement, comparison and adjustment in the feedback loop of management (Fig.1). This difference can be illustrated by the following example: A low conception rate at herd level may lead to adjustments in: 1) preparation for mating, 2) heat detection or 3) mating procedures. In a synchronised mink production system, where females are in heat only for a short period each year, the adjustments are postponed until next year, if a successful mating is not obtained. 1) If the animals were not properly prepared for the mating season, an adjusted feeding and flushing procedure cannot be performed until next year. 2) If heat was not detected and the females could not be mated in the usual mating season, e.g. because artificial light induced an early heat, the light regime cannot be corrected until the following year. 3) If the females were mated as planned, but did not conceive e.g. due to sterile males, a better procedure for checking the males' testicles cannot be applied until a year later. In all three situations, the completion of the feedback-loop is delayed until the following year, because the relevant adjustment is aimed at the previous production period.

In a continuous animal production, where animals come into heat year round, the adjustment in mating preparation, heat detection or mating procedures may be implemented immediately. In all three situations, the feedback loop may be completed at once for animals entering the relevant production period. The adjustments will be relevant as long as the disturbing factor is in effect; e.g. an adjustment in the management of dry cows due to some cases of mastitis in a period may continue for all dry cows to come, until new adjustments are called for.

At the operational level the management cycle does not differ between the continuous and synchronised animal production systems. Short term decisions, as whether and how to react upon an observation (e.g. lack of bedding material, outbreak of disease, a jammed drinking water supply) is independent of the type of production. If the management cycle is completed within the production process in question, that is, if measurement, evaluation and adjustment can take effect immediately, it will not differ between the continuous and synchronous production systems.

Management problems and tools in mink production

A strictly synchronous animal production system

Mink production is strictly seasonal, as all animals follow the same annual production cycle, which is synchronous within a few weeks. Under Danish conditions the mink females may be successfully mated within 3 weeks in March, 95% of all litters are delivered within 2 weeks in April-May and all animals are pelt prime within 3-4 weeks in November. Mink kits join the annual cycle already during the first year, as they are synchronised in terms of body weight and pelt moulting within 3-4 months after birth. As indicated in Fig. 2, mink production can be divided into a series of seasonal production periods.

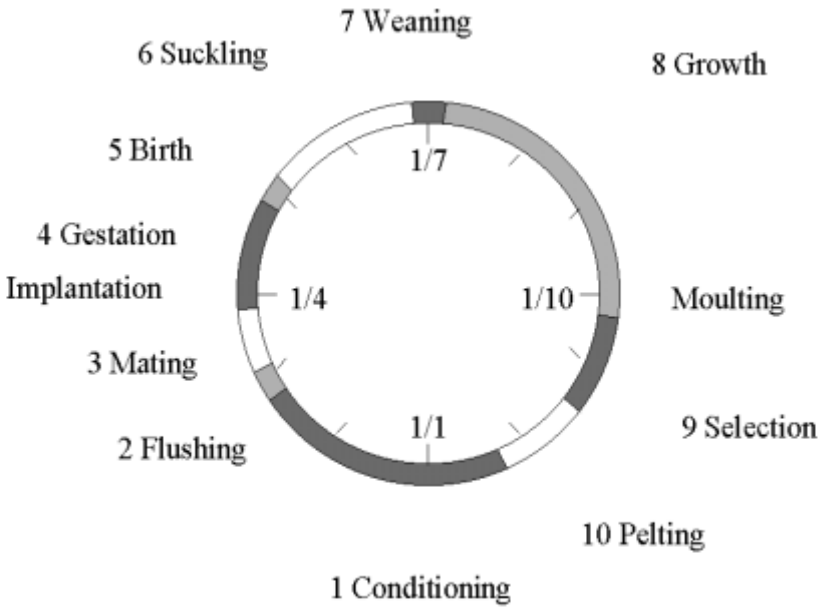

Figure 2. Synchronous periods in the seasonal mink production cycle. Numbered periods indicate a need for special management routines. The centre dial indicates the respective calendar dates.

Management problems to solve

Systems analysis illustrates that due to the strictly synchronised annual mink production there is a significant time lag in the feedback at the tactical level, and a close dependency on the production in the preceding periods. Planning is therefore essential in order to avoid or reduce deviations between production and goals because adjustments may not be possible until next season. Management procedures should be theoretically sound, well documented in practise and robust with regard to the variation in the uncontrollable production factors prevailing in the area. The management routines as well as the labour intensity change dramatically between the different production periods over the year. Especially new farmers therefore need management tools to guide them safely through the production periods as they build up management experience. During labour-intensive periods, part-time farmhands need introduction to the management strategy. Most farmers will therefore benefit from systematic ways to plan and communicate their management routines effectively.

Annual variation in the uncontrollable production parameters (e.g. animals, feed, weather, health, immune status) causes variation in the production result (e.g. litter size, body weight, pelt size). The most important uncontrollable factor is believed to be the climate, although only few direct climatic effects have been documented (Møller, 1991a; 1991b). As such factors will often affect all farms in the area, an evaluation of their effect should involve the result of previous years as well as that of other farms in the area. An annual variation in the uncontrollable factors raises a number of problems regarding the management of mink production. First, it is difficult to determine whether a deviation caused by an uncontrollable factor one year should be adjusted for the next year. Secondly, it is difficult to assess the effect of new management procedures or adjustments, as the production results are not directly comparable between years. Thirdly, it takes many years before a mink farmer has experienced even the most frequent combinations of weather conditions, disease outbreaks, feed complications and management errors occurring during the different production periods. Consequently, experience is gained slowly and stepwise. Mink farmers therefore require tools to help evaluate the need for adjustments from year to year, to evaluate the effect of changes in the management routines and to build up experience effectively.

The systems analysis of the strictly synchronised mink production identified a need for management

tools to help plan and prepare for the next production period, to evaluate the effect of changes in the management and to evaluate the need for adjustments from one year to another. To meet this need three management tools have been developed and evaluated:

1. Systematic Operation Programmes (SOPs)
2. On-farm experiments to develop management
3. Decision support for production results across years and farms

Management tools developed for mink production

1. Systematic Operation Programmes

To meet the needs for well-documented and robust plans a number of Systematic Operation Programmes (SOPs) have been developed for the most labour intensive periods of breeding and nursing. The purpose of the SOPs is to improve management in a certain period of production by systemising management routines in terms of timing, time expenditure and priority (Sørensen & Hindhede, 1987). The central element in an SOP is a number of action plans (Table 1), each describing a set of periods or intervals in which observed situations initiate specific actions. Each situation should be described clearly and unambiguously, making the observation and the action as independent as possible of the judgement of the herdsman (Sørensen & Hindhede, 1987).

Two SOPs have been developed to support the short and labour intensive mating and nursing periods and one for prevention of diarrhoea in suckling mink kits. To ensure the professional as well as the practical qualities of the SOP, an evaluation procedure was carried out as a part of the development activity as described in detail elsewhere (Møller, 1996; 1997; Møller et al., 1997). An English version of the SOPs for the "Mating period" and "Whelping period" is published on the Internet at the URL address:

http://web.agrsci.dk/hsv/shm/Hpmink01/sopintro.htm. In the following an action plan for the mating period is presented as an example of a period-specific management tool (Table 1).

Table 1. Part of the 'Action plan for the mating period' describing a set of periods or intervals in which observed situations initiate specific actions.

Action plan for the mating period (6/3-25/3)

Usual mating plan where young as well as old females are mated from the beginning of March.

Period	Observation	Action
At feeding	=>	Feed females ad libitum.
March 6 - 7	=>	Mating starts. Place the female with the male, and record the time.
Until March 15	Females that have not been exposed to a male, were a little willing yesterday or were tried three days ago.	Try or retry the female. Record the date on the females' card.
Mating attempts	Young males who will not start mating.	Let them try with an old female first.
	The animals fight, mating is not started within 15-20 minutes.	Put the female back in her own cage. Note the date on the card as a mating attempt. The male can be tried again after a break.
	The female is willing. Neck bite is established and mating starts.	Record the time on the card.
	After 10 minutes of mating.	Approve the mating – record the date on the card. Remove the female after the pair has separated. If necessary the mink can be separated after approx. 20 min.
From March 9	=>	Feed also the males ad libitum.

Internet version 1.4 by Steen Møller SteenH.Moller@agrsci.dk

The plans are targeted for the operational level, but as important dependencies between the production periods are included, the plans have an effect at the tactical level of management as well. The plans are

robust in the sense that risky management solutions are avoided, even in situations with high potential benefit.

2. On-farm experiments to develop management

In a strictly synchronous animal production there will be many animals of the same age in each period. This makes it relatively easy to design on-farm experiments defined as experiments carried out under circumstances representing the target group (Sørensen & Hindhede 1997). It is therefore important that the on-farm experiment only affects the factor under investigation and does not in other ways interfere with the management or the environment in a critical manner. On-farm experiments as a way to improve the management in mink production can be illustrated by the following example. The correlation between the body weight and the subsequent pelt length in mink was compared between a group of farms participating in an "experience exchange group". A significant difference between farms gave rise to on-farm experiments including timing of pelting in relation to pelt maturity as an experimental factor. The investigation on pelt length was conducted on 9 private farms during 1995 and 1996, with a total of 480 young male mink of standard, mahogany, wild and pastel colour types. The animals were kept under standard farm conditions and fed a conventional diet delivered daily from two mink feed kitchens. The usual pelting time was based on the farmer's judgement of when the pelt was at its prime within each farm and colour type. At pelting the mink were killed, body weight and length was measured and each skin identified by a numbered plastic tag through the nose cartilage. After skinning, fleshing, stretching on boards and drying, the pelt length was measured from snout to tail base. Of 480 mink in the investigation, 405 were pelted at their prime as defined by the farmer. In 1996, 75 mink from 4 farms were pelted on average 16 days (from 9 to 32 days) later than mink of the same colour type pelted at their prime on each farm. Data were obtained from 23 combinations of year, farm, colour type and pelting time, each consisting of 10 to 25 mink (Møller, 1999). In 1999 the on-farm experiments showed that the regression of pelt length on body weight varied significantly (more than 5 cm for mink of the same weight) between farms and colour types. Within the pelting season from mid November to mid December, the timing of pelting did not affect the average pelt length (Møller, 1999) or fur quality (Møller, 2001). The effect of 100 g in body weight in October/November was 1.26 cm of pelt while the effect of a 100 g weight change during the last month before pelting was 0.55 cm (Møller, 1999). Thus, the effect of a 100 g body weight during the moulting period was more than twice the effect of a 100 g weight change between moulting and pelting, as illustrated in Fig. 3.

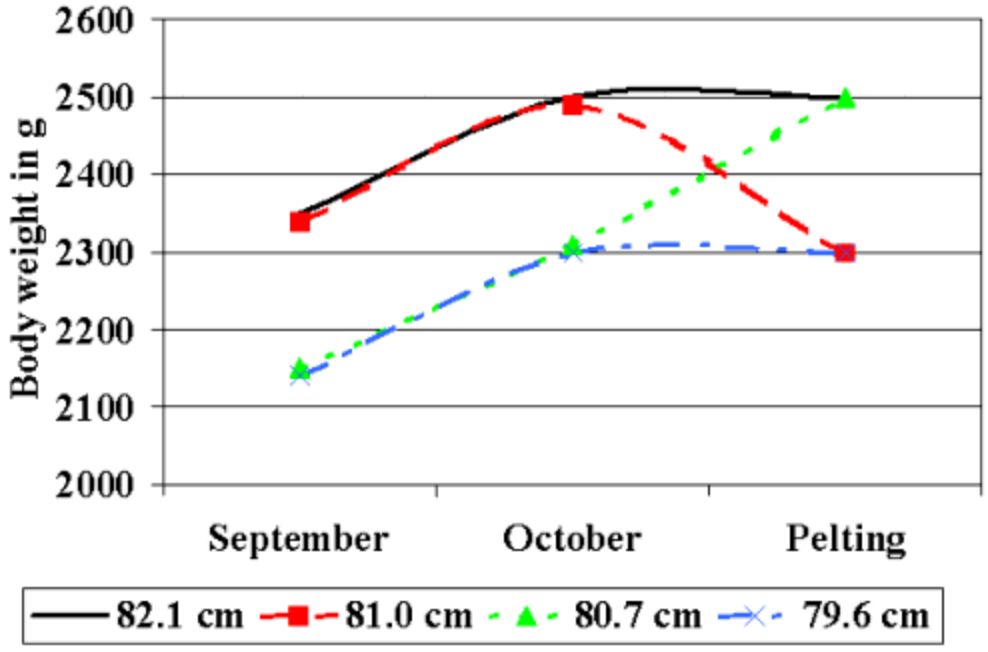

Figure 3. The effect on pelt length of a 100 g body weight modelled in four different scenarios from moulting in October to pelting in November.

Based on these on-farm experiments it could be concluded that pelting time is not an important management factor in relation to pelt length or fur quality, while feeding should focus on body weight at the time of moulting rather than on weight at pelting in order to maximise pelt length. The reason for the differences among farms in the relationship between pelt length and weight, equalling to an estimated value of 12-14% of the pelt price, should be further investigated.

3. Decision support based on production factors across years and farms

As each mink farm is a breeding unit, data on reproduction are registered each year for the selection of breeding animals on the individual farm. In order to extract more information from these data, the extension service developed a database and a set of routine calculations across farms (Sønderup et al., 1992). By use of these data, a decision support (DS) tool for comparison and evaluation of annual reproduction at farm level has been developed. The DS tool supports the management system in figure 1 by facilitating the steps: I. "Measurement of system's behaviour" II. "Comparison with the goal" including "Defining a feasible goal" and III. "Evaluation of adjustment". The DS tool is based on statistical modelling of a database with registrations of the performance of individual mink females from 27 farms in a geographical region serviced by two feed kitchens.

Reproduction data for 1992 to 1997 were extracted from 129,700 litters of the most common Standard brown or black colour types. The results of the DS tool are presented to each of the mink farmers as the actual as well as the potential litter size for the farm each year and separately for each colour type as exemplified for farm 88 in Fig. 4. Furthermore, for each year the regional average litter size for all farms as well as for the best 25% of the farms is presented (Fig. 4). The potential litter size is calculated as least squares means, expressing the expected litter size if the female age, the number of matings per female and the length of gestation had been as the average of all farms. The effect of each

of the variables (age, number of matings and length of gestation) is calculated for each farm as the difference between the actual and the average values of each variable multiplied by the model estimates (Møller, 2000).

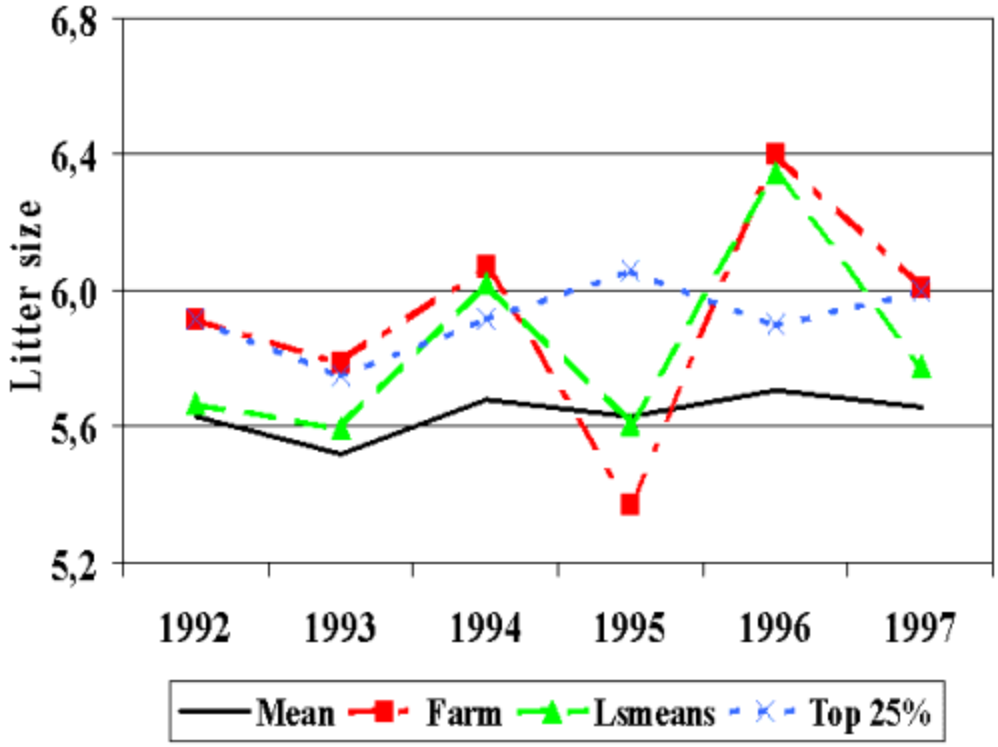

Figure 4. Actual and estimated litter size (least squares means) of farm 88 and average and upper quartile of black colour type mink from 27 farms.

The statistical modelling of the reproduction database constitutes the measurement of system's behaviour at a regional rather than at a farm level. The differences in average litter size for black mink varied from –0,12 to +0,16 between succeeding years, and thus confirm the need for a DS tool in order to define a feasible goal in mink reproduction, e.g. relative to the regional annual litter size (RALS). The DS tool supports the comparison with the goal by information on the potential (lsmeans) litter size, as well as the actual value and estimated effect of litter size index, dam age and length of gestation on the actual farm (Fig. 4). The factors in the model (dam age, reproduction index and length of gestation) explained a major part of the difference between the RALS and farm 88 each year, except in 1994 and 1996. The three factors explained -0.24 out of the -0.26 kits the litter size was below the RALS in 1995, and thus demonstrate the potential benefit of the DS tool in the analysis of a deviation from the goal. Based on this analysis the manager may decide the appropriate adjustment in controllable factors (Møller, 2000). Furthermore, a calculated increase in litter size of 0.09 kits by shortening the length of gestation from 46 to 45 days shows the potential for producing general knowledge through the implementation of these kinds of DS tools.

Discussion

The systems description of the strictly synchronised mink production disclosed specific needs for management tools at the tactical level. The SOP concept is of special interest to synchronous animal production because planning and use of previous experience is important in order to avoid errors that may affect the whole year's production. As each production period takes place only once a year,

synchronous production systems have a special need for systematic and detailed operational planning.

The generality of the SOP concept is reflected by strictly seasonal 'Management calendars' and 'Management plans' for fallow- and red deer management (Vigh-Larsen, 1991). The seasonality of the synchronised animal production is similar to crop production, and SOP have also been developed for the production of sugar beets (Kristensen et al., 1988). Aubry et al. (1998) found that the farmer's planning schedule for winter wheat management had properties similar to the SOP concept.

The stepwise development of management by on-farm experiments in mink production is similar to the development of management known as Evolutionary Operation (EVOP) in the industry. The EVOP is a simple experimental design developed as a tool to improve the allotment of production factors in an operating full-scale process (Box & Draper, 1969). By an ongoing series of changes in few factors at the time, the production is improved step by step. To avoid products with inferior characteristics, only small changes are made in each factor and many repetitions are necessary in order to determine the effect of these changes (Box & Draper, 1969).

In the industrial EVOP design, the different combinations of factors are processed one after the other and each cycle is repeated until a significant result is obtained (Banerjee & Bhattacharyya, 1992; Box & Draper, 1969). With each mink as an experimental unit, the number of observations may be very high on most mink farms, so significant results may be obtained each year. In a strictly synchronous animal production, a large number of animals may be subjected to the different combinations of factors during the same production period and the results may be calculated and evaluated well in advance of the potential implementation next year. Compared to the industrial EVOP all 'cycles' are run at the same time and under the same environmental conditions. The farm experiments or EVOP approach therefore seem well suited as a systematic way to improve the management in mink and other strictly synchronous animal productions. If the experimental factor interacts with annual factors the experiment should be repeated in order to evaluate the interaction. E.g. depending on the average ambient temperature during the lactation period supplementary drinking water may have no or profound effect. A dripping water system have been shown to significantly increase the weight gain of the kits and reduce the weight loss of the females if the ambient temperature was high during the lactation period, while no effect was found when the temperature was low (Møller, 1991a; 1991b)

On-farm experiments will enable the farmer to evaluate the effect of gradual changes in management routines and to take advantage of farm specific factors, which are not included in the common body of experience. By conducting the same experiment simultaneously under different production conditions on a number of farms it is possible to evaluate the generality of the results (Møller, 1999; 2001). Conducting the same experiments within a group of farms is therefore likely to become an economical and systematic instrument in the mink farmer's improvement of production management. As illustrated by the on-farm experiments with pelt length and pelt quality in relation to time of pelting, farm experiments may produce valuable general knowledge of interest to the mink industry (Møller, 1999; 2001).

The need to analyse the production results and the relevance of postponed adjustments in response to variations between goals and results is relevant for all farm animals, but the level of annual variation in results appears to be more pronounced in seasonally synchronous production as exemplified by mink (Møller, 2000). The systematic evaluation of production results in relation to other farmers is similar to the many national control programmes in dairy and pig production. However, in these continuous productions, the aim is often to identify significant changes in production results and the need for adjustment, as soon as possible (Thysen, 1993; Thysen & Enevoldsen, 1994).

Conclusions

It is concluded that due to a time lag in the three steps of the feedback loop of management at the tactical level adjustment of controllable factors is often postponed until the relevant production period next year. Characteristic needs for management support are identified as tools to plan and prepare for the production period to come, to evaluate the effect of changes in management and to evaluate the need for adjustments from year to year. Systematic Operation Programmes, On-farm experiments and Evaluation of production results between years can

be developed to meet the need for management tools in mink production.

References

Aubry, C., Papy, F. & Capillon, A., 1998. Modelling decision-making processes for annual crop management. Agricultural Systems 56, 1, 45-65.

Banerjee, R. & Bhattacharyya,-B.C., 1992. Evolutionary operation (EVOP) to optimize protease biosynthesis by Rhizopus oryzae. Bioprocess Engineering 8, 151-155.

Box, G.E.P. & Draper, N.R., 1969. Evolutionary Operation: A Statistical Method for Process Improvement. New York, John Wiley & Sons.

Huirne, R., 1990. Basic concepts of computerised support for farm management decisions. European Review of Agricultural Economics 17, 69-84.

Kristensen, I., Hermansen, J.E. & Østergaard, V., 1988. "Systematic operation programme for production of fodder sugar beets - Development, analysis and utilisation." Rep. 641, Natl. Inst. Anim. Sci., 115 pp. In Danish with English summary and subtitles.

Mauget, R., 1982. Seasonality of reproduction in the wild boar. In: Cole, D.J.A. & Foxcroft, G.R. (Eds.) Control of pig reproduction, 509-526. Butterworths, London.

Møller, S.H., 1991a. Management and environment in Mink Production. In: Møller, S.H. (Ed). Production of Mink, The influence of various management, environmental and nutritional elements on behaviour, physiology and production in mink. Rep. 688, Natl. Inst. Anim. Sci., 13-60. In English.

Møller, S.H., 1991b. Drinking behaviour of mink in relation to watering system and water temperature. NJF seminar nr 192, Uppsala 6-7 March. 11 pp.

Møller, S.H., 1996. Improvement of mink management by systematic operation programmes. VIth Int. Sci. Congr. in Fur Anim. Prod. Applied Science Reports 27, Polish Society of Animal Production, 151-158.

Møller, S.H., 1997. Systematic operation programmes for the improvement of mink management distributed on the WWW. Proceedings of the First European Conference for Information Tecnology in Agriculture, 15-18 June, Copenhagen, 175-178.

Møller, S.H., 1999. Effects of weight development, pelting time, colour type and farm on skin length in mink. Acta Agriculturae Scandinavica. Section A, Animal Science 49, 121-126.

Møller, S.H., 2000. A decision support tool for litter size management in mink, based on a regional farm reproduction database. Scientifur 24, 3 183-191.

Møller, S.H., 2001. Length and quality of mink skins from early or late pelting season. NJF seminar nr. 331. Snekkersten, Denmark, 1-3 October. 1 pp. abstract.

Møller, S.H., Sørensen, J.T. & Kristensen, A.R., 1997. The concepts of systematic operation programmes (SOPs) applied for the improvement of health and welfare management in mink. Livestock farming systems, More than food production. EAAP Publication No. 89, 277-281.

Møller, S.H., Hansen, S.W. & Sørensen, J.T., 2003. Assessing animal welfare in a strictly synchronous production system: The mink case. Animal Welfare 12, 699-703.

Sharp, P.J., 1993. Photoperiodic control of reproduction in the domestic hen. Poultry Science 72, 897-905.

Sønderup, M., Børsting, E. & Hansen, J., 1992. An adviser based databank for support of commercial breeding programmes. "Vth Int. Sci. Congr. in Fur Anim. Prod.," Norwegian Journal of Agricultural Science, Supp. No. 9, 54-60.

Sørensen, J.T. & Hindhede, J., 1987. Systematic operation programme; Development and analysis. In: Seabrook, M.F. (Ed.) The role of the stockman in livestock productivity and management. Commission of the European Communities. Proceedings of a Seminar in the Community Programme for Co-ordination of Agricultural Research, Brussels, 16-17 December 1986. EUR. 10982 EN, 67-71.

Sørensen, J.T. & Hindhede, J., 1997. On-farm experiments as a research method in animal welfare and health. Proceedings of the fourth international symposium on livestock farming systems. Livestock farming systems, More than food production. EAAP Publication No. 89, 1997, 267-272.

Sørensen, J.T. & Kristensen, E.S., 1992. Systemic modelling: A research methodology in livestock farming. In: Gibon, A. et al. (Eds.) Global appraisal of livestock farming systems and study of their organizational levels: concepts, methodology and results. CEC Proceeding Eur 14479, 45-57.

Thatcher, W.W., 1974. Effects of season, climate and temperature on reproduction and lactation. J. Dairy Sci. 57, 360.

Thysen, I., 1993. Monitoring bulk tank somatic cell counts by a multi-process Kalman filter. Acta Agric. Scand. Sect. A. Animal Science 43, 58-64.

Thysen, I. & Enevoldsen, C., 1994. Visual monitoring of reproduction in dairy herds. Preventive Veterinary Medicine 19, 198-202.

Vigh-Larsen, F., 1991. Deer Production. Biology, management and economy. Fallow- and red deer. Report from the National Institute of Animal Science No. 694. 68 pp. (In Danish with English summary and subtitles).

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