JBAAR
Journal of Basic and Applied Research in Biomedicine
ISSN 2413-7014

Original article

Effect of Feeding Intensity on Metabolic Maintenance, Reproduction and Welfare in Blue Fox (*Vulpes lagopus*)

Hannu T. Korhonen[1], Juhani Sepponen[2], Heli Lindeberg[2], Nita Koskinen[1]

Natural Resources Institute Finland (Luke), Production Systems, [1]Teknologiakatu 7, 67100 Kokkola, Finland, [2]Halolantie 31 A, 71750 Maaninka, Finland

*Corresponding author: hannu.t.korhonen@luke.fi

Received: 27-4-2019
Revised: 2-6-2019
Published: 15-7-2019

Keywords:
Vulpes lagopus;
Hormones;
Energy metabolism;
Reproduction performance;
Feeding

Abstract: The purpose was to evaluate effects of feeding intensity on metabolic maintenance, reproduction and welfare in blue fox vixens. Study groups were: Group 1: heavy slimming. *Ad libitum* feeding during September-November, aimed to produce extremely fat animals. Heavy slimming before breeding season, aim to have animals with normal breeding body condition. Group 2: maintenance of condition. Restricted feeding 35-45% from the level of Group 1 during September-November. Natural slimming to normal breeding condition. Group 3: Rising condition. Restricted feeding 50-60% from the level of Group 1 during September-November. Aim was to produce lean animals. Rising body condition was before breeding season, aimed to have animals with normal breeding body condition. Blood samples were taken regularly throughout the study. Results showed that urea concentration varied seasonally ($P<0.001$). Concentrations were lowest during winter period. In Group 1, heavy slimming before mating season lowered urea concentration significantly ($P<0.001$). During autumn period, concentration was lowest in Group 3 because of pronounced feeding restriction. Creatinine levels were highest during summer period in all groups. Glucose concentration varied seasonally in all groups ($P<0.001$). Intensive feeding clearly affected on triglycerides, glucose and insulin levels ($P<0.01$). Growth hormone (GH) and insulin-like growth factor (IGF-1) levels were highest during autumn period. Leptin concentration was highest in December in all groups. Thereafter, it clearly declined towards summer ($P<0.001$). Concentration of non-esterified fatty acids (NEFA) was highest in Group 1. Prolactin levels were same in all groups until insemination. During pregnancy, levels increased similarly in groups. After whelping, prolactin levels were low ($P<0.05$) in Group 1 compared to other groups. Whelping result in Group 1was very low ($P<0.001$) compared to Groups 2 and 3. It can be concluded that feeding intensity essentially influences on hormonal levels and reproduction in blue fox vixens.

Cite this article as: Korhonen, H.T., Sepponen, J., Lindeberg, H., Koskinen, N. (2019). Effect of Feeding Intensity on Metabolic Maintenance, Reproduction and Welfare in Blue Fox (Vulpes lagopus). Journal of basic and applied Research in Biomedicine, 5(1): 11-20

INTRODUCTION

Juvenile blue foxes (*Vulpes lagopus)* have been traditionally fed *ad libitum* with the autumn's energy-rich feed on farms. The outcome of this is that the majority of animals are obese in early winter (e.g. Kempe 2018; Mononen et al. 2018). Due to intensive feeding and genetic selection, body weights and size of animals have increased dramatically over decades (Korhonen et al. 2017). Correspondingly, reproduction success has declined (Peura et al. 2004, 2007; Pyykönen 2008; Koivula et al. 2009; Koskinen et al. 2009, Peura 2013; Kempe 2018). Before breeding vixens have to be slimmed down. Some studies indicates that slimming down by severely restricting their feeding during a relatively short period before the breeding season may impair the vixens' reproductive success as compared to longer term but less severe restrictive feeding (Koskinen et al. 2008, 2011). Koskinen et al. (2011) studied the breeding success in 228 primiparous blue fox vixens that were fed from August to December either *ad libitum* or 35-45% or 50-60% below the *ad libitum* level. At the beginning of December, the body mass of the blue foxes was approx. 16, 12 and 10 kg, respectively. To obtain an ideal breeding condition, the foxes were then slimmed down. At the beginning of the breeding season, the body mass of the foxes was approximately 9.0, 8.2 and 7.5 kg, respectively. In this experiment, the *ad libitum* fed vixens, i.e. the vixens that were slimmed down most, had the lowest breeding result (1.91, 4.10 and 4.15 weaned cubs per inseminated vixen, respectively). Also in Koskinen et al. (2008), it was observed that young blue fox vixens that were first fed *ad libitum* and thereafter slimmed heavily down before the breeding season lost more cubs after parturition than continuously restrictively fed vixens.

Rapid changes in energy balance are affected on hormonal regulation and reproduction negatively (Tauson et al. 2000, 2002; Tauson and Forsberg

1992; Chagas et al. 2007). In the mink (*Mustela vison*), it has been found that females which are fat during autumn and thereafter slimmed down quickly are having poorer whelping result than those kept in normal body condition throughout production season (Tauson and Alden 1984, 1985). It seems that the reason for declined whelping result recently observed in blue foxes is the same as in the mink: disturbed energy balance due to excessive autumn fattening followed by a heavy slimming before breeding season. Typically energy intake, body condition and reproductive function are highly related to each other (Blance et al. 2003; Nieminen et al. 2004; Asikainen 2013; Mustonen and Nieminen 2017; Kempe 2018). Sufficient balance between energy intake and energy consumption is a prerequisite for a good reproduction capacity. If there is a lack of proper energy intake, energy reservoirs in the body are targeting to most important organ functions (Wade et al. 1996). A negative energy balance may prohibit reproduction mechanisms even totally. Relations between metabolic maintenance, hormonal balance and reproductíve efficiencies are complex, however, including integration of endocrine and metabolic signals controlling metabolism and reproduction. Appetite, mating behavior and hormonal regulation are linked to preoptic area and hypothalamus of brain (Roche 2006; Chagas et al. 2007). That area regulates particularly excretion of gonadotrophic and somatotrophic hormones which are specific related to reproduction mechanisms, growth and nutritional state.

Although seasonal regulation of body weight and metabolism has been somewhat studied in foxes (e.g. Fuglei et al. 2000, 2004; Korhonen 1988; Burlikowska et al. 2008; Korhonen et al. 2017; Mononen et al. 2018), more data are still needed particularly as concerns hormonal balance. The purpose of the present study was to clarify impact of feeding intensity (*ad libitum* vs restricted portions) on metabolic maintenance and reproduction success in blue fox vixens (*Vulpes lagopus*). Metabolic changes were followed by throughout autumn, winter and spring seasons until weaning of kits in summer. Further aim was to evaluate to which extent excessive obesity and heavy slimming are a matter of animal welfare.

MATERIAL AND METHODS

Experimental animals and set-up

The study was performed at Research Station, Kannus, in western Finland (63.54ºN, 23.54 ºE) during the period from September to next June (2010-2011). The use of experimental animals was evaluated and approved by the Animal Care Committee of MTT Agrifood Research Finland. The general health of the animals was checked daily. Health evaluation was based on general appearance of animals, including consistence of faeces. Furthermore, health of eyes and feet were also followed. Evaluations were done visually by the same person.

Treatment groups employed were: Group 1: heavy slimming (HS). *Ad libitum* feeding during Sept-November, aimed to produce extremely fat animals. Heavy slimming before breeding season, aim to have animals with normal breeding body condition. Group 2: maintenance of body condition (MC). Restricted feeding 35-45% from the level of Group 1 during Sept-November. Natural slimming to normal breeding body condition. Group 3: Rising body condition (RC). Restricted feeding 50-60% from the level of Group 1 during Sept-November. This treatment aimed to produce lean animals. Rising body condition before breeding season. Aim here was to have animals with normal breeding body condition. In each group there were 76 juvenile blue fox vixens.

Experimental animals were raised pairwise until Dec 8, 2010. Housing cages were 105 cm long x 115 cm wide x 70 cm high. Each cage had a wire-mesh platform (105 cm long x 25 cm wide) for resting and a wooden block for chewing and playing (diameter 7 cm, length 35 cm). Vixens were artificially inseminated. Daily routine treatments were conducted according to standard farming procedures (Tupeli 2011; Sepponen et al. 2014).

Diets and feeding

Experimental diets used were the same throughout the study periods (Koskinen et al. 2011). Freshly mixed farm fox feed was supplied twice a day. It was manufactured by the research station's feed kitchen. Palatability of feed was checked before the study. The feed was dispensed by a commercial feeding machine. Leftovers were collected the next day. Watering was automatic *ad libitum*. Daily feed portions were adjusted according to the animals' appetite and seasonal standards (Sepponen et al. 2014).

Blood sampling

During September-November blood samples were taken once a month. After the start of slimming in December, samples were taken on days 1, 2, 3 and 7. Thereafter, samples were taken every second week until insemination. Blood samples were taken from mated vixens on weeks 3, 5 and 7 during pregnancy. After whelping, during lactation period, samples were taken when the kits were age of 2 and 4 weeks.

Blood sampling was started every sampling day at 9 a.m. and lasted each time 2-4 hours. Animals

were fasted overnight prior to blood sampling. For sampling, vixens were sedated by using combination of Zoletil 100 (20 ml) and Dormitor (5 ml) (Korhonen and Huuki 2014). Sedative combination of 0.2 ml/animal was injected i.m. to hind leg. Blood was collected from the jugular vein through a 20-gauge needle into a syringe. Plasma was separated by centrifugation. Blood analyses (glucose, NEFA, triglyserides, urea, creatinine) were made by the research laboratory of Eastern Finland (Joensuu) and at the University of Western Australia, Perth (insulin, leptin, GH, IGF-1, prolactin)) according to standard methods (e.g. Khleifat et al., 2002; Sepponen et al. 2014; Korhonen and Huuki 2014; Nieminen et al. 2004).

Statistical analyses

For statistical analyses, entire research period was divided into four shorter periods: (1) autumn period (heavy fattening of Group 1); (2) winter period (heavy slimming of Group 1); (3) spring period (breeding season); and (4) summer period (post-weaning season). Analyses were made separately for each of four periods. There were all together 22 sampling times.

Statistical analyses were carried out by using SAS 9.2. statistical MIXED model procedure (SAS, 2009). The statistical model used was:

$$y_{ijk=}\mu + \tau_i + v_k + \varsigma_{ik} + \varepsilon_{ijk}$$

where μ was the general mean, τ_i was treatment$_i$ effect, v_k was time$_k$ effect, ς_{ik} treatment x time interaction and ε_{ijk} was the residual error.

Because animals were housed pairwise until Dec 8, 2010, mean of cage was used in analyses during autumn period. In order to have homogeneity of variances, log transformation was used for insulin, insulin-like growth factor (IFG-1), prolactin and triglycerides (TG) during all four periods. Log transformation was used for growth hormone (GH) and glucose during winter and spring periods, and for urea during summer period.

RESULTS

Summary of the results are shown in Figs. 1-10. Urea concentration varied seasonally (P<0.001). Concentrations were lowest during winter period. In Group 1, heavy slimming before mating season lowered urea concentration significantly (P<0.001). During autumn period, concentration was lowest in Group 3 because of pronounced feeding restriction (Fig.1). Creatinine levels were highest during summer period in all groups (Fig.2). Glucose concentration varied seasonally in all groups (P<0.001) (Fig.3). Intensive feeding clearly affected on glucose, insulin (Fig.4) and triglycerides (TG; Fig.5) (P<0.01). Leptin concentration (Fig.6) was highest in December in all groups. Thereafter, it clearly declined towards summer (P<0.001).Growth hormone (GH) (Fig.7) and insulin-like growth factor (IGF-1)(Fig.8) levels were highest during autumn period. Concentration of non-esterified fatty acids (NEFA) was highest in Group 1 (Fig.9).

Prolactin levels were same in all groups until insemination (Fig.10). During pregnancy, levels increased similarly in groups. After whelping, prolactin levels in Group 1 were low compared to Groups 2 and 3 (P<0.05). Number of kits at birth (mean ± SE) were in Groups 1, 2 and 3 3.9± 1.2, 7.3 ± 1.3 and 7.0 ± 1.7, respectively (P<0.001). Correspondingly, number of kits at age of two weeks were 0.9 ± 0.6, 5.5 ± 1.2, and 4.4 ± 1.4, respectively (P<0.001). Number of kits at age of four weeks in Groups 1,2 and 3 were 0.9 ± 0.6, 5.4± 1.2 and 3.5± 1.4, respectively (P<0.001).

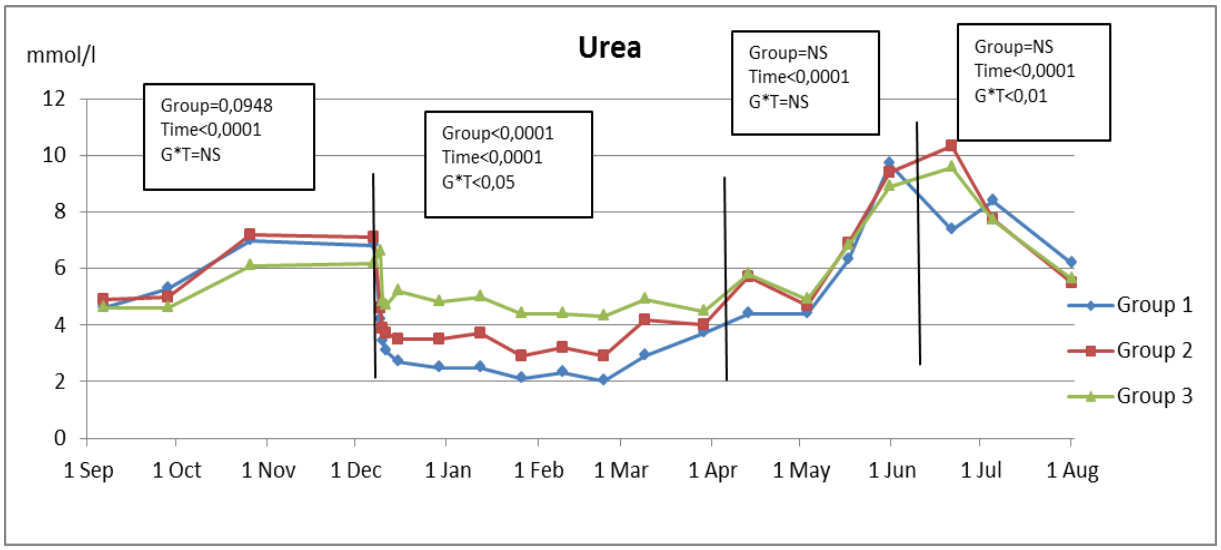

Fig 1. Seasonal changes in urea concentration. G=Group, T=Time. G*T=Group x Time interaction. P-values given when significantly different. NS=not significant.

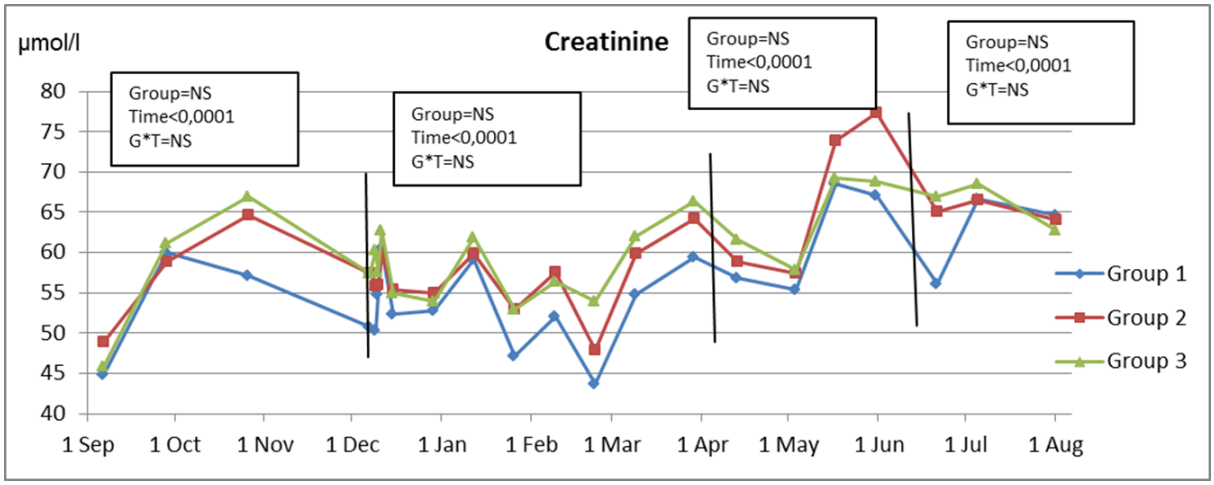

Fig. 2. Seasonal changes in creatinine concentration. G=Group, T=Time. G*T=Group x Time interaction. P-values given when significantly different. NS=not significant.

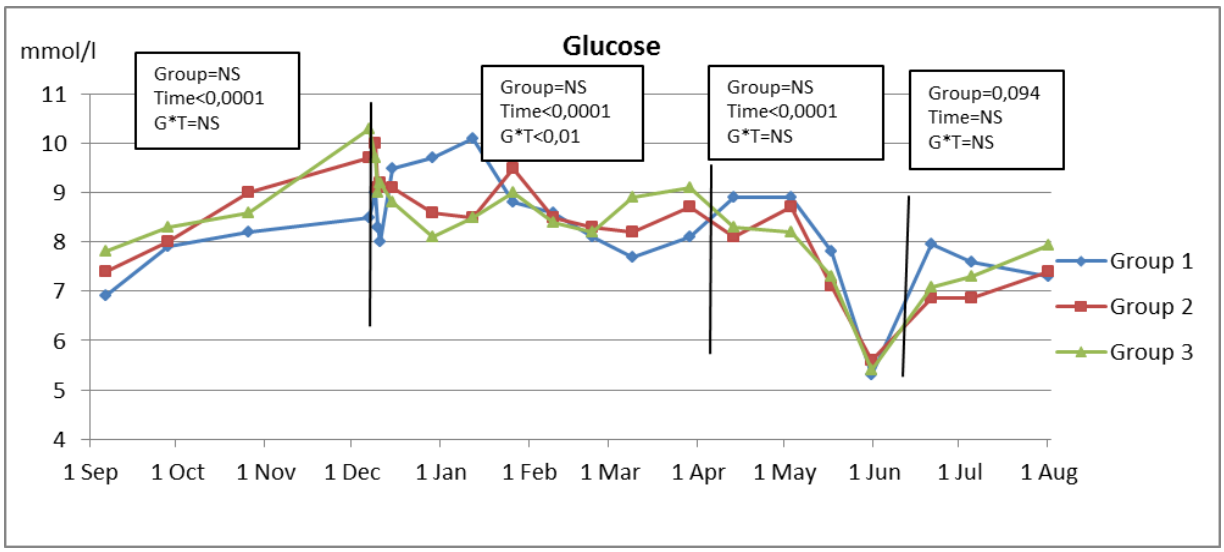

Fig. 3. Seasonal changes in glucose concentration. G=Group, T=Time. G*T=Group x Time interaction. P-values given when significantly different. NS=not significant.

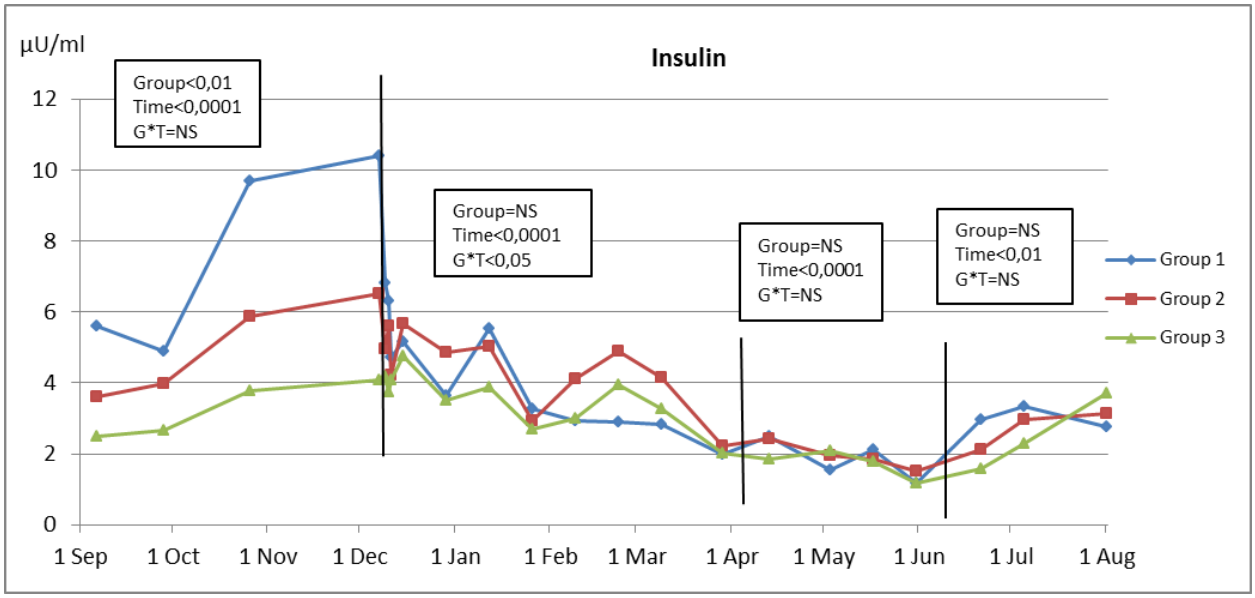

Fig. 4. Seasonal changes in insulin concentration. G=Group, T=Time. G*T=Group x Time interaction. P-values given when significantly different. NS=not significant.

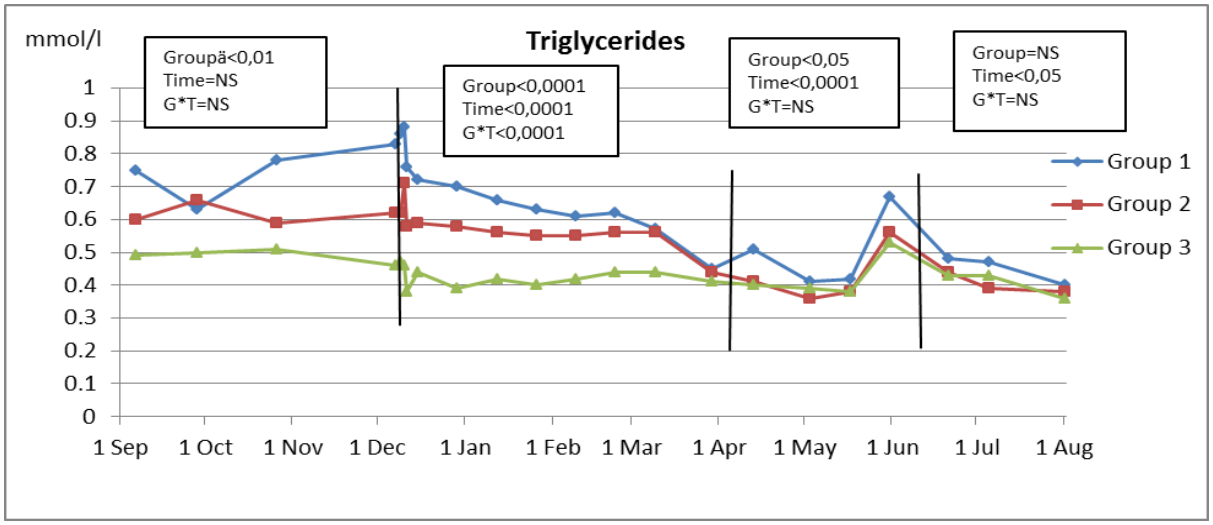

Fig. 5. Seasonal changes in triglyceride concentration. G=Group, T=Time. G*T=Group x Time interaction. P-values given when significantly different. NS=not significant.

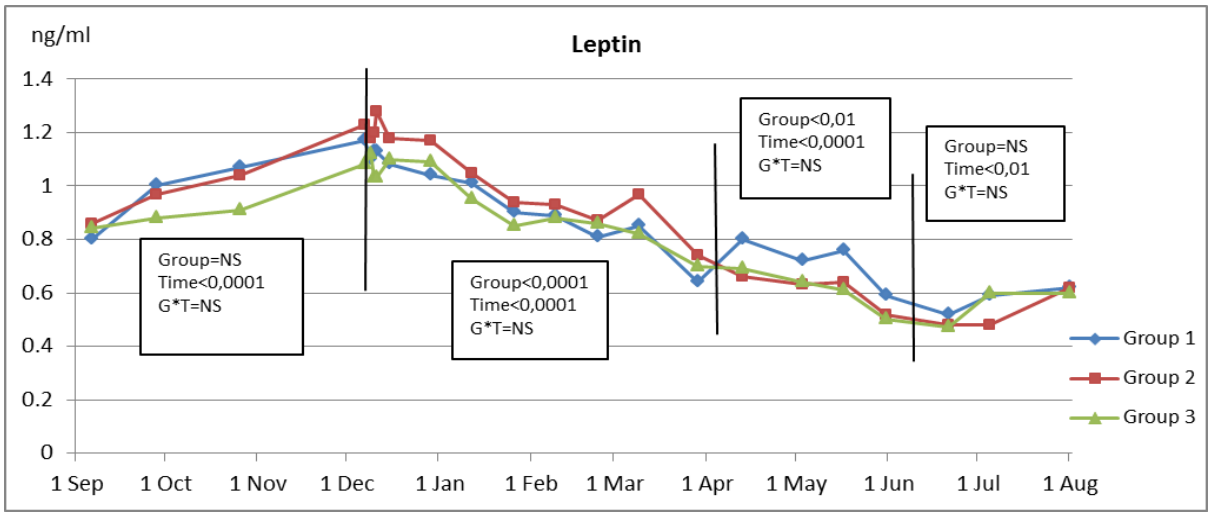

Fig. 6. Seasonal changes in leptin concentration. G=Group, T=Time. G*T=Group x Time interaction. P-values given when significantly different. NS=not significant.

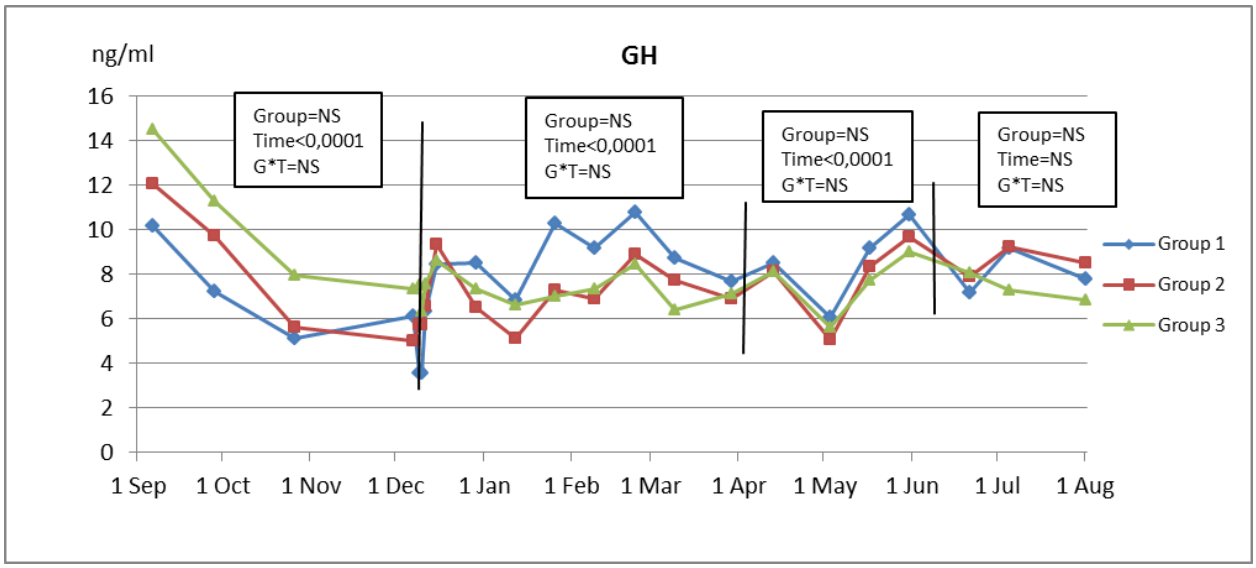

Fig. 7. Seasonal changes in growth hormone (GH) concentration. G=Group, T=Time. G*T=Group x Time interaction. P-values given when significantly different. NS=not significant.

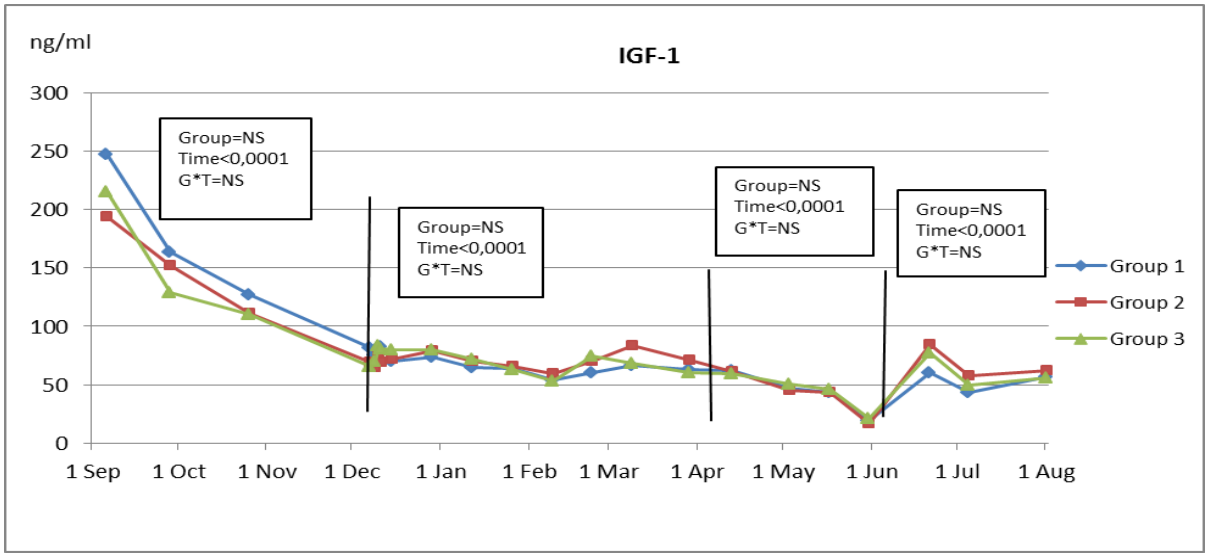

Fig. 8. Seasonal changes in insulin-like growth hormone (IGF-1) concentration. G=Group, T=Time. G*T=Group x Time interaction. P-values given when significantly different. NS=not significant.

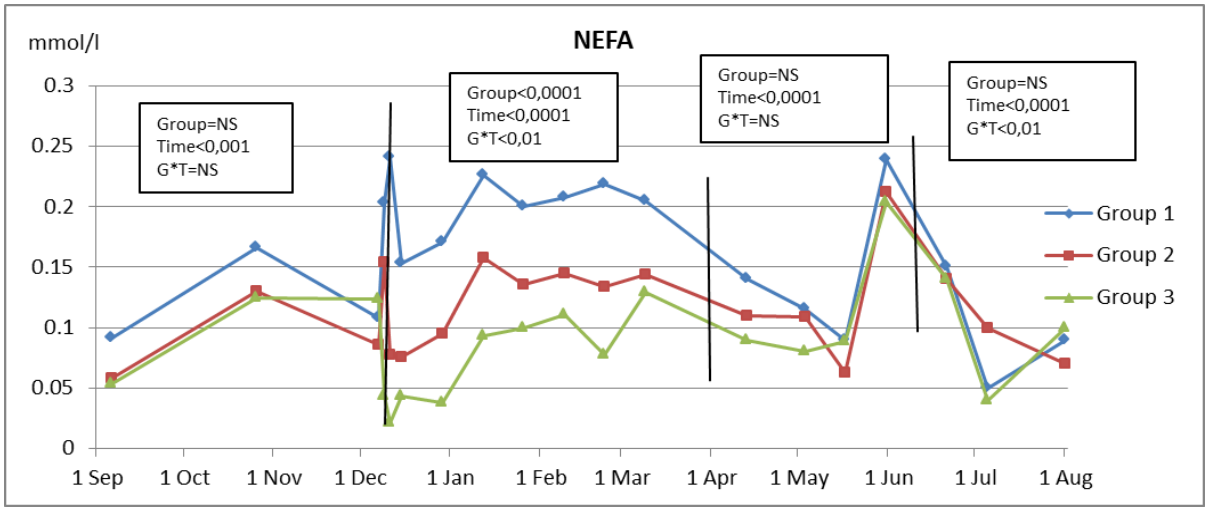

Fig. 9. Seasonal changes in non-esterified fatty acid (NEFA) concentration. G=Group, T=Time. G*T=Group x Time interaction. P-values given when significantly different. NS=not significant.

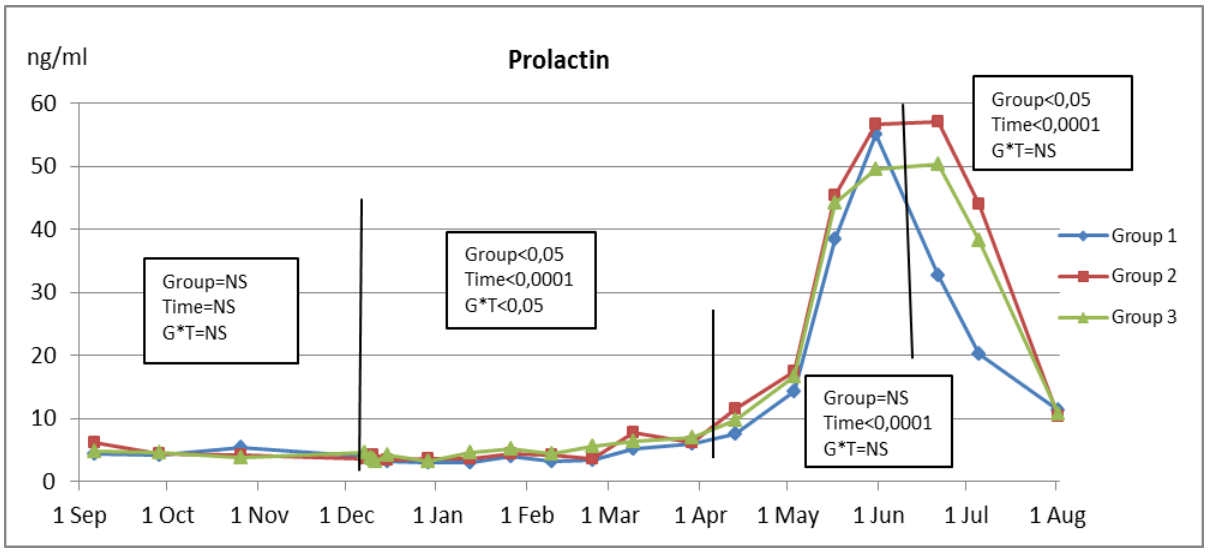

Fig. 10. Seasonal changes in prolactin concentration. G=Group, T=Time. G*T=Group x Time interaction. P-values given when significantly different. NS=not significant.

DISCUSSION

In mammals, the feedback-regulated systems that control the reproductive axis and the somatotropic axis interact at several levels and thus link nutritional and metabolic inputs into the reproductive process (Chagas et al. 2007).The preoptic-hypothalamic continuum is involved in the integration of appetite, estrus behavior and nutritional sensing, and produces the releasing hormones that control the secretion of gonadotropins and somatotropin. The gonadotropic system is the key driver of reproduction, whereas the somatotropic system is the key driver of milk production, lipolysis and tissue maintenance (Chagas et al. 2007). In the present study, effects of feeding intensity on gonadotropic and somatotropic systems were evaluated by following seasonal variation of several essential hormones.

Urea is the end product of nitrogen metabolism produced by liver. Animal body cannot exploit urea, therefore, it has to be removed from the body through kidneys. Urea concentration in the urine is typically affected by liver condition, diet and nutritional state (Ramsay et al. 1991; Charles and Leeming 1998). Low urea concentrations are found during undernourishment and intense fasting (Hori et al. 2006; Khleifat et al., 2006; Lawler et al. 2007). At early state of feeding restriction glucose levels are low and animals utilize fat storage as main energy source. This increases dissolving of fat but declines urea concentration.

In the present study, feed restriction was heaviest in Group 3 until the end of November. Urea concentration was correspondingly lowest in this group during autumn period. Feeding regimens of Group 1 and 2, on the other hand, did not affected on urea concentrations then. These concentrations were comparable to those found previously (Korhonen and Huuki 2014). Urea concentration of Group 1 was lowest during breeding season. Feeding restriction was also heaviest in Group 1 then which explains low concentration. During whelping period, urea concentrations in all groups increased which is explained by increased feeding level.

Creatinine is a breakdown product of creatinine phosphate in muscle, and is usually produced at a fairly constant rate by body (Khleifat et al., 2001; Schoch et al. 2006). Creatinine is removed from the blood chiefly by the kidneys. Creatinine is an important indicator of renal health because it is an easily measured byproduct of muscle metabolism that is excreted unchanged by the kidneys (Charles and Leeming 1998; Hori et al. 2006). Dehydration of body typically increases creatinine levels. Creatinine values found in the present study are normal (Rosen and Kumagai 2008; Yoghem et al. 2008; Korhonen and Huuki 2014; Sepponen et al. 2014) and very similar between study groups. Highest values were found at the end of pregnancy.

Glucose is an energy source of tissues (Verkest et al. 2012). Proper glucose concentration, i.e. blood sugar level, is essential for good brain function and health (Larson et al. 2003). Regular feeding rhythm and feeding intensity typically keep blood sugar levels constant (Lawler et al. 2007; Rosen and Kumagai 2008). If animal is fasting or feed amount is highly restricted, glucose needed is utilized from glycogen reservoirs of the body. In the present study, significant differences in glucose concentrations were not found between study groups. This tempts us to conclude that feeding regimens employed did not have essential influence on blood sugar levels. Measured glucose levels were close to those previously found (Korhonen and Huuki 2014).

Insulin is regulating energy metabolism of the body (Verkest et al. 2012). Particularly it has an essential role in sugar metabolism but it is also involved in regulation of protein and fat metabolism (Kemnitz et al. 1994; Barzilai and Rossett 1995). The amount of blood sugar is most import factor connected to insulin secretion. The amount of insulin secretion declines quickly when blood sugar goes down. In the present study, it was clearly seen that intensive feeding increases insulin but also blood sugar levels. Effect of feeding intensity on insulin levels was pronounced in *ad libitum* feeding (Group 1), i.e. insulin level was high during autumn period. After feed restriction in Dec-Jan, insulin levels declines quickly in Group 1.

Leptin is a protein that is made in the fat cells. It circulates in the bloodstream and goes to the hypothalamus in the brain (Zieba et al. 2005). Via this way fat cells convey information to the brain that the body's energy thermostat is set correctly and that enough energy is stored in fat cells to engage in normal metabolic processes. Leptin typically regulates metabolism, energy consumption and the amount of fat deposits in several mammals during winter (Nieminen et al. 2001; Mustonen et al. 2005; Zieba et al. 2005). Fasting and pronounced exercise declines the amount of excessive leptin levels in blood and tissues (Ishioka et al. 2005; Trisoli et al. 2013). In the present study, leptin levels were highest at the end of the year, i.e. when the animals were most obese. Towards summer levels declined parallel to body weights. The present results were very consistent showing that body weights and the amount of subcutaneous fat significantly influence on leptin levels seasonally.

Growth hormone (GH), also known as somatotrophin, is a peptide hormone that stimulates

growth, cell reproduction and regeneration (Poretsky et al. 1985; Shimizu et al, 2008; Strage et al. 2014). Particularly it affects growth and development of bones and skeleton. GH is a stress hormone that raises the concentration of glucose and free fatty acids but weakens effect of insulin. It also stimulates production of insulin-like growth factor 1(IGF-1). In the present study, GH concentrations were highest in early autumn which was due to intensive growth during that time. Late in the autumn, the concentrations stabilized and remained similar until the end of the year. Significant differences between the groups were not found. Differences in body weights thus were not reflected by changes in GH.

Insulin-like growth factor 1(IGF-1) is a protein which is produced primarily by the liver as an endocrine hormone as well as in target tissues in a paracrine/autocrine fashion (Pedersen et al. 2005). Production is stimulated by growth hormone (GH) and can be retarded by undernourishment. In the present study, IGF-1 levels were highest during early autumn when growth and feeding of animals was most intense. Towards winter the levels declined and reached a steady level. IGF-1 and GH levels were parallel. Feeding regimen did not have any influence on IGF-1 and GH levels.

The fat in the animal body is composed of triclyserides (TG). Its content in blood varies depending on diet composition. Typically high-fat food and intense weight gain increases the levels of blood TG. Fasting, on the other hand, declines TG values (Yochem et al. 2008). In the present study, *ad libitum* feeding significantly increased TG values during growing period. Correspondingly, intensive feeding restriction declined TG values. The present results are in accordance with previous findings regarding relation between feeding regimen and TG values (Sepponen et al. 2014).

Non-esterified fatty acids (NEFA) are main component of triclycerides and functioning as an energy source in several tissues (Vanholder et al. 2006). The plasma concentration increases in fasting as fatty acids are released from adipose tissue as metabolic fuel. NEFA reflects fat mobilization in relation to negative energy balance and stress (Lottati et al. 2008; Restitutti et al. 2012). In the present study, NEFA values were highest in *ad libitum* animals (Group 1) almost throughout the study. Lowest values were found in Group 3 which also had most restricted feeding regimen.

Prolactin (PRL), also known as luteotropic hormone or luteotropin, is a protein that is best known for its role in enabling vixens to produce milk. Prolactin is secreted from the pituitary gland in response to eating, mating, estrogen treatment, ovulation, and nursing (Mondain-Monval et al. 1985; Jöchle 1997). Prolactin is secreted in a pulsatile fashion in between these events. Prolactin also plays an essential role in metabolism, regulation of the immune system, and pancreatic development. In the present study, prolactin levels were low and similar in all study groups during autumn period. Prolactin level increased in all group at the onset of whelping period. In *ad libitum* animals (Group 1), prolactin levels declined quickly after whelping. This kind of decline in prolactin typically lowers whelping result (Sepponen et al. 2014). This was seen also in the present study; whelping result in Group 1 was very poor compared to Groups 2 and 3. Thus, heavy fattening during autumn and heavy slimming during winter have negative effects both on prolactin levels and whelping success.

CONCLUSIONS

It can be concluded that feeding intensity essentially influences on hormonal balance and reproduction in blue fox vixens. Too heavy fattening during autumn period and too heavy slimming before mating significantly declines reproduction result. Too intensive variations in energy and hormone balances may jeopardize animal welfare.

ACKNOWLEDGEMENTS

This study was financially supported by the Finnish Fur Breeders' Association and MTT Agrifood Research Finland. The staff of Research Station is acknowledged for their valuable help in carrying out this experiment. Special thanks to Dr. Dominique Blache (University of Western Australia) for help in analyses.

REFERENCES

Asikainen J. (2013). Wintering strategy of the boreal raccoon dog (*Nyctereutes procyonoides*) – Applications to farming practice. Dissertations in Forestry and Natural Sciences. University of Eastern Finland. pp. 82.

Barzilai N., Rossetti L. (1995). The relationship between changes in body composition and insulin responsiveness in models of the aging rat. Am. J. Physiol. Endocrinol. Metab. 269 (3):591-597.

Blache D., Zhang S., Martin G.B. (2003). Fertility in male sheep: modulators of the acute effects of nutrition on the reproductive axis of male sheep. Reprod. Suppl. 61: 387-402.

Burlikowska K., Szymeczko R., Iben C., Piotrowska A., Boguslawska-Tryk M., Kubacki S. (2008). Lipid metabolism indices in the blood serum of reproductive female polar foxes in relation to age of animals. Medycyna Wet. 64 (7): 879-882.

Chagas L.M., Bass J.J., Blache D., Burke C.R., Kay J.K., Lindsay D.R., Lucy M.C., Martin G.B., Meier S., Rhodes F.M., Roche J.R., Thatcher W.W., Webb R. (2007). Invited review: new perspectives on the

roles of nutrition and metabolic priorities in the subfertility of high-producing dairy cows. J. Dairy Sci. 90:4022-4032.

Charles J.M., Leeming N.M. ,(1998). Chronic dietary toxicity study on 2,4-dichlorophenoxybutyric acid in the dog. Toxicol. Sci. 46:134-142.

Fuglei E., Aanestad M., Berg J.P. (2000). Hormones and metabolites of arctic fox (*Alopex lagopus)* in response to season, starvation and re-feeding. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 126 (2): 287-294.

Fuglei E, Mustonen A.-M., Nieminen P. (2004). Effects of season, food deprivation and re-feeding on leptin, ghrelin and growth hormone in arctic foxes (*Alopex lagopus*) on Svalbard, Norway. Journal of Comparative Physiology B 174: 157-162

Hori Y., Uechi M., Kanakubo K., Sano T., Oyamada T. (2006). Canine ovarian serous papillary adenocarcinoma with neoplastic hypercalcemia. J. Vet. Med. Sci. 68 (9):979-982.

Ishioka K., Hatai H., Komabayashi K., Soliman M.M., Shibata H., Honjoh T., Kimura K., Saito M. (2005). Diurnal variations of serum leptin in dogs: effects of fasting and re-feeding. Vet. J. 169:85-90.

Jöchle W. (1997). Prolactin in canine and feline reproduction. Reprod. Dom. Anim. 32:183-193.

Kemnitz J.W., Roecker E.B., Weindruch R., (1994). Dietary restriction increases insulin sensitivity and lowers blood-glucose in rhesus-monkeys. Am. J. Physiol. 266 (4):540-547.

Kempe R. (2018). Selection for Welfare and Feed Efficiency in Finnish Blue Fox. Ph.D. Thesis. University of Helsinki, Faculty of Agriculture and Forestry. 59 p.

Khleifat, K., Shakhanbeh, J., & Tarawneh, K. A. (2002). The chronic effects of Teucrium polium on some blood parameters and histopathology of liver and kidney in the rat. *Turkish journal of Biology*, *26*(2), 65-71.

Khleifat, K., Homady, M. H., Tarawneh, K. A., & Shakhanbeh, J. (2001). Effect of Ferula hormonis extract on social aggression, fertility and some physiological parameters in prepubertal male mice. *Endocrine journal*, *48*(4), 473-482.

Khleifat, K. M., Abboud, M. M., Omar, S. S., & Al-Kurishy, J. H. (2006). Urinary tract infection in South Jordanian population. *J Med Sci*, *6*, 5-11.

Koivula M, Mäntysaari EA., Strandén I. (2009) New fertility traits in breeding value evaluation of Finnish blue fox. Acta Agriculturae Scandinavica Section A 59: 131-136

Korhonen H. **(**1988). Regulation of energy economy in raccoon dogs and blue foxes: a process of dynamic interactions. Comparative Biochemistry and Physiology 91A: 263-268.

Korhonen H.T., Huuki H. (2014). Serum biochemistry and hematology in blue fox (*Vulpes lagopus*). Open Journal of Veterinary Medicine 4: 255-260.

Korhonen H.T., Eskeli P., Lappi T., Huuki H., Sepponen J. (2014). Effects of feeding intensity and Ca:P ratio on foot welfare in blue foxes (*Vulpes lagopus*). Open Journal of Animal Sciences 4: 153-164.

Korhonen H.T., Ojala E., Moisander-Jylhä A-M., Kempe R., Sepponen J., Koistinen T., Peura J. (2017). Effects of plane of nutrition on foot condition and animal welfare in blue foxes. NJF Seminar 498, Radisson Blur Plaza Hotel, Oslo, Norway, 18- 20 October 2017. Proceedings pp. 55-56.

Koskinen N., Tauson A-H., Sepponen J. Rekilä T. (2008). Feeding history affects cub survival of young breeding blue foxes (*Alopex lagopus*), a field study. International Fur Animal Scientific Association 2008 Congress: August 19-23: Halifax, Nova Scotia, Canada

Koskinen N., Tauson A-H., Sepponen J., Rekilä T. (2009). Siniketun energiatarpeen määrittäminen. In: Rehunvalmistajien luentopäivät 10.-11.2. 2009 Keuruu

Koskinen N., Sepponen J., Tauson A-H. (2011). Feeding intensity and body condition of young blue fox vixens, effect on breeding result. NJF seminar 450, Knivsta, Sweden, 1-2 November, 2011. 2 pp.

Larson B.T., Lawler D.F., Spitznagel E.L., Kealy R.D. (2003). Improved glucose tolerance with lifetime diet restriction favorably affects disease and survival in dogs. J. Nutr. 133:2887-2892.

Lawler D.F., Ballam J.M., Meadows R., Larson BT., Li Q., Stowe H.D., Kealy R.D.(2007). Influence of lifetime food restriction on physiological variables in Labrador retriever dogs. Exp. Geront. 42:204-214.

Lottati M., Kolka C., Dittman J., Yae S., Harrison L.N., Mooradian V., Kirkman .E., Bergman R.(2008).Free fatty acids modulate insulin sensitivity following reduction of visceral fat by greater omentectomy in obese dogs. Obesity 16:62.

Mononen J., Lindeberg H., Korhonen H.T., Kempe R. (2018)**.** Large size of farmed foxes and its effects on animal welfare. Report for Fur Europe. Natural Resources Institute Finland, Luke. 42 p.

Mustonen A-M., Pyykönen T., Asikainen J., Hänninen S., Mononen J., Nieminen P. (2005). Circannual leptin and ghrelin levels of the blue fox (*Alopex lagopus*) in reference to seasonal rhythms of body mass, adiposity and food intake. J. Exp. Zool. 303A:26-36.

Mustonen A-M., Nieminen P. (2017). A review of the physiology of a survival expert of big freeze, deep snow, and an empty stomach: the boreal raccoon dog (*Nyctereutes procyonoides)* Journal of Comparative Physiology B, 1-11. DOI 10.1007/s00360-017-1114-5.

Mondain-Monval M., Møller O.M., Smith A.J,, McNeilly A,S., Scholler R.(1985). Seasonal variations of plasma prolactin and LH concentrations in the female blue fox (*Alopex lagopus*). J. Reprod. Fert. 74:439-448.

Nieminen P., Asikainen J., Hyvärinen H.(2001). Effects of seasonality and fasting on the plasma leptin and thyroxin levels of the raccoon dog (*Nyctereutes procyonoides*) and the blue fox (*Alopex lagopus*). J. Exp. Zool. 289:109-118.

Nieminen P, Saarela S, Pyykönen T, Asikainen J, Mononen J and Mustonen A-M 2004 Endocrine response to fasting in the overwintering captive raccoon dog (*Nyctereutes procyonoides*). Journal of Experimental Zoology 301A: 919-929.

Pedersen H.D., Falk T., Häggström J., Tarnow I., Olsen L.H., Kvart C., Nielsen M.O. (2005). Circulating concentrations of insulin-like growth factor-1 in dogs with naturally occurring mitral regurgitation. J. Vet. Intern. Med. 19:528-532.

Peura J, Strandén I, Smeds K., (2004). Genetics of litter size, age at first insemination and animal size in blue fox *(Alopex lagopus*). In: Urlings B, Spruijt B, Ruis M, Boekhorst L (eds) Proceedings of the VIII International Scientific Congress in Fur Animal Production, Scientifur *28*: 206-210.

Peura J, Strandén I, Mäntysaari AE., (2007) Genetic parameters for Finnish blue fox population: litter size, age at first insemination and pelt size. Agricultural and Food Science 16: 136-146.

Peura J., (2013). Optimization of the current breeding scheme for blue fox. PhD-thesis, Department of Agricultural Sciences Publications 2013:19. Unigrafia, Helsinki, Finland.

Poretsky L., Grigorescu F., Seibel M.,(1985). Distribution and characterization of insulin and insulin-like growth factor-I receptors in normal human-ovary. J. Clin. Endocrin. Metab. 61(4):728-734.

Pyykönen T., (2008) Environmental Factors and Reproduction in Farmed Blue Fox (*Vulpes lagopus*) *Vixens*. PhD-thesis, Kuopio University Publications C. Natural and Environmental Sciences 242. Kopijyvä, Kuopio, Finland.

Ramsay M.A., Nelson R.A., Stirling I. (1991). Seasonal-changes in the ratio of serum urea to creatinine in feeding and fasting polar bears. Can. J. Zool. 69 (2):298-302.

Restitutti F., Raekallio M., Vainionpää O., Kuusela E., Vainio O. (2012). Plasma glucose, insulin, free fatty acids, lactate and cortisol concentrations in dexmedetomidine-sedated dogs with or without MK-467: a peripheral α-2 adrenoceptor antagonist. Vet. J. 193:481-485.

Roche J.F. (2006). The effect of nutritional management of the dairy cow on reproductive efficiency. Anim.Reprod. Sci. 96:282-296.

Rosen D.A.S., Kumagai S. (2008). Hormone changes indicate that winter is a critical period for food shortages in Stellar sea lions. J. Comp. Physiol. B 178:573-583.

SAS Institute Inc. (2009). SAS/STAT® 9.2 User's Guide. Cary, NC: SAS Institute Inc.

Schoch R.D., Willoughby D., Greenwood M. (2006). The regulation and expression of creatinine transporter: a brief review of creatinine supplementation in humans and animals. J. Int. Soc. Sports Nutr. 3: 60-66.

Sepponen, J., Korhonen, H.T., Eskeli, P., Koskinen, N. (2014). Tuotanto- ja siitoskauden ruokinnan vaikutus siniketun rasva-aineenvaihduntaan ja siitoskuntoon. Loppuraportti 12.2.2014. 13 pp.

Shimizu T., Murayama C., Sudo N., Kawashima C., Tetsuka M., Miyamoto A.(2008). Involvement of insulin and growth hormone (GH) during follicular development in the bovine ovary. Anim. Reprod. Sci. 106:143-152.

Strage E.M., Lewitt M.S., Hanson J.M., Olsson U., Norrvik F., Lilliehöök I., Holst B.S., Fall T. (2014). Relationship among insulin resistance, growth hormone, and insulin-like growth factor I concentrations in diestrous Swedish elkhounds. J. Vet. Intern. Med. 28:419-428.

Tauson A.-H., Aldén E. (1984). Pre-Mating body weight changes and reproductive performance in female mink. Acta Agric. Scand. 34: 177-187.

Tauson A.-H., Aldén, E. (1985). Different feeding intensity levels to mink. 2. Effects on female reproductive performance, pre-weaning kit growth and longevity of females. Swedish J. Agric. Res. 15: 97-107.

Tauson A.-H., Forsberg M. (1992). Effects of flushing on LH release in mink, Norw. J. Agric. Sci., Suppl. 9: 235–241.

Tauson, A-H., Fink, R., Forsberg, M., Lagerkvist, G., Wamberg, S. (2000). LH release in mink (*Mustela vison*): pattern of the LH surge and effect of metabolic status. Reprod.Nutr. Dev.40:. 229-247.

Tauson, A-H., Forsberg, M., Chwalibog, A. (2002). Plasma concentrations of leptin mirror changes in body weight but do not influence the pattern of the preovulatory luteinizing hormone surge in mink (*Mustela vison*). J.Nutr. 132: 1790-1792.

Trisolini C., Albrizio M., Roscino M.T., Pantaleo M., Rizzo A., Sciorsci R.L. (2013). Leptin and queen ovary: new insights about ovulation. Res. Vet. Sci. 94:707-710.

Tupeli, R. (2011). Nuorten sinikettunaaraiden tuotantokauden aikaisen ruokinnan, painonkehityksen ja kuntoluokan vaikutus lisääntymistulokseen. Pro gradu -examination, University of Eastern Finland, Department of Biology.

Vanholder T., Leroy J.L. van Soom A., Maes D., Coryn M., Fiers T., de Kruif A., Opsomer G. (2006). Effect of non-esterified fatty acids on bovine theca cell steroidogenesis and proliferation in vitro. Anim. Reprod. Sci. 92:51-63.

Verkest K.R., Rand J.S., Fleeman L.M., Morton J.M. (2012). Spontaneously obese dogs exhibit greater postprandial glucose, triglyceride, and insulin concentrations than lean dogs. Domest. Anim.Endocrin. 42:103-112.

Wade G.N., Schneider J.E., Li H.Y. (1996). Control of fertility by metabolic cues. Am. J. Physiol. Endocrinol. Metab. 270:1–19.

Yochem P.K., Stewart B.S., Mazet J.A.K., Boyce W.M. (2008). Hematologic and serum biochemical profile of the northern elephant seal (*Mirounga angustirostris*): variation with age, sex, and season. J.Wildl. Dis. 44 (4):911-921.

Zieba D.A., Amstalden M., Williams G.L. (2005). Regulatory roles of leptin in reproduction and metabolism: a comparative review. Domest. Anim. Endocrinol. 29:166-185.