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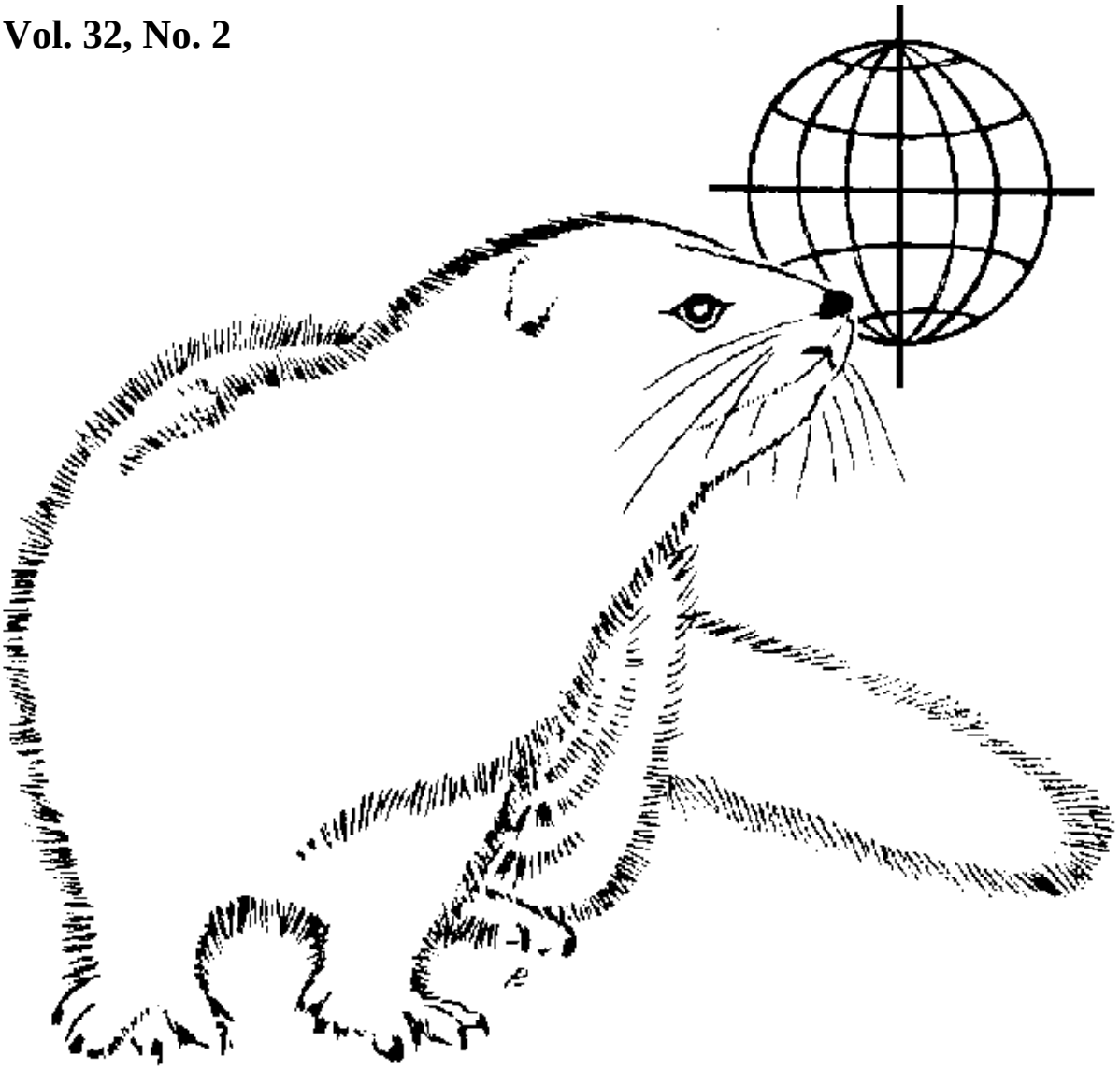

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INTERNATIONAL FUR ANIMAL SCIENTIFIC ASSOCIATION

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Notes from the Group of Editors

This issue of Scientifur, Volume 32, No 2, contains a vast number of abstracts of fur animal articles published in various periodicals by Finnish researchers.

Furthermore, this issue also carries an interesting article on purification methods for mink immunoglobulins as well as a Chinese short communication on the effect of replacing fish and meat meal with different proportions of soybean diets on the metabolism and reproductive performance of raccoon dogs.

For your information the last two issues of Scientifur, Volume 32, will contain the proceedings of the 2008 IFASA Congress, which is to be held in Halifax, Nova Scotia Canada, August 19-23. For more information on the Congress see http://www.ifasanet.org/congress.

On behalf of the
Group of Editors

Birthe Damgaard

A note on stereotyped behaviour in pair and group housed farmed juvenile raccoon dogs

L. Ahola, S. Hänninen, J. Mononen

Farmed fur animals are traditionally housed in cage environment that is often considered as too barren to meet the species-specific needs of these animals. Group housing has been suggested to offer an easy and feasible way to enrich the housing environment of farmed social animal species, including the raccoon dog. Therefore, we assessed the effects of group housing on the behaviour of farmed juvenile raccoon dogs. Farmed raccoon dog litters were housed either traditionally in pairs or in sextets. The space allocation was 0.6 m2 per animal. The present results showed that farmed juvenile raccoon dogs housed in sextets prefer to stay in tight groups all through their growing season. The cubs housed in pairs, although spending less time in active behaviours, performed more stereotyped behaviour especially in August than the sextet-housed cubs. Accordingly, the present finding indicates that group housing creates an enriched farm environment for farmed raccoon dogs and, thus, possibly enhances the welfare of these animals.

Applied Animal Behaviour Science, 2007: 174-180.

Effects of family housing on some behavioural and physiological parameters of juvenile farmed mink (*Mustela vison*)

S. Hänninen, J. Mononen, S. Harjunpää, T. Pyykönen, J. Sepponen, L. Ahola

Farmed mink are traditionally weaned at the age of 6–8 weeks and the kits live in male–female pairs until pelting time (approximately 6 months of age). It is possible that keeping an entire litter together with the dam in a row-cage system until pelting could provide the juvenile mink with some form of social enrichment, lasting maternal contact and a more diverse physical environment. We compared traditional pair housing of mink in standard mink cages (P group, 13 litters) with family housing in row-cage systems where several standard mink cages were connected to each other via openings in the nets separating the cages (F group, 13 litters). The F kits were housed in families of five to nine siblings and their mother. The P kits were housed in male–female pairs. Animal density was the same for both groups. There were altogether 41 F male, 33 F female, 31 P male and 31 P female kits, and 13 F and 13 P dams in the experiment. At the end of the study, the F kits had more bite scars (score 3.5 ± 0.2 for males and 4.3 ± 0.2 for females) than the P kits (males 1.1 ± 0.1, females 1.3 ± 0.1) ($P < 0.05$), indicating that aggression may have been more common in family-housed than pair-housed kits. The mass of adrenals (males F: 107 ± 5 mg versus P: 123 ± 6 mg, females F: 105 ± 5 mg versus P: 107 ± 5 mg, $P < 0.01$) and serum cortisol levels after ACTH administration (439 ± 28 nmol/l versus 448 ± 32 nmol/l, 457 ± 31 nmol/l versus 501 ± 31 nmol/l, respectively, $P = 0.067$) were lower in F than P kits, which might indicate that the F kits had experienced less long-term stress than the P kits. The housing system had no effect on the body mass of the kits at any time points when they were weighed, although feed consumption was lower in the F group than in the P group (209 ± 38 g/(d animal) versus 248 ± 15 g/(d animal)) in November ($P < 0.01$). In the late autumn, with sub-zero temperatures, the F animals typically huddled together in one nest box, which may have provided them with thermoregulatory benefits. These benefits might partially explain the difference in the function of the HPA-axis between the two groups. To conclude, although aggression was a severer problem in family than pair housing, the problems were altogether milder than in some earlier studies. The mink kits housed as families might have been less stressed than the pair-housed kits, but the stress results were rather ambiguous.

Applied Animal Behaviour Science, 2008: 384-395.

Blue foxes' (*Alopex lagopus*) preferences between earth floor and wire mesh floor

T. Koistinen, L. Ahola, J. Mononen

Blue foxes (Alopex lagopus) with access to both earth floor and wire mesh floor have shown a preference, measured as time allocation, for a wire mesh floor. However, in these earlier studies there have been several confounding factors (e.g. with the size and elevation of the floors) that were controlled for in the present preference experiment. In addition to the blue foxes' preference, we measured the foxes' behaviour both on an earth floor and awiremesh floor, and evaluated the effects of an

earth floor deprivation on the behaviour of the foxes. From the cubs' age of 4 weeks (July), 16 fox families (vixen and cubs) were housed in an outdoor fur animal shed in cage systems consisting of two fox cages. At the cubs' age of 8 weeks, the cubs were separated from their mother and thereafter only the experimental male–female sibling pairs were housed in the experimental cages until December. In the eight pairs of the control group, there was a wire mesh floor in both cages,whereas in the eight pairs of the earth group, there was a wiremesh floor in one cage and a 30–40 cm deep earth floor in the other cage. The behaviour and locations of the foxes' were recorded using instantaneous sampling (with a sampling interval of 5 min) for 24 h five times, i.e. in September just before an earth floor deprivation (SEP or BDE), on the 9th day of the 14-day deprivation (DEP), on the 1st day after the deprivation (ADE), in October (OCT) and in December (DEC). The recordings SEP, OCTand DEC were interpreted as a preference setup and, the recordings BDE, DEP and ADE were interpreted as a deprivation setup. The control group used both available cages equally, whereas the earth group used the wire mesh cage more than the earth floor cage for both resting and activity. The earth group showed less oral stereotypic activity than the control group. A rebound effect in digging and sniffing on sand was observed after the earth floor deprivation in the earth group. The results confirm the earlier findings that farm born blue foxes prefer a wire mesh floor to earth floor when measured as time allocation. However, farmed foxes with early experience of an earth floor may value the behaviours enabled by the presence of an earth floor.

Applied Animal Behaviour Science, 2008: 38-53.

Blue foxes' motivation for access to an earth floor measured by operant conditioning

T. Koistinen, L. Ahola, J. Mononen

The aims of the present series of experiments were to measure farmed blue foxes' motivation for access to an earth floor exploiting operant conditioning, and to analyse the foxes' behaviour on the earth floor in different seasons. Six farm-born blue fox males were used in the experiments. The foxes' motivation for access to the earth floor was measured in autumn, winter and spring. In each season, the foxes were exposed to work for access to the earth floor on a fixed ratio (FR) sequence (FR4, FR8, FR16, FR32 and FR64) in operant apparatus designed for the study. The FR sequence was repeated three times in autumn and spring and twice in winter. In each experiment, the foxes were tested every second day in 6-h test sessions. The reward, i.e. the possibility to stay on the earth floor, lasted for 4 min. The slope and intercept of the demand curve were calculated. The foxes' behaviour during the earth floor rewards was analysed using continuous recording. The slopes of the fragment of the demand curves for the earth floor ranged from −0.40 to −0.31. The slope or the intercept of the demand curve revealed no differences between the seasons. However, the foxes' behaviour on the earth floor varied between the seasons. The time spent digging and the number of vole jumps were lowest in winter when the earth floor was frozen. The percentage of play behaviour from the total time spent on the earth floor was highest during autumn, when the foxes were young and the earth floor was not frozen. In conclusion, blue foxes are motivated for access to an earth floor. The motivation for access to the earth floor does not change between the seasons or with age or experience of the foxes. Foxes' behavioural profile on the earth floor varies according to the properties of earth floor and is possibly also dependent on the age of foxes.

Applied Animal Behaviour Science, 2007: 328-341.

Blue foxes' motivation to gain access to solid floors and the effect of the floor material on their behaviour

T. Koistinen, Mononen

Farmed blue foxes are willing to work to gain access to a sand floor from a wire mesh floor. It is not clear whether the foxes work for the sand floor because of its solidity or because it enables them to perform certain behaviours, e.g. exploration and digging. Here, we measured blue foxes' motivation to gain access from a wire mesh floor to a floor with 15–30 cm deep sand, a floor with 3–4 cm deep sand, a solid concrete floor, and another wire mesh floor. In addition, we analysed the foxes' behaviour on these floor materials. Seven male blue foxes were trained and tested in self-constructed operant

apparatuses. In an apparatus, the fox could move a bottomless test cage from a wire mesh floor to a neighbouring, alternative floor material for a 4-min visit by pressing a lever in the test cage for a fixed number of times (Fixed Ratio, FR). The foxes worked for each floor material for 12 days. In each daily test session, the foxes were exposed to work on one of the four workloads (FR 6, 12, 24, 48), for 3 h. The behaviour of the foxes was analysed during the 4-min visits on each floor material. The results showed that there was no difference between the floor materials, either in the demand elasticity of the fragment of the demand curve (ranging from □0.46 to □0.33), or in the intensity of the demand. However, the foxes' behaviour varied between the floor materials. More digging, play, rooting (exploration with the muzzle), and vole jumping were observed on the floor materials with sand, than on the concrete floor and the wire mesh floor. Both the presence and the depth of the sand layer stimulated these behaviours. It is concluded that juvenile blue foxes do not value solid floor materials more than a wire mesh floor. However, the sand floor stimulates more digging, play, vole jumping, and exploration than the concrete floor or wire mesh floor. Furthermore, the depth of sand may be an important factor in eliciting these behaviours. Access to a floor material with sand may improve the welfare of farmed blue foxes by providing the possibility to perform species-specific behaviours.

Applied Animal Behaviour Science, 2007: in press, corrected proof.

Water baths for farmed mink: intraindividual consistency and interindividual variation in swimming behaviour, and effects on stereotyped behaviour

J. Mononen, M. Mohaibes, S. Savolainen, L. Ahola

Swimming behaviour and effects of water baths on stereotyped behaviour in farmed mink (Mustela vison) were studied in three experiments. The singly-housed mink had access from their home cages to extra cages with 20.5 litre water baths. Two short-term experiments aimed to investigate how quickly adult and juvenile mink start using and how consistently they use water baths over 10 days, and whether the extent of the use correlates between dams and their females kits. A four-month experiment was designed to compare the development of stereotyped behaviour in juvenile mink housed with and without swimming opportunity. The behavioural analyses were based on several 24-hour video recordings carried out in all three experiments. There were obvious inter-individual differences and intra-individual consistency in swimming frequency and time. Farmed mink's motivation to swim can be assessed in short-term experiments, and measurement of water losses from the swimming baths and use of instantaneous sampling with 10 min sampling intervals provide quite reliable measures of the amount of swimming. The bath use of the juveniles correlated with that of their dams, indicating that an individual mink's eagerness to swim may have a genetic component. The lower amount of stereotyped behaviour in mink housed with water baths indicates that long-term access to baths may alleviate frustration in singly-housed juvenile farmed mink.

Agricultural and Food Science, 2008: 41-52.

Lipid metabolism in the adipose tissues of a carnivore, the raccoon dog, during prolonged fasting

A.M. Mustonen, R. Käkelä, A. Käkelä, T. Pyykönen, J. Aho, P. Nieminen

Previous studies on laboratory rodents, rabbits, and humans have demonstrated that adipose tissue fatty acid (FA) mobilization is selective, and its efficiency is related to the molecular structure of FAs. This study was undertaken to find out whether such preferences of FA mobilization are a general feature of mammalian white adipose tissue (WAT) and are also manifested in carnivores. Fractional mobilization of a wide spectrum of FAs was studied by gas-liquid chromatography from six subcutaneous (scapular, rump, ventral) and intra-abdominal (omental, mesenteric, retroperitoneal) WAT depots of raccoon dogs (Nyctereutes procyonoides) fed or fasted for 2 months. Fasting stimulated the mobilization of shorter-chain saturated, mono-unsaturated (MUFAs), and polyunsaturated FAs (PUFAs). The effects of unsaturation and the position of the first double bond from the methyl end were more inconsistent. The effect of double-bond position may be due to

chain shortening of longer-chain MUFAs and preferential utilization of n-3 PUFAs over n-6 PUFAs. Moreover, there were site-specific differences in fractional mobilization, the omental adipose tissue being the most divergent. The in vivo FA mobilization from the regional WAT depots of a carnivore was selective, and the molecular structure of the FA affected its efficiency.

Experimental Biology & Medicine, 2007:58-69.

The effect of a combination of permanent breeding cage and low housing density on the reproductive success of farmed blue foxes

T. Pyykönen, S. Hänninen, M. Mohaibes, J. Sepponen, J. Mononen, L. Ahola

Social factors are known to affect the reproduction of many canids both in the wild and in farms. For example, reproduction in farmed silver foxes is regulated by social stress; foxes seem to benefit from noncramped housing conditions and permanent breeding cages. However, no comparable studies have been carried out in farmed blue foxes.

The aim of our experiment was to create an alternative, improved, economically viable and practical housing solution for blue foxes. Therefore, we compared reproductive performance of blue foxes in permanent breeding cages with low animal densities (L group, N = 79) and traditional housing with its changing social environment with high animal density (H group, N = 74). The reproductive data from the L and H groups were compared separately for primiparous and multiparous vixens because the reproductive performance in primiparous vixens was substantially lower ($P < 0.001$) than in multiparous vixens.

Altogether, 41 and 39% of the primiparous vixens in the H and L group whelped ($P > 0.05$), but only 28 and 34%, respectively, weaned at least one cub ($P > 0.05$), i.e., 72 and 66% of the primiparous vixens did not reproduce in the H and L group, respectively ($P > 0.05$). The total reproductive performance, expressed as cubs at weaning per breeding female, was 1.7 ± 3.5 for the H and 1.6 ± 2.9 for the L group ($P > 0.05$). In the primiparous vixens, the only statistically significant difference observed between the two housing systems was that the onset of oestrus occurred five days earlier in the H than in the L group ($P < 0.05$).

All multiparous vixens in the L group exhibited oestrus compared to 94% in the H group ($P > 0.05$). Furthermore, there was a nonsignificant (ns) trend for fewer barren females (9% versus 17%), more successfully reproducing vixens (83% versus 74%) and a higher number of live-born cubs (10.9 ± 4.7 versus 9.4 ± 3.9) in the L than in H group in the multiparous vixens (for all $P > 0.05$). This resulted in 1.7 and 1.4 cubs more per breeding and per mated vixen, respectively, at weaning in the L group (7.3 ± 5.0) compared to the H group (5.6 ± 4.2), but also this difference was nonsignificant.

Although our present results lack statistical significance, they are promising enough to encourage field experiments with sufficiently large number of animals to prove or disprove these preliminary findings that lower housing density and permanent breeding cage, together or separately, may enhance reproduction particularly in multiparous blue fox vixens.

Animal Reproduction Science, 2008:255-264.

Aviation noise does not impair the reproductive success of farmed blue foxes

T. Pyykönen, J. Juntunen, L. Ahola, A. Parri, J. Mononen

The aim of our study was to assess the effects of aviation noise on reproduction and cub mortality in farmed blue foxes. Eighty artificially inseminated blue fox vixens (45 primiparous and 35 multiparous) were exposed to aviation noise on 5 days when they were pregnant or had cubs. The noise during the exposures varied from 85 to 121 dB ($L_{AF\,max}$). Vixens (45 primiparous and 34 multiparous) on a farm without flight action acted as controls. Cubs were counted 1, 3, 7, 14 and 49 days postpartum and at the beginning of July. Litter size (cubs per whelped vixen), reproductive performance (cubs per mated vixen) and cub losses (lost cubs per whelped vixen) were analyzed from both experimental farms (A and C).

The flight action had no effect on reproductive success. Reproductive performance in primiparous

vixens was 4.2 ± 3.8 and 4.3 ± 3.6 cubs (ns, Mann–Whitney U-test) in the control and aviation group, respectively, while in multiparous vixens the corresponding figures were 7.1 ± 4.4 and 7.3 ± 3.8 cubs (ns).

In general, litter size declined from birth to weaning (in primiparous vixens from 8.1 ± 3.8 to 5.4 ± 3.2 cubs, and in multiparous from 9.7 ± 3.8 to 7.2 ± 3.8 cubs, $P < 0.001$, GLM for repeated measures). The decline was greater in primiparous than in multiparous vixens ($P < 0.01$). There were no differences in total cub losses between the experimental groups (ns).

Accordingly, the present results show that exposure to severe and repeated aviation noise does not impair the reproductive success of farmed blue foxes.

Animal Reproduction Science, 2007:128-136.

Physiological adaptations to fasting in an actively wintering canid, the Arctic blue fox (*Alopex lagopus*)

A.M. Mustonen, T. Pyykönen, M. Puukka, J. Asikainen, S. Hänninen, J. Mononen, P. Nieminen

This study investigated the physiological adaptations to fasting using the farmed blue fox (Alopex lagopus) as a model for the endangered wild arctic fox. Sixteen blue foxes were fed throughout the winter and 32 blue foxes were fasted for 22 d in Nov-Dec 2002. Half of the fasted blue foxes were food-deprived again for 22 d in Jan-Feb 2003. The farmed blue fox lost weight at a slower rate (0.97-1.02% body mass d-1) than observed previously in the arctic fox, possibly due to its higher initial body fat content. The animals experienced occasional fasting-induced hypoglycaemia, but their locomotor activity was not affected. The plasma triacylglycerol and glycerol concentrations were elevated during phase II of fasting indicating stimulated lipolysis, probably induced by the high growth hormone concentrations. The total cholesterol, HDL- and LDL-cholesterol, urea, uric acid and total protein levels and the urea:creatinine ratio decreased during fasting. Although the plasma levels of some essential amino acids increased, the blue foxes did not enter phase III of starvation characterized by stimulated proteolysis during either of the 22-d fasting procedures. Instead of excessive protein catabolism, it is liver dysfunction, indicated by the increased plasma bilirubin levels and alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase activities, that may limit the duration of fasting in the species.

Journal of Experimental Zoology Part A: Comparative Experimental Biology, 2005: 32-46.

Selective fatty acid mobilization in the American mink (*Mustela vison*) during food deprivation

P. Nieminen, R. Käkelä, T. Pyykönen, A.M. Mustonen

The mobilization of fatty acids (FAs) during food deprivation is a selective process in laboratory rodents and humans. The site-specific differences in adipose tissue functions - e.g. energy storage versus insulation - should also affect the use of different FAs. To study this, 16 female minks were randomly assigned into the control group or fasted for 5 days. Preferential mobilization of n-3 polyunsaturated FAs (PUFAs) during fasting caused a decrease in the n-3/n-6 PUFA ratio in fat and liver. In addition, the minks utilized short-chain FAs efficiently in all fat depots, but long-chain FAs - 20:0, 20:1n-11, 20:1n-9, 22:1n-11 and 24:1n-9 - were preserved. The number of double bonds in the FA chain correlated positively with mobilization rate in the retroperitoneal fat. The observed negative correlation between mobilization rate and the location of the first double bond from the methyl end may be due to peroxisomal chain-shortening of long-chain FAs and not the double bond position per se. As a result, minks are able to preserve a low melting point and fluidity of the subcutaneous fat depots, which would be essential to a Northern semi-aquatic mammal.

Comparative Biochemistry and Physiology, Part B, Biochemistry and molecular biology, 2006: 81-93.

Endocrine and metabolic alterations in the mink (*Mustela vison*) due to chronic phytoestrogen exposure

A. Ryökkynen, A.M. Mustonen, T. Pyykönen, P. Nieminen

Phytoestrogens are natural components of plant-based food items with beneficial health effects. The aim of the present study was to investigate the chronic effects of dietary phytoestrogens, genistein (8 mg kg(-1) day(-1)) and beta-sitosterol (50 mg kg(-1) day(-1)), on the weight regulation of the mink (Mustela vison). The parental generation was exposed from August 2002 to May-June 2003 to either beta-sitosterol or genistein, while the kits were exposed through gestation and lactation. Food consumption and body masses were monitored monthly. Plasma lipid, glucose, total protein and hormone (ghrelin, leptin, triiodothyronine and thyroxine) concentrations were measured from the parents in August 2002, January 2003 and at the end of the experiment in May-June 2003 when the kits were 21 days of age. Relative food intake was higher in the beta-sitosterol-exposed minks than in the control or genistein minks in September 2002. Plasma leptin and total protein concentrations were lower in the beta-sitosterol kits compared to the control kits. Furthermore, plasma ghrelin levels and liver phosphorylase activities of the mink kits were higher due to genistein exposure. In mink kits, exposure to both phytoestrogens reduced the plasma thyroxine concentrations. The kidney glycogen concentrations and the muscle phosphorylase activities of phytoestrogen-treated adult minks were elevated. The results of this study suggest that minks are sensitive to perinatal phytoestrogen exposure.

Chemosphere, 2006:1753-1760.

Group size and space allocation in farmed juvenile blue foxes (*Alopex lagopus*)

L. Ahola, J. Mononen, T. Pyykönen, M. Mohaibes, S. Hänninen

Farmed juvenile blue foxes were housed either singly, in pairs, or in quartets at a stocking density of either 0.6 m2 or 1.2 m2 per animal. The effects of group size and space allocation on physiological, behavioural and production-related parameters were assessed. The results showed that the larger space allocation, although having only minor effects on the measured parameters, allowed the foxes to maintain their individual space even in the larger group sizes. Social tension within the groups affected the behavioural and production-related parameters to a greater extent than did space allocation. The sex-related dominance order, with males having easier access to feed than females, and females having more bite scars and higher serum cortisol levels than males, appears to be the major factor affecting the general performance of mixed-sex group-housed farmed blue foxes. These results suggest that group housing of farmed juvenile blue foxes could be considered as an alternative, socially enriched way of housing these animals.

Animal Welfare, 2005: 1-9.

Adaptations of the raccoon dog (*Nyctereutes procyonoides*) to wintering – effects of restricted feeding or periodic fasting on lipids, sex steroids and reproduction

J. Asikainen, A.M. Mustonen, T. Pyykönen, S. Hänninen, J. Mononen, P. Nieminen

The raccoon dog (Nyctereutes procyonoides) is an omnivorous canid with autumnal hyperphagia and fattening followed by mid-winter passivity and fasting in boreal latitudes with seasonal snow cover. The effects of two different feeding levels (400 or 200 kcal/animal/d) or fasting (5-week fasting+1-week feeding+3-week fasting) on plasma lipids, sex steroids and reproductive success of farm-bred raccoon dogs (n=60 females and 24 males) were studied in winter. The body masses, body mass indices (BMIs) and levels of plasma triacylglycerols (TG), total cholesterol and low- and high-density lipoprotein cholesterol did not differ between the fed and the restrictively fed animals. During fasting, the plasma TG concentrations increased and the BMIs decreased, indicating the release of fatty acids from adipose tissue. After the fasting periods, the levels of plasma cholesterol and high-density lipoprotein cholesterol increased,

whereas the TG levels decreased indicating the rebuilding of energy reserves. The fact that the different wintertime feeding regimes had no impact on the plasma glucose, total protein, cortisol, estradiol, progesterone or testosterone levels, or on the reproductive success, indicates versatile adaptive capacity in the species.

Journal of Experimental Zoology, Part A, Comparative Experimental Biology, 2005: 861-871.

Circannual leptin and ghrelin levels of the blue fox (*Alopex lagopus*) in reference to seasonal rhythms of body mass, adiposity and food intake

A.M. Mustonen, T. Pyykönen, J. Asikainen, S. Hänninen, J. Mononen, P Nieminen

The aim of the study was to investigate the circannual rhythms of leptin and ghrelin in the blue fox, a variant of the endangered arctic fox, in relation to its seasonal cycles of body mass, adiposity and food intake. Theeffects of long-term fasting and exogenous melatonin treatment on these weight-regulatory hormones were also investigated. The leptin concentrations of the blue fox increased during the autumnal accumulation of fat and decreased during the wintertime and vernal weight loss periods. The leptin levels peaked 2-6 weeks before the maximum values were observed for the body mass indices, voluntary food intake, and body masses. The ghrelin concentrations fluctuated widely during the autumn but decreased in the winter in association with suppression of food intake. Exogenous melatonin advanced the seasonal changes in the food intake of the blue fox but did not affect the seasonal rhythms of leptin and ghrelin concentrations. The leptin concentrations did not respond to the 3-week fasting periods in a consistent way, but the ghrelin levels increased due to food deprivation. In addition to the amount of fat in the body the leptin secretion of the blue fox may be regulated also by other factors. The blue fox may also express seasonal changes in its leptin sensitivity. Our results reinforce the hypothesis that leptin does not function as an acute indicator of body adiposity in seasonal carnivores but rather as a long-term signal of nutritional status.

Journal of Experimental Zoology, 2005: 26-36.

Adiponectin and peptide YY in the fasting blue fox (*Alopex lagopus*)

A.M. Mustonen, T. Pyykönen, P. Nieminen

Adiponectin (Acrp30) and peptide YY (PYY) are weight-regulatory hormones participating in the control of energy homeostasis. This study investigated the effects of long-term wintertime fasting on plasma Acrp30 and PYY levels in the carnivorous blue fox, a farm-bred variant of the arctic fox (Alopex lagopus). Plasma Acrp30 and PYY concentrations were determined with radioimmunoassays during a 22-day period of fasting, which led to a 20.3% reduction in body mass of the animals (n=32). Sixteen fed blue foxes served as the control group. Acrp30 and PYY were present in blue fox plasma at similar or lower levels as reported previously for other mammals. Fasting had no acute effects on Acrp30 or PYY concentrations of the blue foxes. However, the Acrp30 levels of the fasted blue foxes were 24%-48% higher than in the fed animals between days 8-22 of fasting. Fasted blue foxes also had 6.2-fold higher plasma PYY concentrations after 15 days of fasting. Acrp30 and PYY seem to play roles in the body weight-regulation of the blue fox during long-term fasting, but their specific functions and physiological significance remain to be determined.

Comparative Biochemistry and Physiology, Part A, Molecular and Integrative Physology, 2005: 251-256.

Adaptations to fasting in the American mink (*Mustela vison*): nitrogen metabolism

A.M. Mustonen, M. Puukka, T. Pyykönen, P. Nieminen

The aim of this study was to investigate the adaptations of protein metabolism to seasonal fasting in an actively wintering boreal carnivore. Fifty farm-bred male American minks Mustela vison were divided into a fed control group and four experimental groups fasted for 2, 3, 5 or 7 days. The responses of nitrogen metabolism to wintertime food deprivation were determined by measuring the rate of weight loss, the tissue total protein concentrations and the plasma amino acid, urea, ammonia, uric acid and total protein levels. The mink has relatively poor adaptations to food deprivation, as it is not able to prolong phase II of fasting with fat as the major metabolic fuel. Instead, the species has to derive a part of its energy requirements from the breakdown of body proteins. The end product of protein catabolism--urea--accumulates in its circulation, and the mink may not be able to recycle urea-N. Although the mink can still have a high body fat percent at the end of the 7-day fast, it appears to enter phase III of fasting with stimulated proteolysis during this period.

Journal of Comparative Physiology, Part B, Biochemical, Systemic, and Environmental Physiology, 2005:357-363.

Thermoregulatory adaptations of the overwintering captive raccoon dog (*Nyctereutes procyonoides*) in boreal climate

P. Nieminen, E. Hohtola, T. Pyykönen, T. Paakkonen, J. Aho, M. CittováKontu, J. Asikainen, J. Mononen, A. M. Mustonen

The raccoon dog (Nyctereutes procyonoides) is a nocturnal canid thought to utilise passive wintering strategy in the boreal climate. To record the deep body temperature (Tb), 12 farmed raccoon dogs were implanted with intra-abdominal Tb loggers on November 26, 2003. Between December 3, 2003 and January 27, 2004 half of the animals were fasted for 8 weeks. The amplitude of the diurnal Tb oscillations increased due to fasting. However, the mean diurnal Tb was lower in the fasted animals only during two occasions. Unlike observed previously in other species, not only did the raccoon dogs experience hypothermia between 0600 and 1000 hr but also hyperthermia between noon and 1800 hr. The fasted animals were as active as the fed animals measured after 42-43 days of fasting and there was a significant cross-correlation between physical activity and Tb. The nocturnal period of hypothermia is probably an adaptation to save energy during food deprivation. The diurnal hyperthermia could be explained by the opportunistic foraging behaviour of the species. Opposite to the established assumptions, the raccoon dog does not seem to enter winter sleep on fur farms. In the future it is important to determine if true winter sleep occurs in nature in the species.

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Periparturient behaviour in farmed blue foxes (*Alopex lagopus*)

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Recently, there has been criticism of the housing conditions available for farmed foxes. It is claimed that the present farm conditions are not appropriate for foxes and they lead to poor reproduction and abnormal behaviour such as maternal infanticide. Earlier studies have indicated that farmed silver foxes can be infanticidal. The main factors leading to infanticidal behaviour seem to be of a social nature rather than factors related to the physical housing environment. However, this infanticidal behaviour of silver foxes has often been extrapolated to the farmed blue fox although previous studies have indicated that

the situation in farmed blue foxes seems to be quite different.

Our study aimed at describing and quantifying periparturient behaviour in blue fox vixens inside their nest box, with special emphasis on possible infanticidal behaviour. The behaviour of 16 vixens was video-recorded from 7 days before the expected delivery to 7 days postpartum. The behaviour was analysed separately for pre-, peri- and postpartum periods. Moreover, reproductive performance and cub mortality from birth to weaning were evaluated.

The mean litter size at birth was 10.8 cubs, but this had declined to 7.8 cubs at weaning ($P < 0.05$, GLM). Approximately 2% of the cubs were stillborn and 80% of the cub deaths occurred during the first week of life. The postnatal cub mortality was higher among the primiparous than in the multiparous vixens (32.7 and 16.7% of cubs, respectively, $P < 0.05$, $\chi 2$-test), animals which had already undergone selection for their good reproductive capabilities. Despite the neonatal cub losses, the blue fox vixens exhibited proper maternal care. During parturition, the vixens licked their genital area and helped actively in the deliveries whenever necessary. Between the deliveries of individual cubs and after parturition, the vixens showed sufficient cub-care and nursing behaviour. Most importantly, no obvious signs of maternal infanticide were observed. There were no major differences in the periparturient behaviour of the young and old females, although the primiparous vixens were probably more restless than their multiparous counterparts.

Our results confirm that the lack of maternal behaviour in farmed blue foxes is not a common problem. In the present study, behavioural problems such as infanticide were not observed. However, the cub losses were still relatively high. Accordingly, management in blue fox production needs evaluation and improvement to enhance cub survival. Therefore, we wish to emphasize the behavioural differences and farming practices between farmed blue and silver foxes. Moreover, we suggest that one should be cautious of extrapolating results from one species to another, even when those species are closely related.

Applied Animal Behaviour Science, 2005: 133-147.

Phytoestrogens alter the reproductive organ development in the mink (*Mustela vison*)

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The aim of the present study was to examine the reproductive effects of two perorally applied phytoestrogens, genistein (8 mg/kg/day) and β-sitosterol (50 mg/kg/day), on the mink (Mustela vison) at human dietary exposure levels. Parental generations were exposed over 9 months to these phytoestrogens and their offspring were exposed via gestation and lactation. Parents and their offspring were sampled 21 days after the birth of the kits. Sex hormone levels, sperm quality, organ weights, and development of the kits were examined. The exposed females were heavier than the control females at the 1st postnatal day (PND). The control kits were heavier than the exposed kits from the 1st to the 21st PND. Phytoestrogens did not affect the organ weights of the adult minks, but the relative testicular weight of the exposed kits was higher than in the control kits. The relative prostate weight was higher and the relative uterine weight lower in the β-sitosterol-exposed kits than in the control kits. Moreover, the plasma dihydrotestosterone levels were lower in the genistein-exposed male kits compared to the control male kits. This study could not explain the mechanisms behind these alterations. The results indicate that perinatal phytoestrogen

exposures cause alterations in the weight of the reproductive organs of the mink kits.

Toxicology and applied pharmacology , 2005: 132-139.

Effects of fasting and exogenous melatonin on annual rhythms in the blue fox (*Alopex lagopus*)

P. Nieminen, T. Pyykönen, J. Asikainen, J. Mononen, A.M. Mustonen

The arctic fox (Alopex lagopus) is a winter-active inhabitant of the high arctic with extreme fluctuations in photoperiod and food availability. The blue fox is a semi-domesticated variant of the wild arctic fox reared for the fur industry. In this study, 48 blue foxes were followed for a year in order to determine the effects of exogenous melatonin and wintertime food deprivation on their reproductive and thyroid axes. Half of the animals were treated with continuous-release melatonin capsules in July 2002, and in November-January, the animals were divided into three groups and either fed continuously or fasted for one or two 22-day periods. Food deprivation decreased the plasma triiodothyronine and thyroxine concentrations probably in order to preserve energy due to a decreased metabolic rate. The same was observed in the plasma testosterone levels of the males but not in the plasma estradiol concentrations of the females. Exogenous melatonin advanced the autumn moult and seasonal changes in the voluntary food intake. It also advanced the onset of the testosterone peak in the males. The plasma estradiol levels of the females were unaffected, but the progesterone levels peaked more steeply in the sham-operated females. Melatonin exerted a strong influence not only on the reproductive axis of the males but also on the seasonal food intake. The species seemed quite resistant to periodic involuntary food deprivation.

Comparative Biochemistry and Physiology, Part A, Molecular and Integrative Physology, 2004: 183-197.

Endocrine response to fasting in the overwintering captive raccoon dog (*Nyctereutes procyonoides*)

P. Nieminen, S. Saarela, T. Pyykönen, J. Asikainen, J. Mononen, A.M. Mustonen

The raccoon dog (Nyctereutes procyonoides) is an omnivorous canid utilizing the passive wintering strategy in the boreal climate. Farmed raccoon dogs (n=12) were randomly assigned into two study groups on 26 November 2003. Between 3 December 2003 and 27 January 2004, half of the animals were fasted for 8 weeks and plasma weight-regulatory hormone concentrations determined on 26 November and 30 December 2003 and on 27 January 2004. The plasma peptide YY, ghrelin, and growth hormone (GH) concentrations increased due to food deprivation, while the T_4 and Acrp30 concentrations decreased. Furthermore, the plasma GH concentrations were higher in the fasted raccoon dogs than in the fed animals, which had higher plasma insulin, glucagon, and T_4 concentrations. However, fasting had no effect on the plasma leptin concentrations. The results confirm previous findings with unchanged leptin levels in fasting carnivores. Increased GH levels probably contribute to increased lipolysis and mobilization of fat stores. Ghrelin can also enhance lipolysis by increasing the GH levels. The decreased levels of T_4 may reduce the metabolic rate. The plasma dopamine concentrations decreased due to fasting unlike observed previously in rats. Together with the unaffected adrenaline, noradrenaline, and cortisol concentrations, this suggests that food deprivation in winter does not cause stress to the raccoon dog but is an integral part of its natural life history.

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Endocrinologic adaptations to wintertime fasting in the male American mink (*Mustela vison*)

A.M. Mustonen, S. Saarela, T. Pyykönen, P. Nieminen

The aim of this study was to investigate the endocrine response to wintertime starvation in the male American mink (*Mustela vison*) fasted for 16 hrs, 2 days, 3 days, 5 days, or 7 days (n = 10 per group). After 2 days of fasting, the plasma leptin concentrations decreased, along with the triiodothyronine, testosterone, and progesterone levels, and the blood monocyte counts. Leptin also seems to trigger the response to fasting in mustelids by inducing immunosuppression and downregulation of the reproductive and thyroid axes. The dramatic increase in the peptide YY concentrations after 3 days of fasting may be required to suppress gastrointestinal processes during food scarcity. The plasma insulin levels decreased, and those of glucagon increased after 5 days of fasting in association with efficient glucose sparing and lipid mobilization. Body energy stores cannot be wasted for growth during nutritional scarcity and, thus, the growth hormone levels of the minks decreased after 5 days of fasting. The plasma noradrenaline and cortisol concentrations also decreased after 3 and 7 days without food, respectively. The plasma ghrelin, adiponectin, resistin, thyroxine, adrenaline, or estradiol levels did not respond to fasting. The endocrine response to food deprivation is remarkably similar in divergent mammalian orders, indicating that the hormonal signals enhancing survival during nutritional scarcity must be evolutionarily old and well conserved.

Experimental Biology and Medicine, 2005: 612-620.

Hyperthermia and increased physical activity in the fasting American mink (*Mustela vison*)

A.M. Mustonen, T. Pyykönen, J. Aho, P. Nieminen

The aim of this study was to investigate the thermoregulatory adaptations to fasting in a medium-sized mustelid with a high metabolic rate and energetic requirements. Sixteen farm-bred female American minks, Mustela vison, were divided into a fed control group and an experimental group fasted for 5 days. The deep body temperature (Tb) of the minks was registered at 10 min intervals with intraabdominal thermosensitive loggers and the locomotor activity was videotaped continuously for 5 days during the fasting procedure. The Tb of the fasted animals increased during the first day of fasting and decreased during the second day. After 3–4 days of fasting, the levels of physical activity and Tb of the fasted minks increased above the levels of the fed animals. Significant increases in these parameters were observed at the beginning of the working day on the farm, during the feeding of the fed animals and around midnight. It is concluded that the mink differs from previously studied homeotherms in thermoregulatory and behavioral responses to fasting probably due to its high energy requirements and predatory success.

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Group housing of farmed silver fox cubs

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The present study, the effects of social environment on the welfare of farmed silver fox cubs were clarified. After weaning, cubs from silver fox littes were housed (1) singly, (2) in litters until the end of September and thereafter singly, or (3) in litters throughout their growing season. Separating the cubs at the onset of the species' natural dispersal time may not be strictly beneficial for the cubs because it may limit the animals' possibilities to fulfil their needs for social behaviour. However, the lower incidence of bite wounds in both the single housed cubs and the cubs from litters that were split in autumn showed some beneficial effects of separating the cubs. The cubs that were group housed in litters for the whole time were focussed on their own social system, were more averse to human presence and showed greater responses to acute stress that the cubs that were single housed for at least part of the time. However, the serum cortisol level following adrenocorticotropic hormone administration suggested that cubs that were group housed in litters were less stressed over the long-term compared with the cubs that were single housed for at least part of the time; the low incidence of stereotypic behaviour in the cubs raised litters also supports this hypothesis. Accordingly, and despite some unsolved questions regarding interpretation of the hypothalamic-pituitary-adrenal axis activity, the results from this present study show that social contacts were important for the welfare of silver fox cubs, and suggest that farmed silver fox cubs could possibly be raised in litters without jeopardising their welfare or deteriorating their fur quality.

Animal Welfare, 2006: 39-47.

Purification, characterization and ELISA detection of mink immunoglobulins

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Abstract

This study describes easy purification methods for mink IgG, IgA and IgM immunoglobulins. IgG and IgM were purified from normal mink serum, while IgA was purified from mink bile from healthy animals. By SDS-polyacrylamid-gel-electrophoresis (SDS-PAGE) and immunoblotting under reducing conditions the estimated molecular weights of the immunoglobulin gamma, alpha and mu heavy chains were found to be 54 kDa, 69 kDa and 83 kDa respectively. The purities of purified IgG, IgM and IgA were estimated by immunoglobulin class specific ELISAs to be more than 90% for IgG and IgM, and more than 80% for IgA.

Introduction

Studies on the immune system of minks have received special attention due to two interesting virus infections, Aleutian disease of mink (also known as mink plasmacytosis, Aasted 1985) and the mink distemper infection (Blixenkrone-Moller 1993) . Mink IgG, IgA and IgM immunoglobulins were characterized already in 1972 and the immunoglobulin classes were described to have the same physicochemical properties as other mammalian immunoglobulins (Coe and Hadlow, 1972). Evidence for 4 subclasses of IgG immunoglobulins has also been presented (Tabel & Ingram, 1972). The light chain distribution of mink immunoglobulins was originally found to be exclusively of the lambda type (Hood et al. 1967), but later studies indicated that mink have lambda and kappa light chains in a ratio of 55/45 (Bovkun et al., 1993). There seems to be multiple C-lambda genes in mink of which at least 3 are functional, and a single C kappa gene has been found (Najakshin et al. 1993, Bovkun et al., 1993). Southern blot analyses indicated that there are from 5-7 C gamma genes (including pseudogenes, Najakshin et al. 1996). To our knowledge there are no easy mink immunoglobulin purification methods described in the literature. Also it is difficult to obtain commercially available reagents with defined specificities against mink immunoglobulins. This also accounts for the monoclonal antibodies to heavy and light chains of mink IgG that Peremislov et al. reported on in 1992. In this report we design an easy way to purify mink IgG, IgA and IgM and we also describe immunoglobulin class specific ELISAs.

Materials and methods

Mink

Healthy mink (*Mustella vison*) were of black genotype (Scanblack). They were housed in separate cages and fed a standard mink diet. Mink blood was taken by heart puncture and a serum pool of 200 ml was established.

IgG purification

Two ml of the mink serum pool were passed through a column packed with 2 ml of Protein G Sepharose 4 fast flow (GE Healthcare,Bio-Sciences, Uppsala, Sweden). The column was washed extensively with 0.1M Na-acetate, pH. 5.0 and eluted with 0.1M glycine/HCl buffer, pH 2.6. The eluted fractions were analysed by SDS-PAGE (10% Tris-HCl gels, BIO-RAD, Hercules CA), and IgG-containing fractions were pooled, dialysed against PBS and concentrated on Microcon centrifugal filter

devices (YM-100 Millipore Corporation, Bedford, MA, USA).

Mink IgM purification
The IgG depleted flow through fraction from the above mentioned purification procedure was chromatographed on a Sephacryl S-300 column (78 cm long and 2 cm in diameter, Pharmacia, Uppsala, Sweden), and the fractions were analysed by SDS-PAGE (10% gels). A 2 ml sample of normal mink serum (i.e. non IgG depleted) was also chromatographed on a Sephacryl S-300 column (see results section).

Purification of mink IgA from bile
A pool of mink bile was made out of selected bile samples with low yields of IgG and IgM. This pool was dialysed against a buffer consisting of 0,5M NaCl, 0,02M Tris/HCl 1 mM CaCl2, 1mM MnCl2, 0.02 M NaN3, pH: 7.4. Precipitate created during dialysis was removed by centrifugation for 10 min at 14000 g. The dialysed and centrifuged pool was then passed through a column packed with Protein A Sepharose 4 fast flow (GE Healthcare). The Protein A Sepharose column was washed extensively with 0.1M Na-acetate, pH. 5.0 and eluted with 0.1M glycine/HCl buffer, pH 2.6. The eluted fractions were analysed by SDS-PAGE (10% Tris-HCl gels), and IgA containing fractions were pooled, dialysed against PBS and concentrated (Microcon filters, se above).

Production of a rabbit antiserum to mink IgG
A rabbit antiserum to purified mink IgG was prepared essentially as described before (Aasted, 1989). In short, six rabbits were immunized bimonthly with 0.5mg of the mink IgG preparation in Freund's incomplete adjuvant. Blood was drawn 10 days after the fourth immunization and serum isolated. A pool of the antisera was established. This antiserum pool was strongly reacting with mink IgG, but was also reacting to mink IgA and IgM due to antibodies to the common light chains. In order to make the serum pool specific for mink IgG, mink IgA and IgM immunosorbents were constructed by coupling 5 mg of mink IgA and 5 mg of mink IgM to 2 ml of cyanogen-bromide-activated Sepharose (according to manufacturers instructions, GE Healthcare). One ml of the antiserum pool was then absorbed with 0.1 ml of immunosorbent material. This procedure was repeated until all mink light chain reactivity was removed (as verified by ELISA, see below). Thereafter the rabbit immunoglobulin fraction was isolated from the absorbed rabbit anti-mink IgG immunoglobulin pool by the same Protein G Sepharose chromatography technique as described above (IgG purification section). Part of this rabbit anti-mink IgG immunoglobulin pool was biotinylated with sulfo-NHS-biotin (Sigma-Aldrich, USA) according to the manufacturer's instructions.

ELISAs
Our own absorbed rabbit anti-mink IgG immunoglobulin serum, a commercial rabbit anti-human IgM (Dako, Copenhagen, Denmark A0425) cross-reacting with mink IgM (Porter et al. 1984) and a goat antibody to canine IgA (AbD Serotec, Oxford, UK AAI31) cross reacting with mink IgA, were diluted in 50mM carbonate buffer (pH 9.6) to a concentration of 2µg/ml or 200ng/well in MaxiSorp Immuno Plates (Nunc, Roskilde, Denmark). The antibodies were allowed to absorb overnight at 4°C. The wells were then emptied and blocked for 30 minutes with 1% Bovine Serum Albumin (Sigma) in PBST (PBS with 0.1% Tween 20) followed by 3 times wash. Immunoglobulin preparations to be analysed were two-fold titrated in PBST with 0.1% BSA and added to the ELISA wells and incubated for 1h at room temperature with shaking followed by another wash procedure. For the IgG ELISA, a 1:1000 dilution of our own biotinylated rabbit anti-mink IgG preparation (see above) was added to the ELISA plate wells and incubated for 1 hour at room temperature followed by 3 times wash in PBST. Finally a 1:1000 dilution of Horseradish peroxidase conjugated Streptavidin (Dako P0397) was added to the Wells (IgG ELISA plates) and incubated for 1 h at room temperature with shaking. For the mink IgM ELISA detection a 1:1000 dilution of a mink IgM cross reacting peroxidase labelled rabbit anti-human IgM preparation (Dako P0215) was added to the wells and for the IgA ELISA detection a 1:1000 dilution of a mink IgA cross reacting peroxidase labelled goat anti-canine IgA (Serotec AAI31P) was used. Peroxidase activity was measured by normal OPD staining and stop procedure. The staining intensities for all three ELISAs were measured by an ELISA reader (Easy Reader EAR 400, SLT-Labinstruments, Austria). The reactions were read at a wavelength of 492 nm with a reference wavelength of 620 nm.

SDS-PAGE and Immunoblotting
Protein fractions from the Sephacryl S-300 Sepharose gel fractionation were analysed by SDS-PAGE and immunoblotting. SDS-PAGE

electrophoresis were performed with 10% SDS-Polyacrylamide gels (Bio Rad, Hercules, Calif. USA). After electrophoresis, the proteins were transferred to Nitrocellulose (NC) membranes in a semi-dry blot chamber (JKA, Biotech, Denmark). After transfer, the NC membranes were blocked with 10% non-fat milk in PBST for 1 h at room temperature followed by four times wash in PBST (5 minutes per wash). The immunological detection systems for IgG, IgM and IgA localisation were identical to the ones used in above mentioned for ELISAs. The peroxidase-labelled reagents were then added in a dilution of 1:500 in PBST with 0.1% Bovine Serum Albumin (BSA, Fraction V, Sigma, St. Louis, MO, USA) and incubated for either two hours at room temperature or overnight at 4°C with shaking. After another 4 washes the membranes were stained with 2 mM DAB (Dako) staining solution in 50mM Tris buffer (pH 7.6) containing 0.01% hydrogen peroxide. Occasionally this staining solution was enhanced with 2 mM nickel (II) chloride.

Results

Sephacryl S-300 fractionation of a pool of mink serum.

Mink serum immunoglobulins can be separated from each other on a Sephacryl S-300 (high resolution) fractionation column (figure 1).

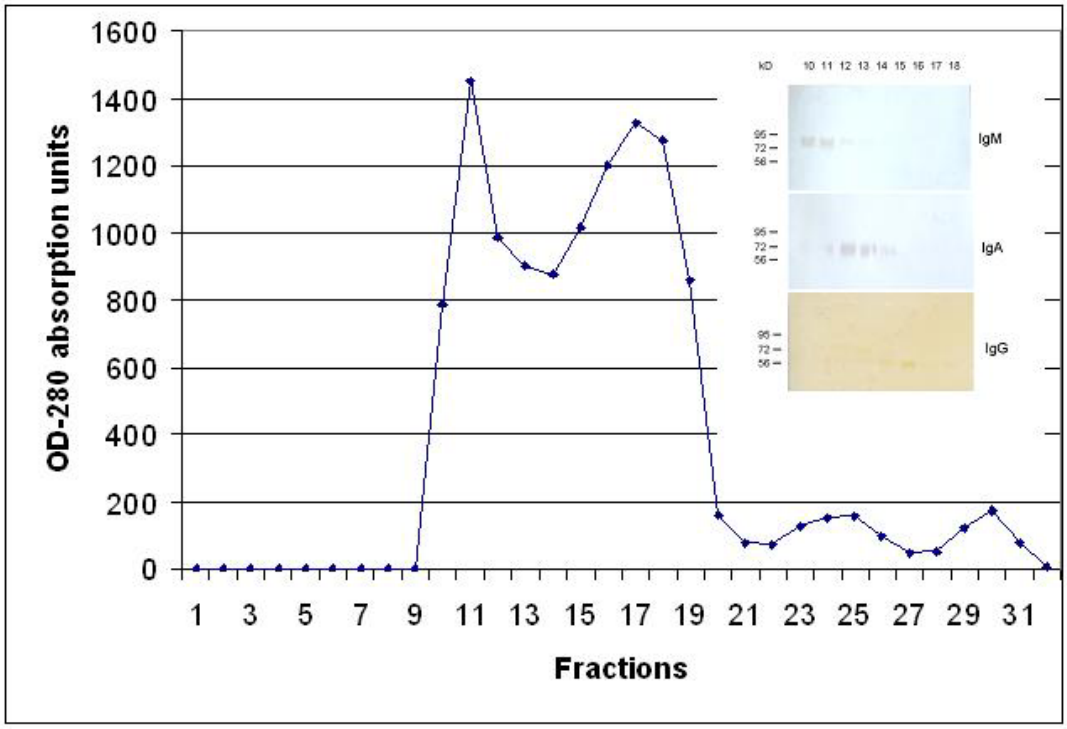

Figure 1. Separation of 2 ml of normal mink serum on a Sephacryl S-300 (high resolution) fractionation column (78 cm long and 2 cm in diameter). The X axis indicate fractions collected (of 5.7 ml). The y-axis indicate protein content of the fractions as measured by OD-280 absorption units. In the upper right corner of the figure immune blots of samples numbers 10-18 from the Sephacryl S-300 fractionation experiment are shown. The nitrocellulose blots were developed with 3 mink class specific antibody reagents: a mink IgM cross-reactive rabbit antiserum to human IgM (upper blot), a mink IgA cross-reactive goat antiserum to canine IgA (middle blot) and our own rabbit antibody to mink IgG (lower blot). The position of the relevant molecular weight marker proteins (95 – 56 kD) are indicated to the left of the blots.

Proof of separation of IgM from IgA from IgG is illustrated by immunoblotting in the 3 upper right blots in figure 1 using mink class specific antibody reagents (the anti-IgM and anti-IgA were cross reacting antibodies). As shown, the separation of the immunoglobulins from each other are not total, and more importantly such Sephacryl S-300 fractionation experiments have indicated, that IgG containing immune complexes may be present in normal sera. Such immune complexes co-migrate with the IgM containing fractions (can barely be seen in figure 1 lowest (IgG) blot). To prevent this potential contamination risk of high molecular IgM preparations with IgG complexes, it is therefore an

advantage to remove IgG from the serum sample by Protein G Sepharose affinity chromatography before Sephacryl S-300 fractionation of the IgG depleted serum sample. This procedure not only prevents the contamination risk of an IgM preparation containing IgG immune complexes but Protein G Sepharose affinity chromatograph does also provide an excellent source of almost pure serum IgG (see materials and methods). Besides immunoblotting, all protein containing Sephacryl S-300 fractions were analysed by SDS-PAGE. The results from these SDS-gels indicated that the very first protein containing fraction contained a mixture of the high molecular weight proteins IgM and alpha-2 macroglobulin and that it was devoid of IgA (see immunoblotting results in figure 1). In order to remove the alpha-2 macroglobulin, the fraction was passed through a column packed with Protein A Sepharose 4 fast flow (GE Healthcare), which binds IgM but not alpha-2 macroglobulin (protein A also binds IgA, but the fraction did not contain IgA). The column was washed extensively with 0.1M Na-acetate, pH. 5.0 and eluted with 0.1M glycine/HCl buffer, pH 2.6. The eluted protein containing fractions were dialysed against PBS and concentrated (Microcon centrifugal filters, se above).

Mink bile provides a fairly pure source of secretory IgA.

Mink bile contains a high concentration of secretory IgA. This is illustrated in figure 2 where 3 different individual bile samples were separated by SDS-PAGE (samples 1-3).

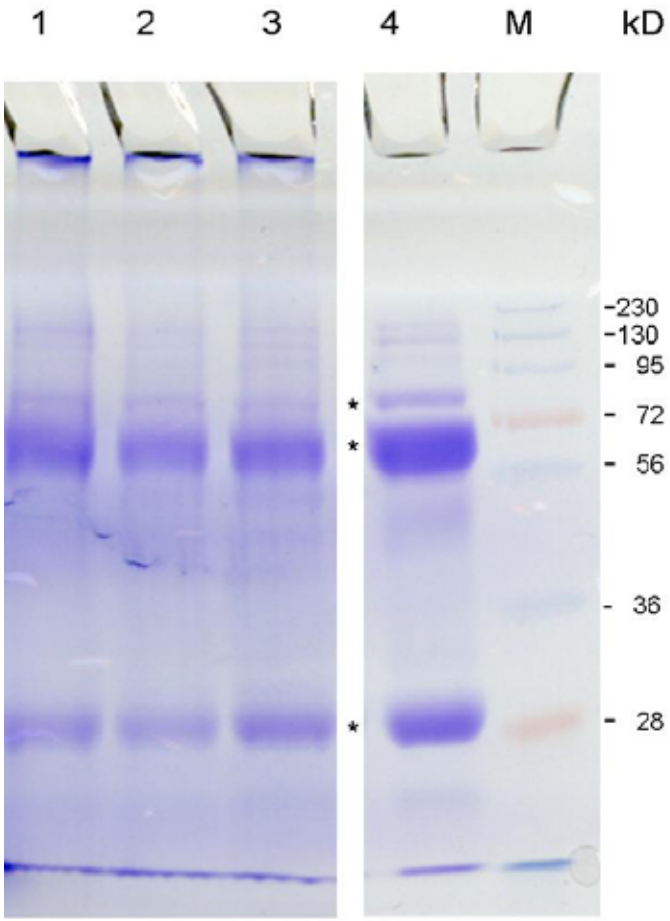

Figure 2. SDS-polyacrylamid gel electrophoresis (SDS-PAGE) of 3 individual bile samples (lanes numbers1-3) and an IgA preparation purified by Protein A Sepharose affinity chromatography (lane number 4). Lane number 5 contains molecular marker protein with the indicated molecular weights to the right of the figure (spanning from 230 – 28 kD). The 3 marked (*) polypeptide bands are from above: Secretory component (molecular weight of 80 kD), The alpha (heavy) chains of IgA (molecular weight 69 kD) and finally the kappa and lambda light chains of IgA (molecular weights of 29-30 kD).

There might be few non-IgA protein impurities in the bile. To remove these impurities we absorbed the secretory bile IgA to Protein A Sepharose and eluted it under acid conditions as described in the material and methods section. Such a purified IgA preparation can be seen in lane 4 in figure 2. The 3 marked (*) polypeptide bands in the purified IgA preparation correspond to the secretory component (molecular weight of 80 kD), alpha immunoglobulin heavy chains (molecular weight 69 kD) and kappa and lambda immunoglobulin light chains of 29-30 kD.

Purity of mink IgM, IgA and IgG as measured by SDS-PAGE.

Figure 3 shows purified mink IgG, IgA and IgM preparations, all of which contained few contaminants.

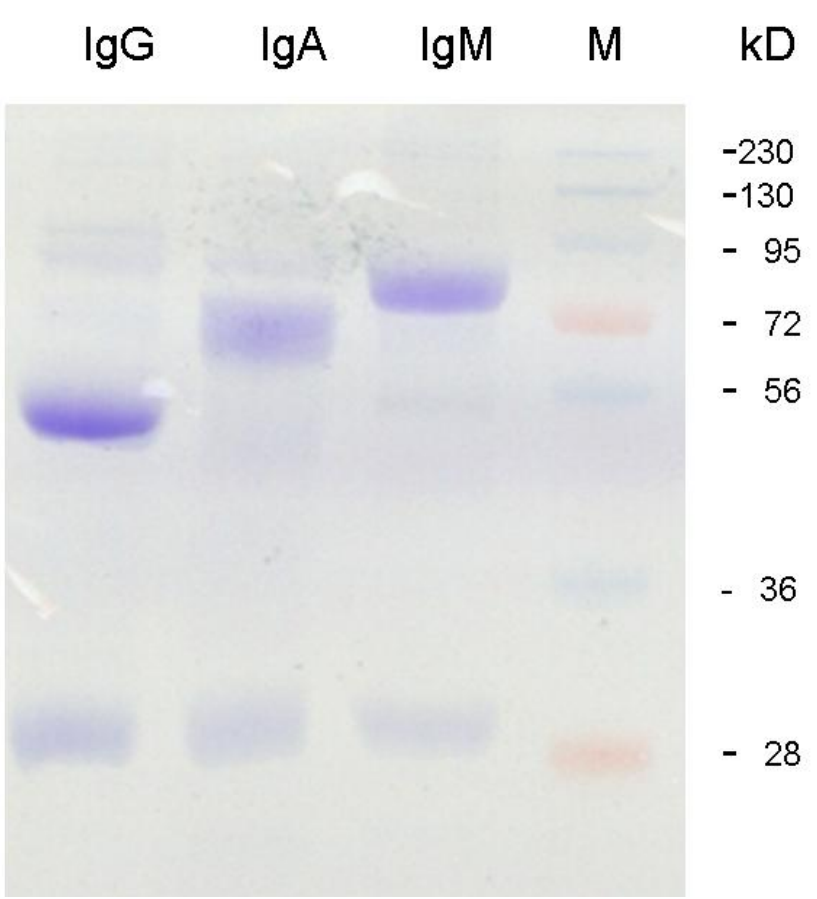

Figure 3. SDS-polyacrylamid gel electrophoresis (SDS-PAGE) of purified mink IgG, IgA and IgM preparations. The estimated molecular weights of the gamma heavy chains, the alpha heavy chains and my heavy chains were 54 kDa, 69 kDa and 83 kDa respectively. The light immunoglobulin chains had molecular weights from 29-30 kDa. Lane M contains molecular marker protein with the indicated molecular weights to the right of the figure (spanning from 230 – 28 kD).

The estimated molecular weights of the gamma heavy chain, the alpha heavy chain and the mu heavy chain were 54 kDa, 69 kDa and 83 kDa, respectively. The light immunoglobulin chains had molecular weights from 29-30 kDa. As molecular weight protein marker kit we used Fermantas prestained PageRuler Protein ladder plus (Fermentas Life Sciences).

Purity of mink IgM, IgA and IgG as measured by ELISA.

We produced a rabbit antiserum pool against purified mink IgG as explained in the materials and methods section. We also produced rabbit antisera against mink IgM and IgA, but these two antisera were found to be inferior to two mink immunoglobulin cross-reactive commercially

available antisera: a goat antiserum to canine IgA and a rabbit antiserum to human IgM. The antisera to mink IgG, IgA and IgM enabled us to construct mink immunoglobulin class-specific ELISAs as illustrated in figure 4.

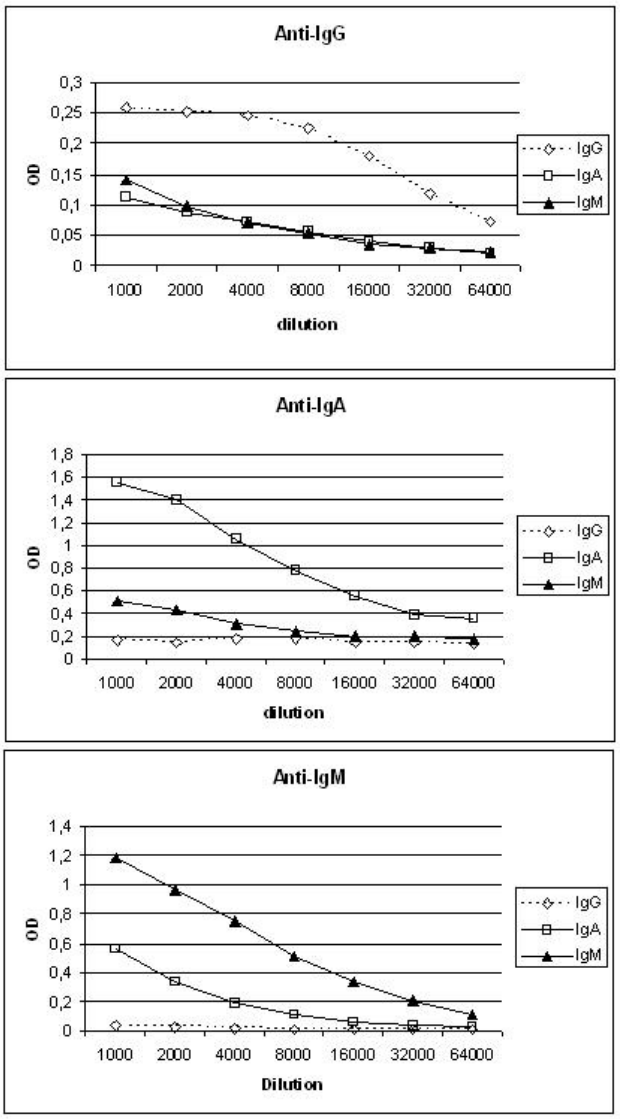

Figure 4. ELISA of the 3 purified mink immunoglobulin preparations (IgG, IgA and IgM) using mink immunoglobulin class specific reagents. Coating of the plates was made with a commercial anti-canine IgA, anti-human IgM or absorbed anti-mink IgG. Immunoglobulin preparations were tested at a starting dilution of 1:1000, and from there at serial two-fold dilutions.

The results indicated that indeed the mink immunoglobulin ELISAs were specific for the different immunoglobulin classes and we estimate our IgM and IgG preparations to be more than 90% pure, and the IgA preparation more than 80% pure.

Estimation of immunoglobulin levels in bile samples
We quantified the IgG, IgA and IgM immunoglobulin levels of 2 bile samples from normal minks and found quantities in the range of 3-5 mg/ml for IgA, 2mg/ml for IgG and 0.25 mg/ml for IgM.

Discussion

This study describes easy purification methods for mink IgG, IgA and IgM immunoglobulins and analyses of the purity of the preparations by ELISA using mink immunoglobulin class specific reagents. It is our experience that there are currently no easy mink immunoglobulin purification methods

described in the literature for mink IgG, IgA and IgM. Furthermore it is difficult in the literature to identify suitable reagents for detection of all 3 main classes of mink immunoglobulins. Not only are pure immunoglobulins needed as control preparations in various ELISA setups, but they are also necessary reagents for investigation of the specificities of serological reagents used to measure mink immunoglobulins (antisera).

The most straightforward purification method was for IgG purification a Protein G Sepharose matrix column. This method is well established for IgG purification from most higher animal species. In an earlier work (Aasted 1989) we used Protein A Sepharose chromatography for mink IgG purification, but found traces of contamination with IgM and IgA. Protein G Sepharose has the advantage that it is known to be more specific in IgG binding properties.

For IgA purification, we originally tried to purify it from serum, but as shown in figure 1, serum IgA is fractionated in between the IgM and IgG containing fractions, and we rarely found IgA rich fractions which were devoid of either IgM or IgG contamination. Therefore we utilized bile as an easy source of highly concentrated secretory IgA (Yamada et al. 1992) which we occasionally found to be low in concentration of both IgM and IgG (figure 2). It is therefore important to collect the bile absolutely free of blood contamination. Eventual IgG contaminants can be removed by Protein G-Sepharose absorption. Few other protein impurities which may be present in bile IgA samples can be removed by absorption of secretory bile IgA on a Protein A Sepharose matrix and elution under acid conditions as described in the material and methods section. Such purified IgA preparations can be seen in figure 2 and figure 3.

For IgM purification we utilized IgMs high molecular mass around 900 kD, which made size separation chromatography (Sephacryl S-300 Sepharose) ideal as an isolation method. In the first experiments we had problems with IgM fractions contaminated with IgG containing immune complexes, but this problem was solved by IgG depletion of the serum samples by affinity chromatography on Protein G Sepharose before Sephacryl S-300 fractionation.

The next problem was alpha-2 macroglobulin contamination of the IgM containing fraction which could not be avoided by size chromatography. This problem could however be solved by absorption of IgM on a Protein A Sepharose matrix and acid elution, which incidentally gave seemingly pure IgM preparations (figure 3).

In order to detect mink IgG, IgA and IgM in different blood-plasma or mucosal preparations, we originally produced rabbit antiserum pools against purified mink IgG, IgA and IgM preparations. The IgG reactive antiserum was found suitable for further studies, but our own IgA and IgM reactive antisera were found to be inferior to commercially available cross-reactive antisera. The antisera to mink IgG, IgA and IgM were used to construct mink immunoglobulin class specific ELISAs as illustrated in figure 4. The results indicated that indeed the mink immunoglobulin ELISAs were specific for the different immunoglobulin classes.

Acknowledgement
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Effect of the replacement of fish meal and meat meal with different proportions of extruded soybean diets on metabolism and reproductive performance of raccoon dog (*Nyctereutes procyonoides* M.)

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Abstract

Raccoon dog (*Nyctereutes procyonoides* M.) is an economic canid with preference to omnivorous food. To reduce the feeding cost, fish meal and meat meal were replaced with different proportions of extruded soybean diets and the effects on metabolism and reproductive performance of raccoon dogs were determined. Forty female raccoon dogs, randomly divided into four groups, were fed with the diet containing extruded-maize, distillers dried grains with soluble, maize oil, lysine, methionine, dicalcium hydrogenphosphate, additives, and fish meal + meat meal replaced with 0 (Diet 1), 10 (Diet 2), 20 (Diet 3) and 30% (Diet 4) extruded-soybean, respectively. The results were: 1 During the pre-mating and pregnancy periods, there was no difference in crude protein apparent digestibility, crude protein metabolic rate and ether extract apparent digestibility among four groups (P>0.05). The dry matter intake of Diet 1 was significantly lower than that of the other diets (P<0.05) during the pre-mating period, but had no difference in pregnancy (P>0.05). The apparent digestibility of calcium and phosphorus of Diet 4 were higher than those of other groups (P<0.05), respectively, during both periods. 2 During the pregnancy and lactation periods, there was no difference in the dry matter intake and reproductive performance among four groups (P>0.05). The number of cubs per litter and cub weaning survival rate of Diet 1 4 were 7.11 and 85.9%, 5.38 and 83.7%, 6.75 and 90.7%, and 6.11 and 98.2%, respectively. The average weight gains of Diet 1 4 during 45 days were 21.33, 31.33, 28.22 and 23.56 g/day, respectively. 3 The results showed that the diets of replacing fish meal and meat meal with extruded soybean feed raccoon dogs successfully during the reproduction periods, and the large use of the plant feed can receive the successful reproduction performance.

Key words: Digestion and metabolism; Extruded soybean; Raccoon dog; Reproductive performance

Introduction

With the increasing requirement of fur ornaments, about twelve millions of raccoon dogs are being raised in China. The low feed cost and successful

reproduction can bring farmers more profits. The raccoon dog is truly omnivorous, and the diet of wild raccoon dog consists mainly of invertebrates, frogs, lizards, rodents and birds along with seeds and berries (Kauhala et al., 1993). The food preference of raised raccoon dogs is meat, fish or their byproducts, which is becoming scarce and expensive in China. Raising practices in China showed that raccoon dogs could be fed with wide plants such as seeds, fruits and berries. Replacing such feed with extruded maize and soybean also works well without damage to health and fur quality (Li & Yang, 2006).

Compared with most plant proteins with methionine as the first limiting amino acid (Storebakken et al., 2000), soybean protein has a well-balanced and relatively constant amino acid composition (Porter & Jones, 2003) and high yield of 204 million tons in 2004 (FAOSTAT, 2004). However, raw soybeans contain anti-nutritional factors, including trypsin inhibitors, hemaglutinins, lectins and saponins, which could cause ineffective food utilization, thereby depressing the growth rate of animals (Hensen et al., 1987; Liener, 1994; Vandergrift et al., 1983). Osborne and Mendel (1917) first proposed the necessity of heating raw soybeans to improve their nutritional values, since extruded-soybean could inactivate such anti-nutritional factors (Camire et al., 1990). Some studies have shown that soybeans treated with extrusion yielded higher weight gain and nutrient digestibility (Faber & Zimmerman, 1973; White et al., 1967).

Whether feeding more plant feed would influence the reproduction of raccoon dog, however, is unknown. In this study the extruded soybean was used to to substitute different proportions of fish meal and meat meal of pelleted diets, and the raising trails and balance experiments were carried out during the reproduction period of female raccoon dogs. The aim of the study was i) to offer the scientific data for the application of extruded soybean in rearing raccoon dogs; (ii) to compare the effects of different diets on reproductive performance of raccoon dogs; (iii) to investigate the effects of using a large amount of plant feed on the metabolism and cub's growth. Such indexes, the estrus rate, mating rate, litter number and cub survival rate were determined, and the digestibility and metabolic rate of different diets were evaluated.

Materials and methods

Forty adult healthy reproducible female raccoon dogs were selected with similar weight, and randomly assigned to fed with 4 diets (10 female raccoon dogs per diet group), respectively. The experiment was conducted at the Research Farm for Fur Animals of Institute of Special Economic Animals and Plants, Chinese Academy of Agricultural Sciences (44 _N; 126_E), in Northeast China from Nov 2006 to June 2007. Raccoon dogs were housed outdoors in individual standard rearing cage (100 cm long ×70 cm wide × 70 cm high) with a wooden nest box (60 cm long × 50 cm wide × 50 cm high) filled with straw for the winter's rest. The animals were exposed to natural temperature and photoperiod.

The experimental diets consisted of extruded corn, distillers dried grains with soluble (DDGS), plant oils, calcium hydrogenphosphate, fish meal, meat meal, extruded soybean, lysine, methionine, vitamin and mineral additives. Fish meal and meat meal were substituted by extruded soybean in the proportion of 0, 10, 20 and 30%, respectively. The four diets were processed into pellets (5~10 mm in length and 4 mm in diameter). The detailed composition and nutritive values of each diet were shown in Table 1.

Table 1. Composition and nutritive value of different diets for raccoon dogs.

	Diet 1	Diet 2	Diet 3	Diet 4
Ingredient, %				
Extruded maize	55.30	44.18	32.61	21.51
Extruded soybean	0	10	20	30
Fish meal	15	10	5	0
Meat meal	15	10	5	0
DDGS[1]	8.0	20.0	32.4	44.3
Maize oil	4.68	3.12	1.56	0
Calcium hydrogenphosphate	0.90	1.50	2.12	2.80
Lysine	0.10	0.16	0.23	0.29
Methionine	0.02	0.04	0.08	0.10
Additives[2]	1.00	1.00	1.00	1.00
Nutritive value, %				
Dry matter	88.3	88.6	89.1	88.7
Crude protein	26.5	28.8	27.8	28.6
Ether extract	8.73	10.0	9.65	10.3
Calcium	2.79	3.10	2.40	2.55
Phosphorus	1.55	1.52	1.37	1.21

[1] Distillers dried grains with soluble.

[2] Containing Vitamin-mineral premix, maize protein meal and salt.

The animals were fed with pelleted diets two times per day at 9:00 am and 14:00 pm and drank water ad libitum. In the mating period all of the experimental animals participated in the mating normally with reproductive male raccoon dogs. Raccoon dogs adopted the form of continuous mating in three days and the mating ratio of male and female was 1: 3, according to gestation duration of raccoon dogs (average 60 days) plus date of the last mating, calculating exact littering date.

The balance trails were carried out in the pre-mating (Jan 22~25) and pregnancy period (Mar 30~April 3), respectively. The feces and urine were collected every 24 hours but only measured for the last 3 days of the trail. During the trails the surplus feed and dry matter intake (DMI) were measured. The raising trails lasted for about 7 months (from Nov 2006 to June2007) including pre-mating, mating, pregnancy and lactation periods. Daily intake of the animals was recorded at the beginning of their successful mating. The estrus rate, mating rate, litter number and cub survival rate was recorded, respectively, and the cubs were weighed per 15 days with an accuracy of 5 g from birth to 45 days old. The health condition of the cubs and female raccoon dogs was also noted.

Table 2. The digestibility and metabolic rate of raccoon dogs fed with different diets during the pre-mating and pregnancy periods.

	During the pre-mating period				During the pregnancy period			
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 1	Diet 2	Diet 3	Diet 4
Dry matter intake, g/day	101±23.6[a]	161±44.1[b]	182±24.7[b]	151±29.9[b]	173±28.6[a]	169±29.0[a]	154±51.6[a]	172±19.7[a]
CP[2] digestibility, %	80.1±6.7[a]	82.7±6.2[a]	80.0±8.6[a]	79.6±3.3[a]	75.3±1.6[a]	75.3±5.3[a]	80.1±4.5[a]	74.6±4.6[a]
CP metabolic rate, %	49.8±13.9[a]	43.3±10.8[a]	41.9±8.2[a]	46.1±12.6[a]	48.3±19.8[a]	45.2±3.1[a]	45.9±3.3[a]	47.4±17.9[a]
EE[3] digestibility, %	94.1±1.6[a]	92.8±4.4[a]	92.0±3.4[a]	92.4±1.6[a]	94.1±3.7[a]	94.0±3.7[a]	92.9±3.5[a]	94.2±3.2[a]
Calcium, %	37.8±14.0[a]	47.0±16.2[ab]	47.4±10.0[ab]	66.1±10.9[b]	33.9±1.9[a]	40.2±11.0[ab]	42.1±4.7[ab]	59.0±7.7[b]
Phosphorus, %	41.3±11.8[a]	44.0±18.0[ab]	50.0±9.2[ab]	63.1±8.0[b]	37.9±3.3[a]	45.2±2.4[b]	49.2±5.4[b]	57.2±2.8[c]

[1] Means within the same rows under the same subtitle with the different letters are significantly different (P<0.05). Each value is the mean ± S.D. of eight replicates.
[2] Crude protein.
[3] *E*ther extract.

Feces were dried in hot air oven at 65°C for 72 h, ground in a mill to pass a 1-mm screen, and subject to analysis of dry matter (DM), crude protein (CP), ether extract (EE), calcium (Ca) and phosphorus (P). Twenty percent of the daily urine output was collected in plastic barrels containing 20 mL of 25% sulphuric acid and frozen for each of the 3 days. At the end of the 3 days, the urine was thawed, composited, and a 20 mL sample collected and frozen at 20°C for nitrogen (N) analysis.

Feed, feed refusal and faecal samples were dried to a constant weight at 65°C for 72 h in hot air oven. Then samples were ground in a mill to pass a 1-mm screen prior to chemical determination. DM in feed and faecal samples was determined following the methods of AOAC (1990). The N content in feed, feed refusal, fecal and urine samples was determined using micro-Kjeldahl method (AOAC, 1984). EE of feed, feed refusal faecal samples were measured with Tecnal TE–044/1 (Piracicaba, São Paulo, Brazil) according to the method of AOAC (1990). The contents of Ca and P in feed and fecal samples were assayed photometrically by the vanadate–molybdate method according to Naumann and Bassler (1976).

The coefficient of nutrients was calculated as follows:

CP metabolic rate (%) = 100×[Intake (kg)×feed CP (%)-Fecal output (kg)×fecal CP (%)-urine output (kg) ×urinary CP (%)]/Intake (kg) ×feed CP (%)
CP digestibility (%) = 100×[Intake (kg)×feed CP (%)-Fecal output (kg)×fecal CP (%)]/Intake (kg)×feed CP (%)

The digestibility of EE, Ca and P was also determined the same as described above.

Data were analyzed using ANOVA and GLM procedure of the Statistical Analysis Systems

Institute (SAS, 1997). The Duncan's multiple comparison test was used to determine differences among means of diets. A probability value ($P < 0.05$) was taken to indicate statistical significance.

Results

1. Effect of different diets on the nutritive digestion and metabolism of raccoon dogs during the pre-mating and pregnancy period

Table 2 showed that there was no difference in CP apparent digestibility and metabolic rate and EE apparent digestibility among different diets in the pre-mating and pregnancy period ($P>0.05$).DMI of Diet 1 was lower than that of the other diet groups in the pre-mating period ($P<0.05$), but had no difference in the pregnancy period ($P>0.05$). The digestibility of Ca and P of Diet 4 was higher than that of Diet 1 in the pre-mating and preganacy periods ($P<0.05$). In the pregnancy period, even Diet 2&3 had higher P digestibility than Diet 1 ($P<0.05$).

2. Effect of different diets on the reproductive performance of raccoon dogs during the pregnancy and lactation periods

Table 3 showed that DMI mean and reproductive performance had no difference among different diet groups ($P>0.05$) during the pregnancy and lactation periods. DMI mean during the lactation period was higher than that during the pregnancy period because the data was the sum of mother and cub's feed consumption. Cubs started to eat the pellet feed 20 days later after birth. In our trails the mean DMI of female raccoon dogs and cubs during the lactation period was the average dry matter intake of the mother raccoon dog and their babies from the cub born day to the weaning day.

Table 3. The effects of different diets on the reproductive performance of raccoon dogs during the pregnancy and lactation periods.

	Diet 1	Diet 2	Diet 3	Diet 4
DMI mean of female raccoon dogs during the pregnancy periods (g/day) [1]	156±33.2	172±27.6	162±30.1	150±41.7
DMI mean of female raccoon dogs and cubs during the lactation period (g/day) [2]	484±105.2	472±90.5	421±84.3	421±112.8
Number of females	10	10	10	10
Number of whelping females	9	8	8	9
Number of born cubs	64	43	54	55
Number of reared cubs	55	36	49	54
Number of cubs per litter	7.11	5.38	6.75	6.11
Weaning survival rate, %	85.9	83.7	90.7	98.2

[1] DMI mean of female raccoon dogs during the pregnancy periods was determined based on the average dry matter intake from the last mating day to cub birthday.

[2] DMI mean of female raccoon dogs and cubs during the lactation period was determined based on the average dry matter intake of mother raccoon dogs and their babies from cub birthday to the weaning day.

3. Comparison of average body weight and daily weight gain of cubs fed with different diets

The average body weight gains during 45 days after birth were 21.33, 31.33, 28.22 and 23.56 g/day for groups of Diet 1 4, respectively. Except for Diet 1, the average body weight gain decreased along with the increasing proportions of extruded soybean in diet. The low average body weight gain in Diet 1 was due to the high mating rate and the litter number. The growth curves of cubs from birth until 45 days old fed with different diets showed that the less number of cubs per litter, the more weight the cubs gained.

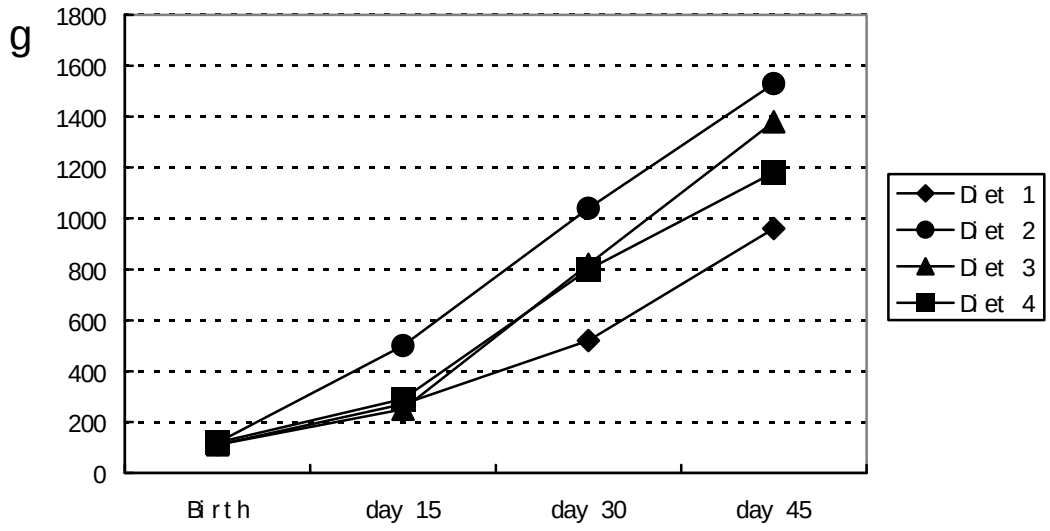

Figure 1. The growth curves of cubs from birth until 45 days old fed with their mother's milk and different diets consisting of fish meal and meat meal substituted by extruded soybean in the proportions of 0, 10, 20 and 30 , respectively.

Discussion

The wild raccoon dog goes through autumn fattening followed by winter sleep before the reproduction period. Raised raccoon dogs also exhibit autumn fattening but no winter sleep (Asikainen, 2002). During winter, approximately from Nov. to Feb., raised raccoon dogs reduce the feed consumption and largely live off their substantive fat reserves (Korhonen, 1988). Our experimental raccoon dogs had the highest weight and built up a reserve of nutrition underneath their skins before the pre-mating period. Our results showed that the feed consumption of all female raccoon dogs was lower compared to our previous research in Summer. The group of Diet 1 had the lowest DMI in the pregnancy period, which might be due to raccoon dogs in Diet 1 group mated earlier than other groups. The feed consumption decreased during mating period. It has been reported that the rut can affect the appetite of raccoon dogs (Korhonen, 1988). This pattern was closely connected to voluntary regulation of feed consumption. During autumn, to enhance gradual deposition of excessive fat reserves (Mustonen et al., 2007), the appetite of animals was good and then decreased in the winter (Nieminen et al., 2002).

DMI, CP digestibility, metabolic rate and fat digestibility of raccoon dogs had no significant difference when the fish meal and meat meal were replaced with extruded soybean during the pre-mating and pregnancy periods, but the digestibility of Ca and P in different diets were influenced by the origins of Ca and P. By comparison of the feed intake between pre-mating and pregnancy periods, we found that the higher dry matter intake was associated with the poorer apparent digestibility of CP, Ca and P. This phenomenon has been reported by others (Korhonen et al., 1986).

Our trails indicated that all the CP metabolic rates in the pre-mating and pregnancy periods were positive. Use of balance experiments to estimate the N (or CP) requirement is not completely accurate. The main reason is that it was difficult to collect all the excretion in our experiments, and it was easy to over-calculate the feed consumption because of the missed feed. The potential paradox of mass loss with positive N balance has been observed in other animals (Holter et al., 1979; Murphy, 1993). Despite its shortcomings, the balance trails remain the best available method for estimating N requirements and can continue to provide useful and reasonable estimates (Murphy, 1993).

The digestibility of Ca and P increased gradually from Diet 1 to 4 with the increasing proportion of extruded soybean in the diets. The reason is that the digestibility of Ca and P in the fish meal and meat meal was lower than those in the complementary calcium hydrogenphosphate in the different diets, which the similar results was observed by Xiong (2004) and He (2001) in pig and poultry.

The raccoon dog is a seasonal breeder with the mating period from late-January to middle-February. Pregnancy lasts about 2 months and the females give birth to five to eight cubs. The reproductive performance of the Chinese raised raccoon dogs were, on the average, 5.2 cubs per mated female in 2004 (Li & Yang, 2006), and our experimental results was 5~8, which was reasonable change bounds. The cub weaning survival rate depend on various factors including the nutrition, the management, the ammonia concentrations in the nests of farmed raccoon dogs (Korhonen & Harri, 1986), the number of cubs per litter and competition among cubs(Ahola, 2007) and so on. However, the nutrition is the crucial factor to get success.

DDGS, the maize distillers, has been widely used in feed industry of raccoon dogs in China although it needs further scientific evaluation. In this study DDGS was used to regulate the level of CP.

In conclusion, our results suggested that the diets replacing fish meal and meat meal with extruded soybean could feed raccoon dogs successfully during the reproduction period, and higher proportions of plant feed in diet had no negative effect on the reproductive performance of raccoon dogs. Thus it's economically important for farmers.

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