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„Effects of dietary fatty acids on reproductive performance in guinea pigs (*Cavia aperea f. porcellus*)"

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Bettina Schragner, BSc

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Contents

1. Introduction

Fatty acids are important components of cell membranes and show high concentrations in the brain (Yehuda 2005). The composition of fatty acids in cell membranes depends on the types of fatty acids ingested via the diet (Yehuda 2005). Two main types of fatty acids can be distinguished: unsaturated fatty acids (UFAs) and saturated fatty acids (SFAs). UFAs contain double bonds, while SFAs only contain single bonds. UFAs are essential nutrients which cannot be synthesized by the body and therefore must be provided in the diet, while SFAs are nonessential and can be synthesized by all organs (Bourre 2004, Lunn & Theobald 2006). Among unsaturated fatty acids monounsaturated fatty acids (MUFAs), with only one double bond and polyunsaturated fatty acids (PUFAs), with more than one double bond are distinguished. PUFAs can be further classified as omega-3 (n-3), omega-6 (n-6) or omega 9 (n-9) fatty acids, depending on the location of the first double bond (3, 6, or 9 carbons from the methyl end of the molecule), (Lunn & Theobald 2006, Wathes et al. 2007).

Several studies have shown different effects of unsaturated and saturated fatty acids. Dietary UFAs had a positive impact on behaviour of guinea pigs, rats and mice. Animals fed on a diet high in UFAs had improved cognitive abilities, were less anxious, more active, less aggressive and showed more sociopositive behaviour than control animals (Nemeth et al. 2015, Nemeth et al. 2016, Federova & Salem 2006, Frances et al. 1996). Dietary SFAs, on the other hand, had negative effects on behaviour. Rodents that received a diet high in SFAs had worse cognitive abilities and were more anxious than animals fed on a normal diet (Granholm et al. 2008, Moon et al. 2014). Juvenile male guinea pigs fed on a diet high in SFAs were more aggressive compared to males that received dietary UFAs. Positive impacts of unsaturated fatty acids could be due to the double bonds, increasing the fluidity of the cell membrane, leading to a higher receptor density and an elevated release of neurotransmitters (Yehuda 2005).

Fatty acids can also play an important role in reproduction. Reproduction is an energy demanding process in mammalian females and dietary fatty acids provide important energy reserves (Nemeth et al. 2017). However, fatty acids can also affect reproduction

in other aspects. Dietary fatty acids lead to increased steroid hormone secretion, influencing ovarian function and thus can positively affect pregnancy rates (Mattos et al. 2000). Studies on cattle showed that cows fed with n-3 PUFAs had higher oestradiol concentrations during oestrus and lower progesterone concentrations during dioestrus than control animals (Robinson et al. 2002). Dietary n-3 PUFAs increased the number of released ova in rats (Broughton et al. 2010) and caused higher pregnancy rates and lower pregnancy losses in cows compared to a diet high in saturated fatty acids (Gulliver et al. 2012). In guinea pigs PUFAs led to larger litter sizes, while animals fed with SFAs had smaller litters and a lower total litter mass (Nemeth et al. 2017). The reason for these effects could be the fact that animals fed on PUFAs adapted the strategy of accumulation of additional energy reserves during gestation. PUFAs can also affect male fertility because unsaturated fatty acids are components of sperm plasma membranes and enable the membrane fluidity needed for fertilization (Wathes et al. 2007).

Several studies examined influences of dietary PUFAs, especially n-3 and n-6 fatty acids on reproduction, but much less is known about the effects of SFAs. The different types of fatty acids could have different effects on reproductive success.
UFAs and SFAs could also affect the oestrus cycle and reproductive behaviour of animals, but very few studies on this topic exist.

Domestic guinea pigs (*Cavia porcellus f. aperea*) are an ideal species for studies investigating oestrus cycles because with 16 to 18 days they have a relatively long cycle duration compared to other rodents (Joshi et al. 1973). The oestrus cycle consists of four stages: pro-oestrus, oestrus, metoestrus and dioestrus (Lilley et al. 1997) and is characterized by hormonal changes with the sexual hormones progesterone and oestradiol playing a major role. Oestrogens, like oestradiol, trigger oestrus (Bauer et al. 2008). The oestradiol concentration show two peaks during the oestrus cycle in guinea pigs. The first peak is reached during pro-oestrus, the second peak around oestrus, which reflects the biphasic follicular growth in the oestrus cycle of guinea pigs (Bauer et al. 2008, Bland 1980). Progesterone is mainly released by the corpus luteum. Progesterone is responsible for the establishment and maintenance of pregnancy,

ovulation and the development of mammary glands (Arck et al 2007, Michel & Bonnet 2014). Concentrations during oestrus are low and increase in dioestrus (Bauer et al. 2008). Glucocorticoid concentrations also change during the reproductive cycle. Carey et al 1995 showed that corticosterone peaked in late pro-oestrus in female rats. In guinea pigs elevated cortisol levels were also observed during pro-oestrus (Garris 1986). Glucocorticoids like cortisol are released in stressful situations like e.g. changes in environmental conditions and trigger physiological stress reactions to enable the animal to cope with the situation (Nemeth et al. 2016; Schöpper et al. 2011, Michel & Bonnet 2014). In short-term stress situations glucocorticoids improve the energy supply of the animal and by that enable adequate responses like flight or fight. Long-term stress can lead to reduced reproductive success and survival (Möstl & Palme 2002). Elevated stress during pregnancy can influence the fetus, because glucocorticoids of the mother are transferred via the placenta (Schöpper et al. 2011, Schöpper et al. 2012).

Domestic guinea pigs are a highly social species (Rood 1972) with a polygynous mating system (Ades et al. 2004). In such mating systems males compete for females. Male guinea pigs have a stable straight- line dominance hierarchy, in which the alpha male displays more aggressive behaviour than the other males and has exclusive access to resources and reproduction (Rood 1972, Sachser et al. 1998). Also females build up a linear rank- order, but they are less aggressive than males (Sachser 1998). The dominance hierarchy of male guinea pigs depends on population size. In small populations, the dominant male monopolizes all females and is very aggressive towards subdominant males. In larger populations, guinea pigs form subunits in which dominant males still have more reproductive success than lower-ranking males, but tolerate social contact between subdominant males and females (Machatschke et al. 2008).

The aim of this study was to examine the effects of dietary saturated and unsaturated fatty acids on sociopositive and agonistic behaviour of female guinea pigs as well as hormone concentrations during the oestrus cycle. We examined the effects when females were kept with and without males. When females had access to males we

further compared sexual interactions and behaviour among males between the diet groups.

2. Methods

2.1. Animals and housing conditions

All guinea pigs (26 females and 12 males) used in this experiment originated from the F1-generation of a study analysing influences of dietary fatty acids on guinea pig behaviour and physiology. Already their parents (F0-generation) received the same experimental diets. The animals were bred at the Department of Behavioural Biology at the University of Vienna. They were habituated to the daily contact with humans, sexually intact and 1.5 years old when the experiment started. All guinea pigs could be identified individually by natural fur colorations. The animals were assigned to three different feeding regimes and housed in single-sexed groups. The control and the SFA group consisted of nine females; the UFA group of eight females. The enclosures' size was 2.0 m x 1.6 m for each group and were enriched with two shelters. The floor was covered with standard woodchip bedding material. The guinea pigs were housed at a temperature of 20 ± 2°C, 50 ± 5 % humidity and a light-dark cycle of 12 hours each with lights on at 07:00 a.m.

2.2. Dietary regimes

Animals were daily fed with guinea pig pellets (ssniff V2233, ssniff Spezialdiäten GmbH, Soest, Germany) and 50 g hay per group. The food pellets of the UFA- and SFA group were additionally mixed with fatty acids. Walnut oil (Manako Walnussöl, Makana Produktion und Vertrieb GmbH, Offenbach a.d. Queich, Germany. 100g walnut oil contain 9.9 g saturated fatty acids, 15.8 g mono unsaturated fatty acids and 73.9 g poly unsaturated fatty acids on average) was used as supplement rich in unsaturated fatty

acids (UFA-group). The SFA group additionally received coconut oil (Manako Bio Kokosöl, Makana Produktion und Vertrieb GmbH, Offenbach a.d. Queich, Germany. 100g coconut oil contain 92 g saturated fatty acids, 6.5 g mono unsaturated fatty acids and 1.5 g poly unsaturated fatty acids on average). The oils and the pellets were mixed in a freezer bag in a relationship of 10 % fatty acids once a week. The control group was fed with untreated food pellets. In all three diet groups, the pellets were provided ad libitum in two feeding dishes per group, so that food was always available for the females. Water was provided in three drinking bottles per group and was available ad libitum.

2.3. Experimental procedure

At the onset of the study the guinea pigs were housed in their single-sexed groups, this period was defined as the premating phase. After a month, when every female went through at least one complete oestrus cycle, the mating phase started. The females of each feeding group were divided into two mating-groups (Control group: five and four females, UFA group four and four females, SFA group five and four females). Each mating group was kept with two males of the same diet group for four months. This resulted in six mating-groups, two for each diet. In each mating group one male was more aggressive than the other. Based on the aggressive interactions between the males, they were classified as dominant and subdominant male. Pregnancy was determined based on regular body mass gain after an oestrus phase when housed together with males. Pregnant females were removed from the mating group and housed together with other pregnant females of the same diet group. After the first four months, the remaining non-pregnant females were kept with two other males of the same diet group for further three months.

Some of the females did not become pregnant during the experiment. These females were defined as non-reproductive females for analysis and compared to the reproductive females which successfully gave birth.

The experimental groups were video recorded each morning. Thereafter measurements were taken (see below) and afterwards Open-Field Tests were conducted (see below). This procedure was the same during premating and mating phase.

2.4. Video recordings

The video recordings started each morning at 09:00 a.m. and lasted 30 minutes. The guinea pigs were filmed in their social group to analyse behaviour during oestrus and dioestrus. The cameras (GoPro Hero 3, resolution of 5 MP, wide angle of 170°) were located at the ceiling above the enclosures. During video recording shelters were removed, so that the animals could not hide under them.

2.5. Measurements

Both during the premating and mating period the oestrus cycle phase of each female was checked daily. For this reason the vagina membrane was visually inspected. The vagina opens during oestrus for 1-6 days (Young et al. 1935). Oestrus was defined as the first day when the vagina was open. Dioestrus was defined as day nine after oestrus. Additionally, body mass of each guinea pig was recorded each morning.

2.6. OpenField test

OpenField tests were conducted to determine if the separation from the social group would lead to elevated glucocorticoid secretion and to enable the collection of individual fecal samples. Each guinea pig was separated from its group for ten minutes and transferred to an unstructured area (1.44 m²). The floor of the Open Field was also covered with wood chips. During the premating mating phase each female was tested once in oestrus and once in dioestrus. During the mating phase OpenField tests were carried out every two weeks.

2.7. Behavioural analysis

Videos were analysed using the software Solomon Coder beta 15.11.19. For each female four videos were analysed, one day in oestrus and one day in dioestrus during the premating as well as during the mating phase. For the males videos of the same days were analysed to enable male behaviour during female oestrus and dioestrus. Recorded behavioural parameters based on Rood (1972) were: (1) movement: walking, running. (2) grooming: face wipes, scratching, nibbling, licking, nosing. (3) side-by-side: sitting together with physical contact. (4) total sociopositive behaviour: social grooming, nose nose and anogenital inspection. (4a) social grooming: nibble another's animals pelage. (4b) nose nose: two animals touching noses. (4c) anogenital inspection: a female sniffs, licks of nuzzles the ano-genital region another female. (5) total aggressive behaviour: head thrust, stand threat, bite, kick back, rumba rumble, displacement, chase, fight, harassment and mounting. (5a) head thrust: jabbing its head towards another individual. (5b) stand threat: animals adopt a curved body posture, orientated broadside to each other. (5c) bite: attacking another animal with its teeth. (5d) kick back: kicking back both hind feet at another animal. (5e) rumba rumble: slowly approaching another individual rhythmically oscillating the hindquarters from side to side and emitting a characteristic burbling vocalization, in an agonistic context. (5f) displacement: taking the place of another animal by chasing it away. (5g) chase: run after another animal and driving it off. (5h) fight: violent confrontation between two individuals. (5i) harassment: repeated agonistic behaviour in short time against the same individual. (5j) mounting: a male climbing on the back of another male. (6) total sexual behaviour: sexual anogenital inspection, rumba rumble, chin-rump-follow, sexual mounting, sexual harassment. (6a) anogenital inspection: a male sniffs, licks of nuzzles the ano-genital region of a female. (6b) rumba rumble: a male slowly approaching a female rhythmically oscillating the hindquarters from side to side and emitting a characteristic burbling vocalization. (6c) chin-rump-follow: a male presses the underside of its head against the rump of a female and follows her. (6d) sexual mounting: a male climbing on the back of a female. (6e) sexual harassment: repeated sexual behaviour in short time against the same female.

Movement and side by side were measured as durations, sociopositive, aggressive and sexual behaviour as well as grooming as frequencies. The interval was set at one second. Behaviour was recorded for female-female interactions, female-male interactions, male – female interactions and male-male interactions.

2.8. Fecal and saliva sampling procedures

After the OpenField test, fecal samples were collected directly from the bedding material. We only collected those samples, which were not contaminated with urine. After each test, the bedding material was replaced. The samples were stored at -20°C until further analysis.

Basal saliva samples were collected during the premating phase. During the mating phase saliva samples were collected before and after the female was transferred to the OpenField. A standard cotton bud was inserted into the cheek pouch of the guinea pig for 30 seconds. Saliva was extracted from the cotton buds by centrifugation (5 min, 10.000 rpm) and stored at -20°C until further analysis.

2.9. Hormone analysis

Hormone analyses were done using biotin-streptavidin enzyme-linked immunoassays (Palme & Möstl 1993; Palme & Möstl 1997). Enzymes and antibodies were provided by the University of Veterinary Medicine, Vienna, Austria. Hormone analyses were run in duplicates. The coefficient of variance (CV) was ≤15% for duplicates (in CVs >15% samples were excluded from analysis). The CV for sample duplicates was calculated as the percentage of standard deviation of duplicates on the mean of duplicates. Progesterone and oestradiol were analysed from fecal samples, cortisol from saliva samples.

Fecal samples were dehumidified at 60°C and crushed. 0.1 g of the samples was suspended in 2 ml methanol (80%). The samples were centrifuged (15 min, 3,000 rpm,

825 x g), diluted 1:20 and analysed using an 11-oxoetiocholanolone antibody measuring FGMs (Palme & Möstl 1997). Saliva samples did not require any extraction procedures. Saliva was diluted 1:50 and analysed using a cortisol-specific antibody (Palme & Möstl 1997). Intra- and inter-CVs were as follow: cortisol 11.61% (intra) and 0.37% (inter), progesterone 12.44% (intra) and 9.59% (inter), oestradiol 7.84% (intra) and 7.66% (inter).

2.10. Statistical analysis

Statistical analyses were carried out using IBM SPSS Statistics 20. Data were tested for normal distribution by Shapiro-Wilk-tests. In case of normal distributions parametric tests were used, otherwise non-parametric tests were applied. Comparisons between behaviour, body weight and hormone concentrations in oestrus and dioestrus as well as before and during mating were executed with t-tests or Wilcoxon-tests depending on data distribution. Comparisons between dominant and subdominant males as well as reproductive and non-reproductive females were analysed with t-tests or Mann-Whitney U-tests. Comparisons between the diet groups were carried out with ANOVA, post-hoc tests with Bonferroni tests and Kruskal-Wallis tests, post-hoc tests with Mann-Whitney U-tests. Significance was set at $p \leq 0.05$.

3. Results

3.1. Behavioural analysis

3.1.1. Movement

SFA females moved less than controls during oestrus in the premating period ($p = 0.038$). Between the other groups, no differences in movement duration were found. During the mating phase movement also did not differ between the three groups during both oestrus phases (table 1).

Differences in movement between the premating and mating phase were only found during oestrus in control females ($p = 0.036$, Tab.1) which moved less during the mating phase. In the UFA- and SFA group, no differences in movement were found during oestrus. During dioestrus movement duration did not differ between the premating and mating phase in any of the diet groups (table 1).

Comparing movement between the oestrus phases in each group no differences were detected during premating. During the mating phase, however, the control females moved more during dioestrus than during oestrus ($p = 0.042$). In UFA- and SFA females movement duration did not differ between oestrus and dioestrus during the mating phase (table 1).

Table 1: movement duration (sec) in both mating and oestrus phases (means ± SE)

diet group	premating phase		mating phase	
	oestrus	dioestrus	oestrus	dioestrus
Control	104.0 ± 16.5	79.1 ± 7.7	55.7 ± 9.3	72.7 ± 13.7
UFA	70.7 ± 14.9	82.1 ± 20.9	52.4 ± 15.7	100.5 ± 36.8
SFA	62.4 ± 10.5	51.4 ± 8.2	66.0 ± 15.3	82.2 ± 25.5

3.1.2. Female – Female interactions

Sociopositive behaviour was observed more frequently during the premating phase, than during the mating phase in each group both during oestrus and dioestrus (Fig. 1). During the premating phase, oestrus UFA females tended to show less sociopositive behaviour towards other females than controls ($p = 0.053$), while during the mating phase sociopositive interactions among oestrus UFA females occurred significantly less frequently compared to the control group. In dioestrus no differences between the diet groups were detected (Fig. 1).

A comparison of sociopositive behaviour between the oestrus phases showed that SFA females tended to have lower frequencies during oestrus than during dioestrus in the premating phase ($p = 0.079$). In the Control- and UFA group no differences between the oestrus phases were detected. During the mating phase, no differences in sociopositive behaviour between oestrus and dioestrus were found in any of the diet groups (Fig. 1).

Oestrous SFA females showed side-by side behaviour for longer periods than Control- ($p = 0.047$) and UFA females ($p = 0.027$) during the premating phase, while no differences were found between UFA- and Control- females. In dioestrus, SFA females also showed longer side-by-side durations than Control females ($p = 0.036$), but no differences between UFA- and SFA females were detected. During the mating phase UFA females in dioestrus spent more time side-by-side than controls ($p = 0.024$). No group differences in side-by-side duration were found between all diet groups during the oestrus phase (table 2).

UFA females did not show any side-by-side behaviour during oestrus in the mating phase. In the Control- and SFA group, no difference between the oestrus phases was detected. During the premating phase no significant differences between oestrus and dioestrus were found (table 2). In addition, side-by-side duration did not differ between the premating and mating phase in any of the diet groups (table 2).

Table 2 side-by-side duration (sec) in both mating and oestrus phases (means ± SE)

diet group	premating phase		mating phase	
	oestrus	dioestrus	oestrus	dioestrus
Control	53.3 ± 17.8	27.6 ± 13.1	127.1 ± 113.5	11.0 ± 7.2
UFA	29.8 ± 14.1	127.7 ± 74.6	0.0 ± 0.0	206.8 ± 143.6
SFA	282.2 ± 90.9	350.4 ± 160.3	179.5 ± 112.8	50.2 ± 35.3

In each group, aggressive female-female interactions occurred more frequently during the premating phase than during mating but only when the individuals were in oestrus. During dioestrus only the Control females showed more aggressive interactions in the premating compared to the mating phase (Fig. 2).

Between the diet groups, aggressive behaviour among females did not differ, neither before nor during the mating phase. During the premating phase, the females of the SFA group tended to show less aggressive behaviour when in dioestrus than the controls (Fig. 2). No differences between oestrus and dioestrus neither before nor during the mating phase were found in any of the diet groups (Fig. 2).

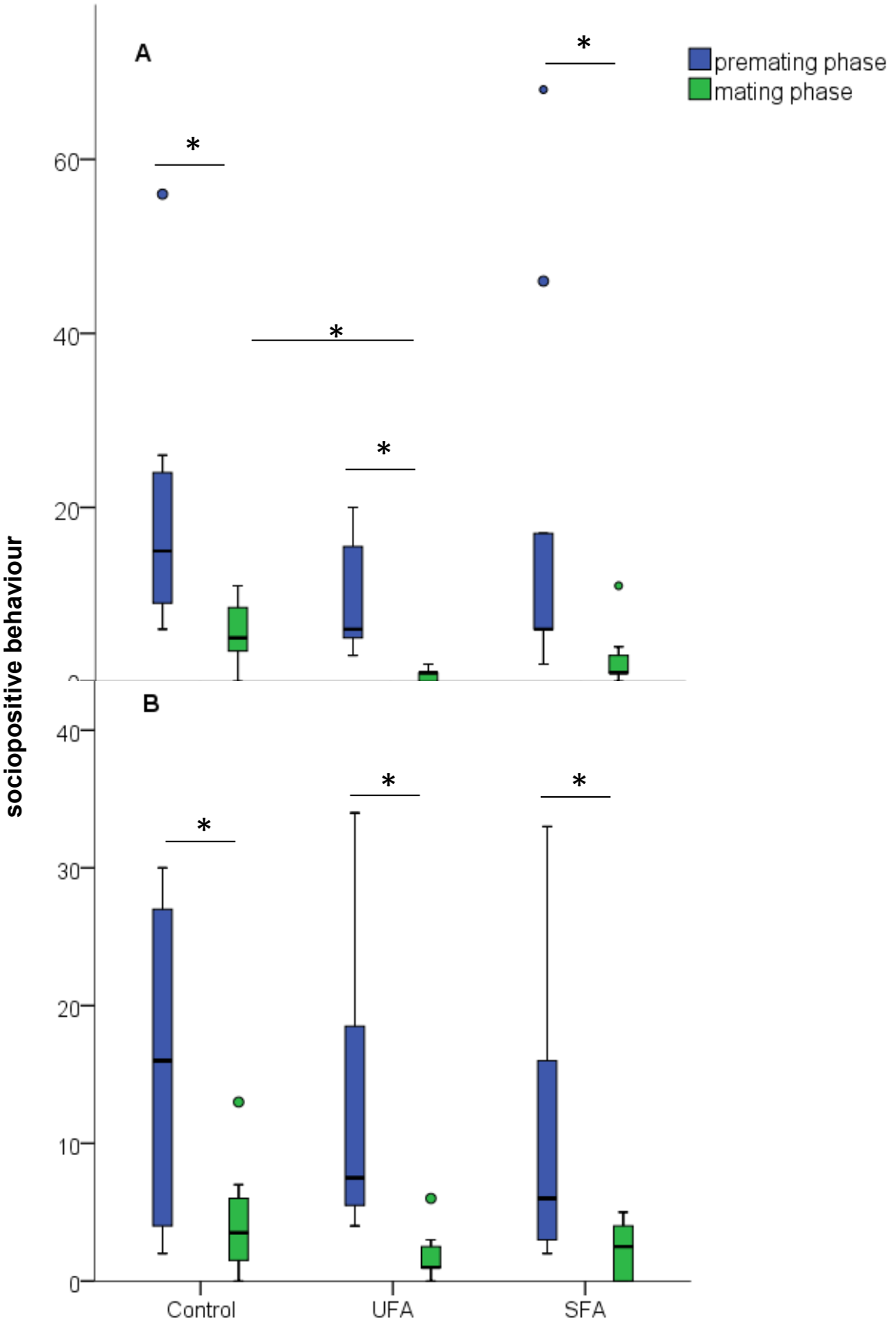


Fig. 1: sociopositive behaviour among females of the three diet groups during oestrus (A) and dioestrus (B) before and during the mating phase (oestrus: n = 8/5/7, dioestrus: n = 8/7/8). (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001)

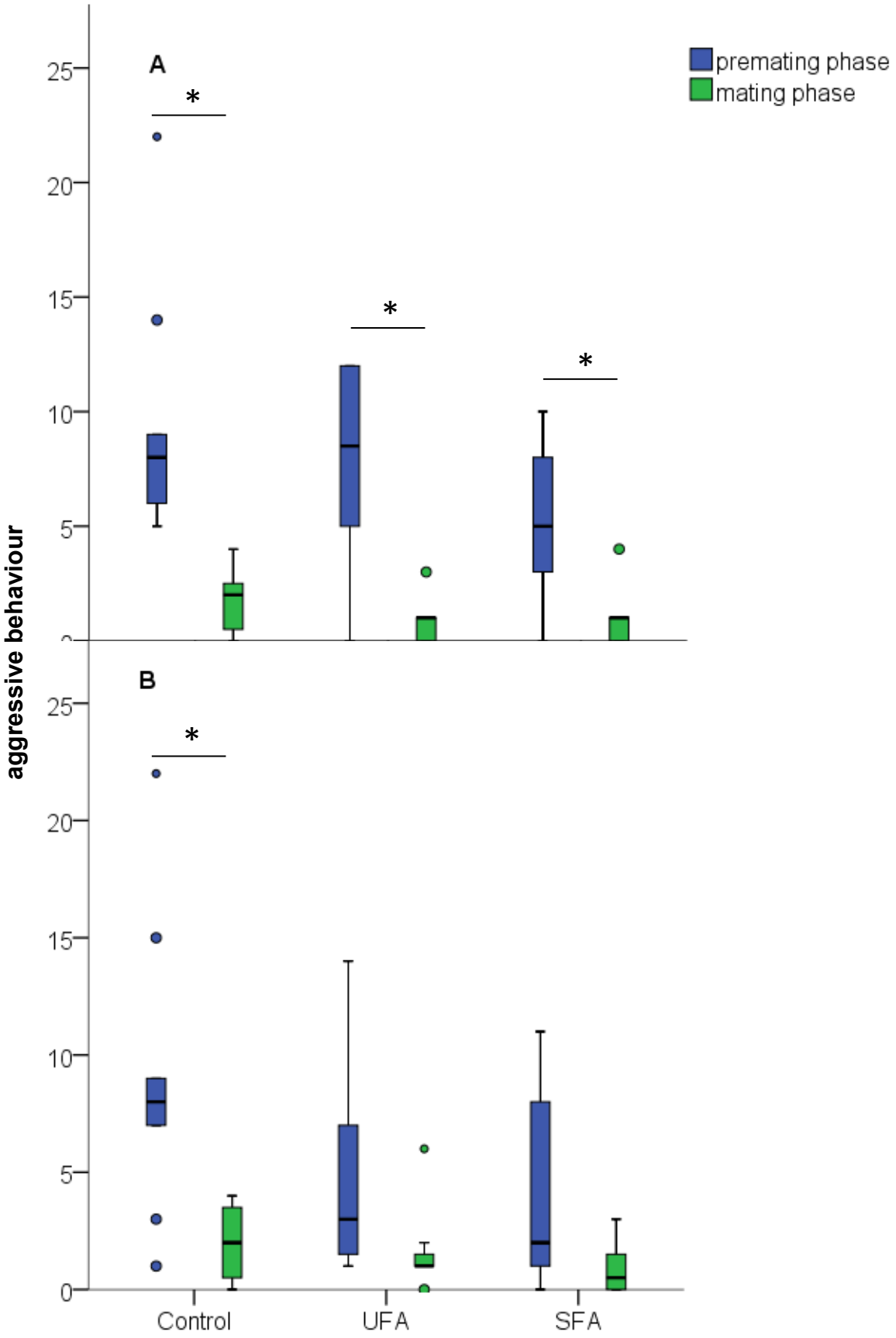


Fig. 2: aggressive behaviour among females of the three diet groups during oestrus (A) and dioestrus (B) before and during the mating phase (oestrus: n = 8/5/7, dioestrus: n = 8/7/8) (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001).

3.1.3. Female – Male interactions

UFA females showed less sociopositive behaviour towards males during dioestrus compared to controls ($p = 0.031$). No difference was found between Control- and SFA females. During oestrus no differences in sociopositive female – male interactions were detected between the diet groups. In addition, no significant difference in social behaviour of females towards males was observed in all diet groups between oestrus and dioestrus (table 3).

Table 3: sociopositive behaviour of females towards males during oestrus and dioestrus (means ± SE)

diet group	oestrus	dioestrus
Control	1.3 ± 0.6	4.1 ± 1.0
UFA	1.2 ± 0.2	1.3 ± 0.3
SFA	0.8 ± 0.5	2.5 ± 1.2

SFA females tended to remain side-by-side to males for longer periods during dioestrus than the Control- ($p = 0.064$) and UFA females ($p = 0.082$), which showed no side-by-side towards males in dioestrus. During oestrus no significant differences in side-by-side duration were detected between the diet groups. Side-by side duration did not differ between the oestrus phases in any of the groups (table 4).

Table 4: side-by-side duration (sec) of females with males during oestrus and dioestrus (means ± SE)

diet group	oestrus	dioestrus
Control	16.5 ± 16.5	0.0 ± 0.0
UFA	12.8 ± 12.8	0.0 ± 0.0
SFA	151.7 ± 144.6	85.1 ± 78.8

SFA females behaved less aggressively towards males during oestrus than UFA females. No differences were found between the other groups. In addition, aggressive behaviour towards males did not differ between oestrus and dioestrus (Fig.3).

Aggressive interactions of females against males mainly consisted in bite and head thrust, which occurred less frequently in the SFA compared to the UFA group during oestrus (bite $p = 0.036$; head thrust $p = 0.018$), SFA females also showed fewer bites towards males during oestrus than the controls ($p = 0.027$). No differences were found between UFA- and Control females. During dioestrus biting did not differ significantly between the diet groups (table 4). Furthermore, SFA females showed fewer head thrusts towards males than UFA females during both oestrus phases (oestrus $p = 0.018$; dioestrus $p = 0.034$). No significant difference was found when compared to the controls. During dioestrus, the females of the SFA group tended to show fewer head thrusts towards males than the controls ($p = 0.064$). No differences between UFA and Control females were detected (table 5).

Table 5: aggressive behaviours bite and head thrust of females towards males in both oestrus phases (means ± SE)

diet group	bite		head thrust	
	oestrus	dioestrus	oestrus	dioestrus
Control	2.0 ± 0.6	2.0 ± 0.9	0.7 ± 0.3	3.2 ± 1.0
UFA	2.4 ± 1.0	3.0 ± 1.0	2.2 ± 0.8	1.7 ± 0.3
SFA	0.2 ± 0.1	1.2 ± 0.5	0.1 ± 0.1	0.6 ± 0.2

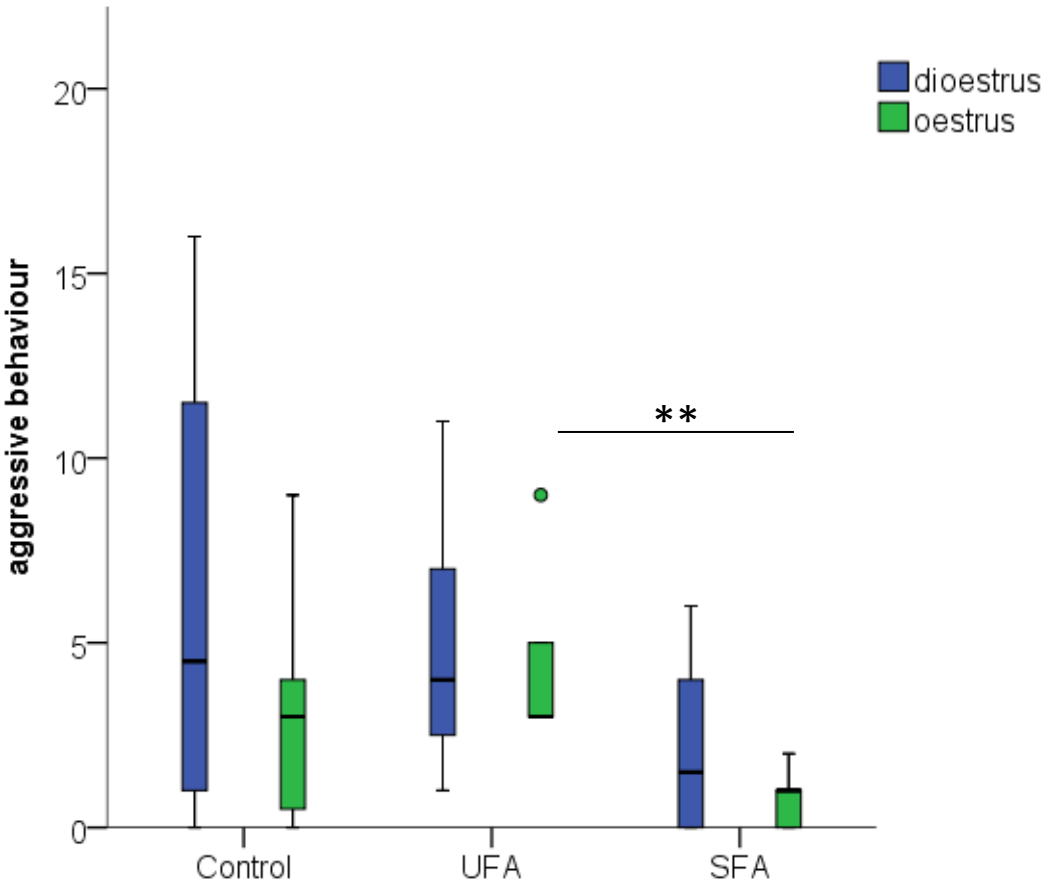


Fig. 3: aggressive behaviour of females towards males during oestrus and dioestrus of all three diet groups (oestrus: n = 8/5/7, dioestrus n = 8/7/8)

3.1.5. Male – Female interactions

Dominant males showed more sexual behaviour than subdominant males both during oestrus and dioestrus. In addition, in female dioestrus more sexual interactions of dominant males were observed compared to the oestrus phase, whereas no such differences were detected in subdominant males. (Fig. 4).

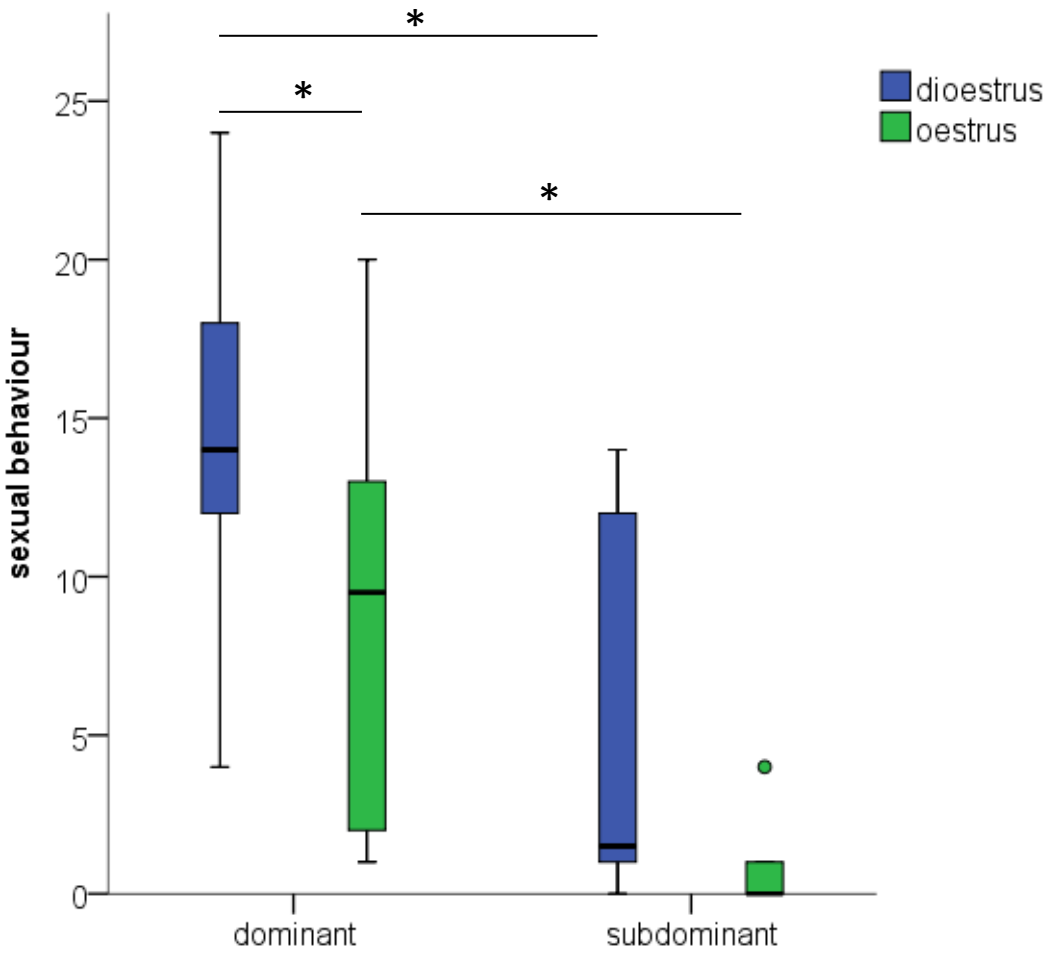


Fig. 4: sexual behaviour of dominant and subdominant males (diet groups combined) towards females during oestrus and dioestrus
(oestrus: n = 6/6, dioestrus: n = 6/6 (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001).

During female oestrus sexual mounting, chin-rump-follow and sexual harassment were only shown by dominant males. The most common sexual behaviour of subdominant males during female oestrus was anogenital inspection (table 6).

Table 6: sexual behaviour and side-by-side duration of dominant and subdominant males during female oestrus and female dioestrus (means ± SE)

behaviour	dominant male		subdomiant male	
	female oestrus	female dioestrus	female oestrus	female dioestrus
rumba rumble	0.6 ± 0.2	1.6 ± 0.6	0.2 ± 0.2	0.5 ± 0.3
sexual anogenital inspection	6.6 ± 1.8	11.1 ± 2.0	0.8 ± 0.8	4.0 ± 2.0
sexual mounting	1.1 ± 0.9	0.6 ± 0.6	0.0 ± 0.0	0.0 ± 0.0
chin-rump-follow	0.3 ± 0.2	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
sexual harassment	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
side-by-side duration	8.8 ± 5.6	3.3 ± 2.3	9.2 ± 6.1	17.5 ± 11.0

Dominant males inspected the anogenital region of females more frequently during oestrus than subdominant males ($p = 0.015$). No difference was observed in the dioestrus phase (table 6).

Anogenital inspection by dominant males also occurred more frequently during female dioestrus than during female oestrus ($p = 0.027$), while no such difference was found in subdominant males (table 6).

The duration of side-by-side with females did neither differ between dominant and subdominant males nor between the oestrus phases (table 6).

3.2. Body Mass

Female body mass did not differ between the diet groups neither before nor during the mating phase both in oestrus and dioestrus (table 7).

In UFA females, however, body mass was higher during oestrus than during dioestrus ($p = 0.033$) in the mating phase. No such differences were detected in the Control- and SFA group. In each diet group, body mass did not differ significantly between the premating and mating phase (table 7).

Table 7: body mass of females in both mating and oestrus phases (means ± SE)

diet group	premating phase		mating phase	
	dioestrus	oestrus	dioestrus	oestrus
Control	1017.2 ± 26.9	1004.4 ± 26.0	1007.8 ± 29.3	1009.0 ± 30.5
UFA	1086.7 ± 29.3	1063.0 ± 29.0	1047.1 ± 38.6	1086.2 ± 37.0
SFA	1030.1 ± 60.4	1023.8 ± 60.0	1034.5 ± 69.5	1019.5 ± 56.5

During the mating phase, UFA females gained body mass from dioestrus to oestrus, while the controls lost mass ($p = 0.035$) (table 7). Correspondingly body mass in UFA females tended to be higher compared to the SFA females during oestrus ($p = 0.070$), while between SFA group and controls no difference was detected. During the premating phase body mass changes between oestrus and dioestrus did not differ between the diet groups (table7).

UFA females lost body mass during oestrus during the premating phase ($p = 0.028$) but gained mass during oestrus in the mating phase (Fig. 5). In the other groups no differences between mating and premating were observed.

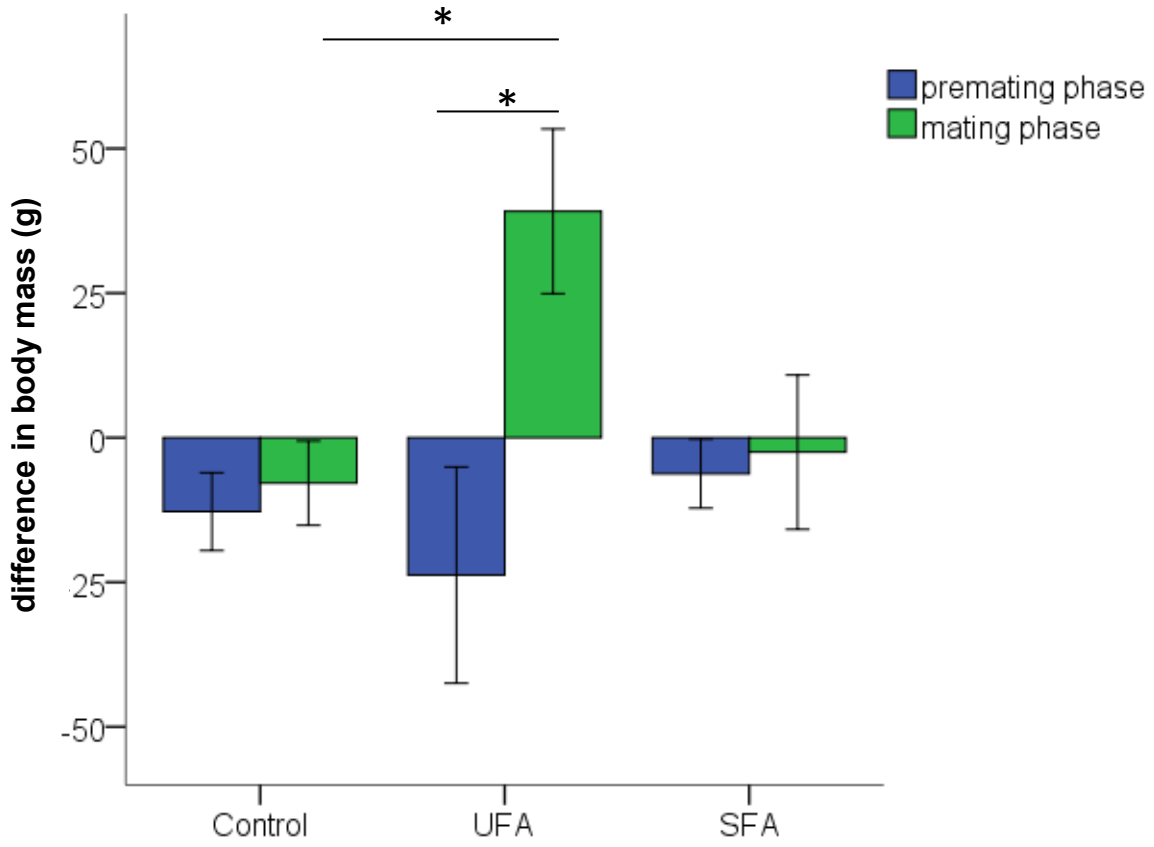


Fig. 5: body mass changes in females from dioestrus to oestrus during the premating and mating phase (premating phase: n = 9/8/9, mating phase: n = 9/8/9) (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001).

3.3. Hormones

3.3.1. Progesterone

The SFA females tended to have lower fecal progesterone metabolite concentrations (FPMs) than the controls during dioestrus in the premating phase. FPMs did not differ between the diet groups before and during the mating phase in both oestrus phases. Differences during oestrus in the mating phase could not be determined due to the low sample size (table 8).

Table 8: fecal progesterone metabolites (ng/ml) in both mating and oestrus phases (means ± SE)

diet group	premating phase		mating phase	
	oestrus	dioestrus	oestrus	dioestrus
Control	306.2 ± 41.3	755.7 ± 185.4	418.8 ± 43.8	437.4 ± 111.7
UFA	202.0 ± 74.2	415.3 ± 77.5	323.6 ± 103.9	416.7 ± 47.1
SFA	290.4 ± 78.3	357.1 ± 36.4	246.4	249.0 ± 46.5

FPMs of all females combined were higher during dioestrus than during oestrus ($p = 0.009$) (Fig. 6). FPMs during dioestrus, in the premating phase were higher than during the mating phase ($p = 0.049$).

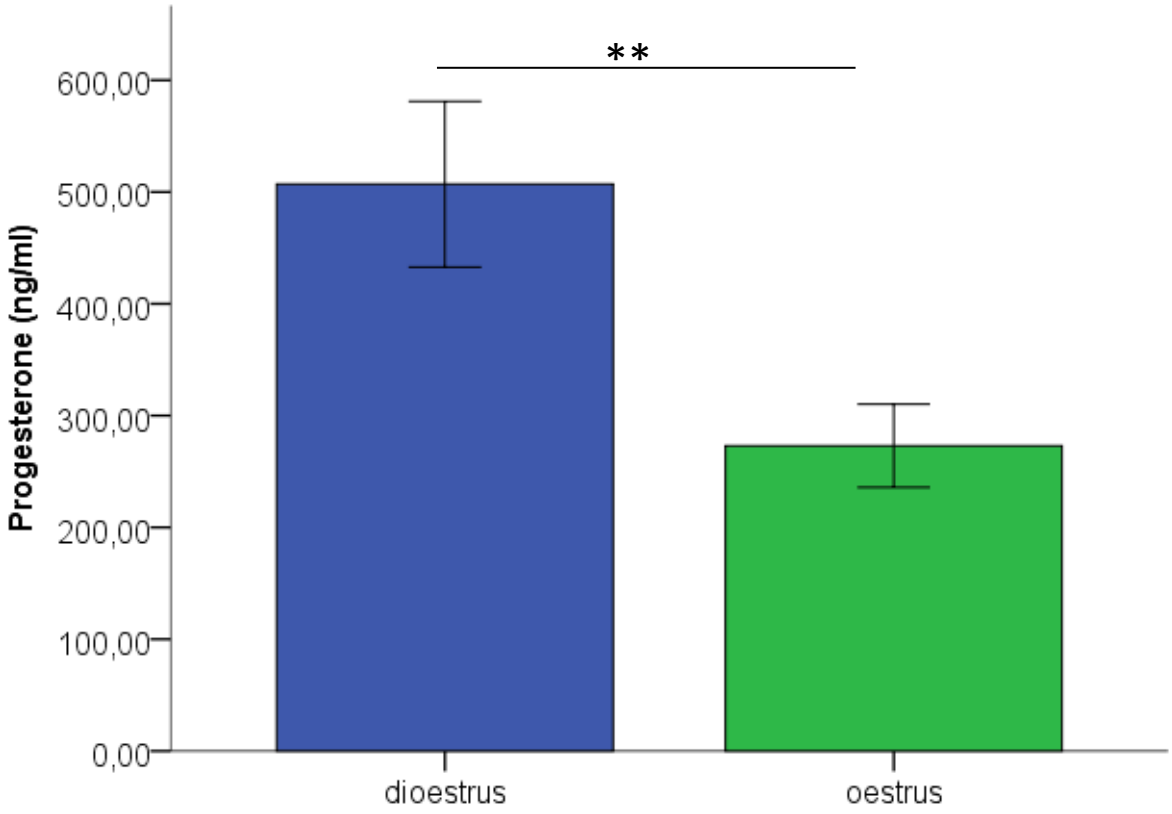


Fig. 6: fecal progesterone metabolite concentrations (diet groups combined) of females during oestrus and dioestrus during the premating phase (n = 19/19) (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001).

3.3.2. Oestradiol

Controls had lower fecal oestradiol metabolite concentrations (FOEMs) during oestrus in the premating phase than UFA- ($p = 0.003$) and SFA females ($p = 0.044$), while no difference was found between the UFA- and SFA group. During dioestrus FOEMs did not differ between the diet groups. In the mating phase no group differences were found during dioestrus (table 9).

Table 9: fecal oestradiol metabolites (ng/ml) in both mating and oestrus phases (means ± SE)

diet group	premating phase		mating phase	
	oestrus	dioestrus	oestrus	dioestrus
Control	60.2 ± 10.9	69.2 ± 19.7	50.8 ± 10.5	115.7 ± 56.5
UFA	147.9 ± 24.7	94.8 ± 10.1	96.8 ± 14.0	83.8 ± 10.6
SFA	112.8 ± 10.8	113.3 ± 8.7	308.5	93.3 ± 11.6

FOEMs differed neither between oestrus and dioestrus nor between the premating and mating phase (table 9).

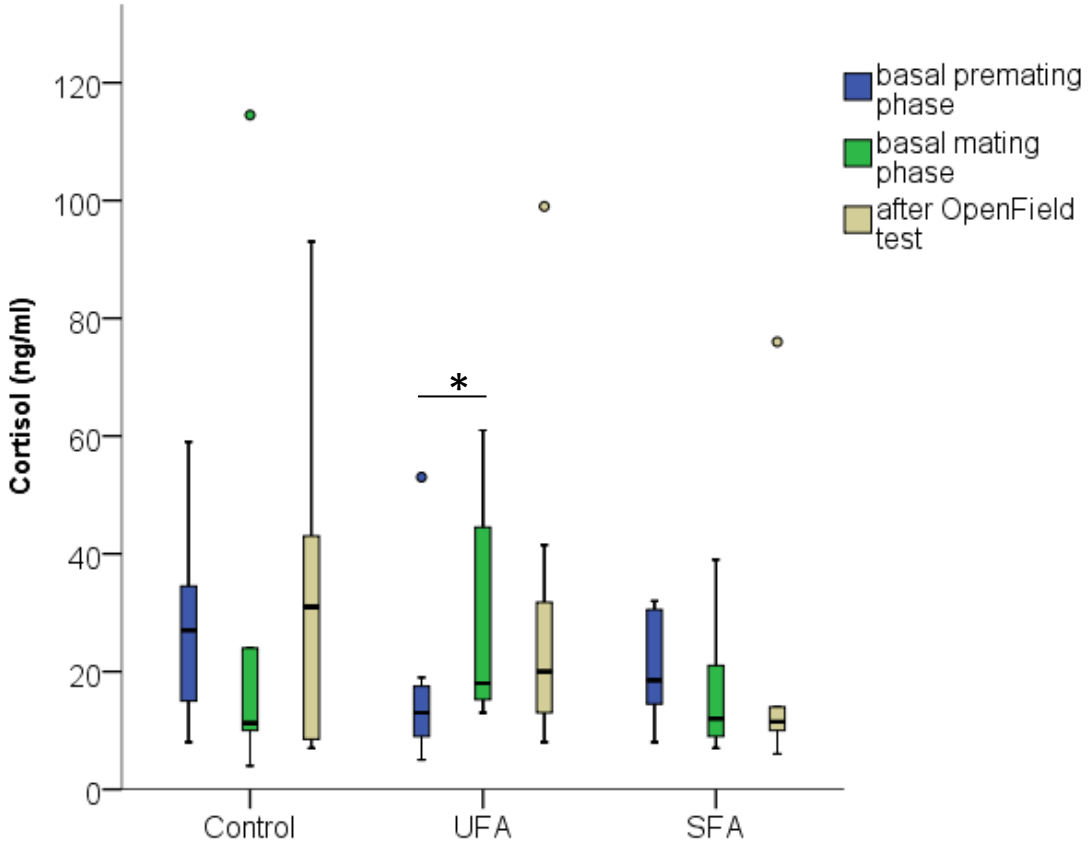
3.3.3. Cortisol

Basal cortisol concentrations did not differ between the diet groups during the premating phase when females were in dioestrus. During oestrus, the sample size was too small to compare individual groups. In the mating phase UFA females tended to have a higher basal cortisol concentration during dioestrus than the controls. No differences in basal cortisol concentrations were found between the other groups. After the OpenField test cortisol concentrations did not differ between the diet groups during dioestrus. The sample size of females in oestrus was too small to compare individual groups (table 10).

Table 10: basal cortisol concentrations in both mating phases and oestrus phases and cortisol concentration after OpenField test

diet group	basal premating phase		basal mating phase		after OpenField test	
	oestrus	dioestrus	oestrus	dioestrus	oestrus	dioestrus
Control	23.5 ± 12.5	28.8 ± 7.0	82.0 ± 67.0	10.8 ± 4.0	44.6 ± 25.3	20.0 ± 9.5
UFA		17.7 ± 6.1	39.5 ± 23.0	24.8 ± 6.7	26.0 ± 13.5	28.1 ± 12.3
SFA	16.0	21.5 ± 3.6		16.6 ± 4.9		21.5 ± 10.9

When pooling oestrus phases, the UFA group had higher basal cortisol concentrations during the mating phase than during premating ($p = 0.046$). Comparisons of basal cortisol concentrations and cortisol concentrations after the OpenField test during the mating phase revealed no significant differences (Fig. 7).



3.4 **Fig. 7:** basal cortisol-concentration of females during premating and mating phase and after the OpenField test (n = 5/6/5) (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001).

The duration of the oestrus cycle did not differ between the diet groups, neither before nor during the mating phase (table 11).

Table 11: cycle duration (days) of females before and during the mating phase combined (means ± SE)

diet group	cycle duration
Control	19.3 ± 0.9
UFA	16.6 ± 1.3
SFA	15.5 ± 1.5

Most of the SFA females became pregnant during their first oestrus period with males, while controls mainly became pregnant during their second oestrus or later. Only two UFA females reproduced successfully, one of them became pregnant during the first oestrus with males, the second one later (table 12).

Table 12: number of females of each diet group, which became pregnant during their first, second, third or later oestrus

diet group	pregnant in first oestrus	pregnant in second oestrus	pregnant in third oestrus and later
Control	2	3	2
UFA	1	0	1
SFA	6	1	0

3.5. Comparison of reproductive and non-reproductive females

Seven out of nine females became pregnant in the Control- and SFA group. In the UFA group rates were much lower with two out of eight females. These females were defined as reproductive females; the females, which did not become pregnant were referred to as non-reproductive females.

The body mass of reproductive and non-reproductive females did not differ during both oestrus phases before and during the mating phase.

Reproductive and non-reproductive females of all groups showed no differences in cycle duration between the premating and mating phase.

3.5.1. Female – Male interactions

No differences in sociopositive and aggressive behaviour of females towards males were found between reproductive and non-reproductive females during both oestrus phases. Reproductive females, however, tended to show more sociopositive ($p = 0.063$) and aggressive behaviour ($p = 0.074$) towards males during dioestrus than during oestrus (table 13).

Table 13: sociopositive and aggressive behaviour of reproductive and non-reproductive females towards males in both oestrus phases (means ± SE)

diet group	reproductive females		non-reproductive females	
	oestrus	dioestrus	oestrus	dioestrus
sociopositive behaviour	0.7 ± 0.2	2.6 ± 0.7	1.8 ± 0.7	2.7 ± 0.9
aggressive behaviour	2.5 ± 0.6	4.7 ± 1.3	2.7 ± 1.2	3.8 ± 1.2

3.5.2. Male – Female interactions

Males tended to show more aggressive behaviour towards reproductive females than towards non-reproductive females during oestrus. Sociopositive and sexual behaviour of males towards females did not differ between reproductive and non-reproductive females in oestrus. No differences in sociopositive, aggressive or sexual behaviour of males towards reproductive and non-reproductive females were detected during dioestrus (table 14).

Males showed more sexual behaviour towards non-reproductive females during dioestrus than during oestrus ($p = 0.018$), while no such difference was observed towards reproductive females. Sociopositive and aggressive behaviour of males towards females did not differ between reproductive and non-reproductive females (table 14).

Table 14: sociopositive, aggressive and sexual behaviour of males towards reproductive and non-reproductive females in both oestrus phases (means ± SE)

diet group	reproductive females		non-reproductive females	
	oestrus	dioestrus	oestrus	dioestrus
sociopositive behaviour	1.3 ± 0.4	2.0 ± 0.7	0.7 ± 0.3	0.8 ± 0.4
aggressive behaviour	0.3 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.1 ± 0.1
sexual behaviour	3.6 ± 1.5	6.2 ± 1.8	2.9 ± 1.9	7.3 ± 2.5

3.5.3. Hormones

Reproductive females had higher FPMs than non-reproductive females during oestrus ($p = 0.024$). The FPMs of reproductive and non-reproductive females did not differ during dioestrus in the premating phase. No differences were detected in FOEMs between reproductive and non-reproductive females during the premating phase, neither during oestrus nor during dioestrus. During the mating phase the sample size was too small to compare reproductive and non-reproductive females.

During the mating phase, non-reproductive females of all diet groups pooled had higher basal cortisol-concentrations ($p = 0.025$) and cortisol levels after the OpenField test ($p = 0.010$) than reproductive females. No difference in basal cortisol concentrations between reproductive and non-reproductive females could be found during the premating phase (Fig. 8).

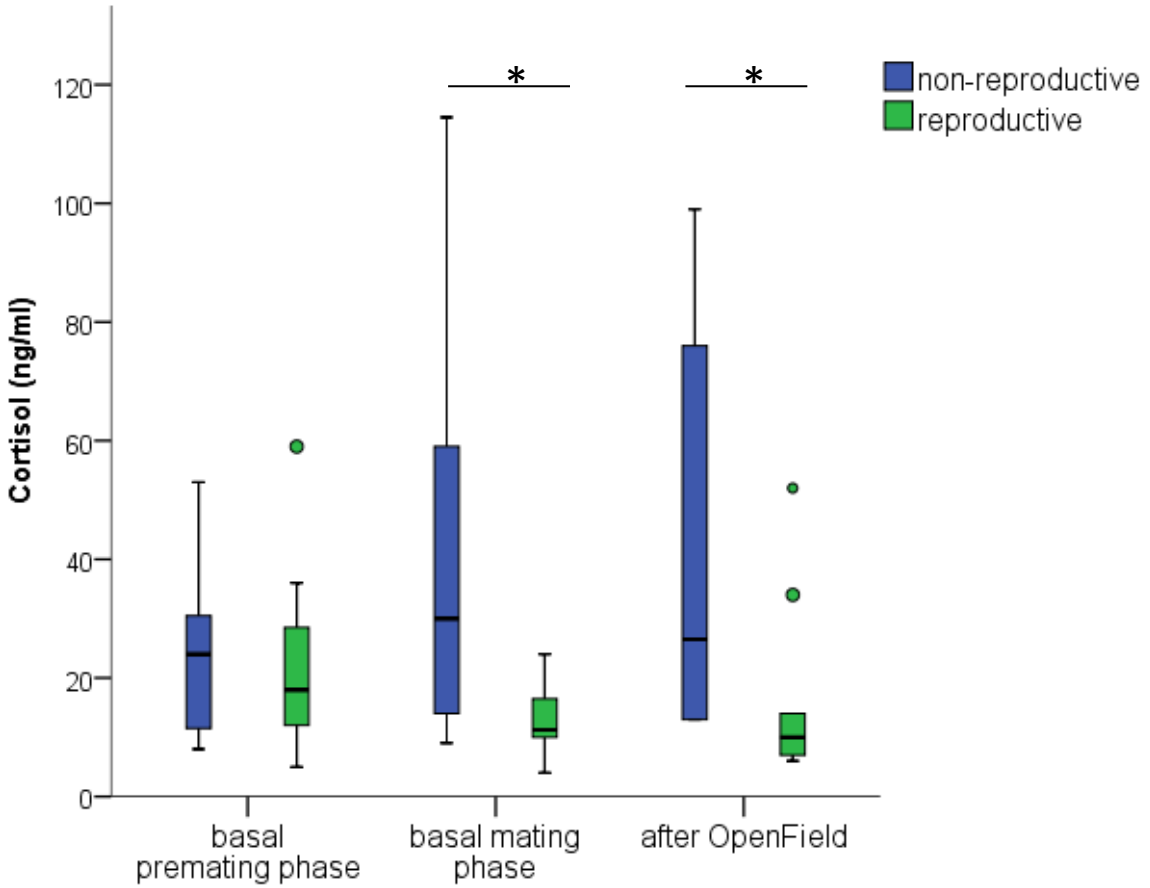


Fig. 8: basal cortisol concentrations (ng/ml) during the premating and mating phase and cortisol concentration (ng/ml) after OpenField test of reproductive and non-reproductive females (diet groups combined) (premating phase: n = 15/8, mating phase: n = 10/9, after OpenField test: n = 10/10) (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001).

4. Discussion

The present study was designed to analyse the effects of dietary fatty acids on reproduction in female guinea pigs. Based on previous studies (Nemeth et al 2016, Nemeth et al 2017), a diet supplemented with UFAs was expected to positively affect social behaviour and reproductive success, whereas SFAs were assumed to have negative effects. In this experiment, however, neither positive influences of dietary UFAs, nor negative influences of dietary SFAs in females could be determined. This could be related to sex differences in dietary effects on behaviour. Previous studies (Nemeth et al. 2016) already demonstrated that the effects of dietary fatty acids on social behaviour of guinea pigs were stronger in males than in females. Males of all diet

groups were more aggressive in group housing and showed more activity during social confrontations with unfamiliar individuals than females of the same diet groups (Nemeth et al. 2016). Thus the effects of dietary fatty acids on aggressive behaviour and movement in males could be caused by generally higher conflict rates and higher activity levels compared to females (Nemeth et al 2014). Although female guinea pigs also build up a linear dominance hierarchy (Sachser 1998), aggressive interactions are not as common as in males (Rood 1972). In addition, different effects of dietary SFAs and UFAs were already observed during juvenile development in male guinea pigs. More aggressive and less sociopositive interactions were observed among SFA males compared to animals fed on a diet high in unsaturated fatty acids (Schuster 2016).

This study, however, indicated positive effects of SFAs on mating in females, which remained in spatial cohesion with males for longer periods than the others. SFA females also were less aggressive towards males than UFA females in both oestrus phases and correspondingly showed fewer bites and head thrusts against males than the other groups. This could indicate faster acceptance of male partners and was also reflected in improved reproductive performance. Seven out of nine SFA females became pregnant and nearly all of them during their first oestrus with access to males, while only two UFA females became pregnant, at all. Furthermore, seven out of nine control females became pregnant, but only two of them during their first oestrus.

Sociopositive and aggressive interactions among SFA females had similar expression rates compared to interactions among females of the other diet groups. The females in each diet group interacted less frequently during the mating phase than during the premating phase. This could be due to the presence of males, as females interacted more with males than with other females. Another possible reason could be that each diet group was split up into two mating groups. This caused smaller female groups during the mating phase than during the premating phase and by that influenced behaviour among females.

A continuative study (Klinc 2017) on the same individuals analysed the effects of dietary fatty acids on gestation and lactation and again, sociopositive behaviour of SFA females

was similar to that shown in control females. As only two UFA females became pregnant, pregnancy and lactation could not be compared to the other groups. SFAs also appeared to have positive effects on body condition as SFA females had a higher body mass during gestation and lactation than the controls. The positive effects during gestation were assumed to be caused by the higher energy reserves of SFA females compared to controls (Klinc 2017).

In contrast to Nemeth et al (2017), reproductive performance of UFA females in this study was lowest compared to SFA's and controls. Only two out of eight females in the UFA group became pregnant, one of them during its first oestrus in the mating phase. However, the continuative study indicated that the two reproductive UFA females were among the best in terms of reproductive output. Compared to control- and SFA females litter sizes were larger and all pups were born alive (Klinc 2017).

The comparison of females that reproduced successfully and those that did not revealed some parameters, which could have negatively affected reproductive performance in the UFA group. UFA females gained body mass from dioestrus to oestrus, when kept with males, while all other lost mass in this period, independent of male presence. This body mass gain was unexpected, because also previous studies had documented that guinea pigs lose weight during oestrus due to the increased oestradiol concentration. In guinea pigs treated with oestradiol injections reduced water and food intake, led to lower body mass compared to controls (Czaja et al. 1983). In this study, oestradiol concentrations during oestrus could not be compared due to low number of fecal samples. Another factor that could have influenced reproductive performance of the UFA females is stress, as indicated by elevated cortisol secretion. In contrast to the SFA females and controls, UFA females had higher basal cortisol concentrations during the mating phase than during the premating phase. Previous studies detected effects of dietary fatty acids on the hypothalamic-pituitary-adrenal-axis. Guinea pigs fed on a diet high in unsaturated fatty acids had lower saliva cortisol levels after social confrontations than animals fed on a control-diet (Nemeth et al 2014). The n-3 PUFAs ALA or DHA also were associated with lower glucocorticoid secretions in rats (Ferraz et al 2011), while SFAs led to higher concentrations of cortisol in guinea

pigs (Nemeth et al 2016) and humans (Manzanares et al 2014). Positive effects of PUFAs on cortisol levels could, however, not be observed in this experiment. Cortisol-concentrations in UFA females were even higher during the mating phase than before. Differences in cortisol-concentrations were also detected between reproductive and non-reproductive females. Like the UFA females, non-reproductive females of all diet groups had higher cortisol levels during the mating phase than during premating. As the elevated cortisol levels were only observed during the mating phase, it could be assumed that the presence of males caused stress for the females. We first assumed that the selected males were not accepted by the females, but changing the males did not increase reproductive success. Six females in the UFA group did not become pregnant, but also four females of the other diet groups remained non-reproductive. As no differences between reproductive and non-reproductive females were observed in all other parameters, it can be assumed that the elevated stress impaired pregnancy in all groups. Stress could also have been caused by incomplete oestrus cycles, like divergent hormone secretion, and could have led consequently to the rejection of male mating attempts. Successful reproduction depends on precisely timed hormone secretion and behaviour during the oestrus cycle. Stress can impair pregnancy by influencing endocrine events of the late follicular phase due to the elevated concentration of oestrogens (Turner et al 2005). As oestrogens support the activity of the HPA-axis in rats (da Silva 1995), the following oestrus phase and ovulation is particularly susceptible to the effects of stress (Turner et al 2005). Oestrus is characterized by elevated concentrations of oestradiol (Bauer et al 2008), which could unfortunately not be compared. The cycle phase of each female was inspected visually by checking the vagina membrane. The membrane of the UFA females opened regularly, indicating that the females were in oestrus. Concerning oestrus cycle duration no differences between the UFA females and the other diet groups were detected. On the other hand it is also possible that the elevated stress during the mating phase had other reasons leading to incomplete oestrus cycles and therefore impaired pregnancy. However, immobility, a behavioural stress reaction of guinea pigs (Machatschke et al 2004, Nemeth et al 2016) could not be observed and the activity levels of UFA females did not differ during both mating phases. As guinea pigs live in social hierarchies (Sachser 1998), an unstable hierarchy, which led to more aggressive interactions

among females, could be another possible explanation for elevated stress of the UFA females, but neither before nor during the mating phase more aggressiveness was detected.

The results of this study differed from the study of Nemeth et al 2017, in which the F0-generation of guinea pigs was mated to analyse effects of short-term feeding of dietary fatty acids on reproduction. Compared to this study, all guinea pigs of the F0-generation became pregnant, most of them during their first oestrus during the mating phase. Body mass and litter size of SFA-females was lower compared to controls and UFA females, and no differences in cortisol concentration at conception could be detected. The reasons for the poor reproductive performance of the UFA females in this study compared to previous results (Nemeth et al 2017) remains unclear.

Dietary fatty acids did not affect progesterone and oestradiol secretion before the mating phase. As expected progesterone concentrations were generally higher during dioestrus than during oestrus, because progesterone is responsible for the establishment of pregnancy, preparing the uterus for the embryo (Michel et al. 2014). Although females tended to higher oestradiol concentrations during oestrus, no significant differences between oestrus and dioestrus were detected. The reason could be the time of sample collection. The oestrus phase is only a short period in female guinea pigs. Young et al. (1935) found that oestrus usually occurred during night and the mean length of oestrus was 8.21 ± 0.07 hours. The vagina membrane ruptures due to the increased concentration of oestradiol, which is involved in the induction of ovulation (Czaja et al 1983, Blatchley et al 1976) and remains open for 2 to 7 days (Czaja et al 1983). The samples were collected on the first morning the membrane was found open, but it is possible that the receptive phase was already over at that time and oestradiol concentrations were decreasing.

In males, clear dominance relationships were detected. As expected one male of each mating group was dominant, as documented in higher initiated conflict rates. The mating groups consisted of four respectively five females and two males. The guinea pigs showed the typical social organization they adopt when individual numbers are low

(Sachser et al. 1998). More sexual behaviour of dominant males compared to subdominant males was detected both during female oestrus and dioestrus in each group. This led to the assumption that dominant males had exclusive access to reproduction and only these males mated. Sexual behaviour of dominant males occurred more frequently during female dioestrus and not as expected during oestrus. As most copulations took place during the night-time, when ovulation usually occurs in guinea pigs (Young et al. 1935), it can be assumed that the males had already mated in the morning and therefore less sexual behaviour could be observed during the behavioural recordings. Another possible reason could be that dominant males paid more attention to subdominant males during female oestrus to keep the subdominant away from receptive females. Dominant males behaved more aggressive in male – male interactions when females were in oestrus.

Conclusions

Dietary fatty acids did not seem to affect interactions among females, body mass changes and hormone secretion in female guinea pigs. Positive effects of SFAs were observed especially with regard to female mating behaviour and correspondingly in earlier pregnancy. The positive effects of SFAs on reproduction could be caused by high fat intake and therefore a better availability of energy reserves compared to controls. Although UFAs also led to high fat intake providing energy no positive effects on reproduction were detected. In contrast, UFA females had higher cortisol levels during the mating phase and the lowest proportion of reproductive females. Potential reasons for increased stress levels of UFAs females during the mating phase and their poor reproductive performance are discussed but still remain unclear.

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6. Abstract

Fatty acids are important components of cell membranes and can be classified as unsaturated (UFAs) and saturated fatty acids (SFAs). Previous studies showed that dietary UFAs had positively affected social behaviour and reproductive performance in some rodent species, while SFAs led to negative effects. Fatty acids also play an important role in reproduction, because dietary fatty acids provide important reserves for this energy demanding process. In this study the effects of dietary fatty acids on sociopositive and agonistic behaviour of female guinea pigs as well as hormone concentrations during the oestrus cycle, before and during the females had access to males were examined. When females were kept with males, we further compared sexual interactions. Female guinea pigs were divided into three diet groups (control, UFA, SFA) and mated with males. Animals were daily fed with guinea pig pellets. The food pellets of the UFA- and SFA group were additionally mixed with fatty acids in a relationship of 10 % oil. Walnut oil was used as supplement rich in unsaturated fatty acids (UFA-group). The SFA group additionally received coconut oil. The control group was fed with untreated food pellets. Behaviour, body condition and hormone concentrations were compared between and within the diet groups during the premating phase, when females were kept without males and the mating phase, when they had access to males, and during oestrus and dioestrus. In SFA females positive effects on mating behaviour were observed. These females behaved less aggressively and outwent the others in reproductive performance. In UFA females negative effects were detected. The individuals were more aggressive towards males and had higher cortisol levels during the mating phase. Only two UFA females became pregnant, while seven SFA- and seven Control-females mated successfully. No effects of dietary fatty acids on progesterone and oestradiol concentrations as well as social behaviour among females were observed. All females interacted more frequently during the premating phase compared to the mating phase. Clear dominance positions were detected in males, leading to more sexual behaviour in dominant males. In summary the results indicate that SFAs positively affected reproductive performance in females, which could be caused by high fat intake and therefore a better availability of energy reserves compared to controls. UFA females appeared to have higher stress levels in the

presence of males, but the reasons for their poor reproductive performance remained unclear.

guinea pig, reproduction, dietary fatty acids, social behaviour, cortisol

Zusammenfassung

Fettsäuren sind ein wichtiger Bestandteil der Zellmembranen und werden in ungesättigte (UFAs) und gesättigte (SFAs) Fettsäuren unterteilt, die unterschiedliche Einflüsse auf das Verhalten von Tieren haben können. Studien zeigten, dass UFAs positive Auswirkungen auf das Sozialverhalten einiger Nagetierarten haben, während SFAs eher zu negativen Effekten führen. Fettsäuren haben auch einen bedeutenden Einfluss auf die Reproduktion, da sie wichtige Reserven für diesen energieaufwändigen Prozess zur Verfügung stellen. In dieser Studie wurden die Einflüsse von gesättigten und ungesättigten Fettsäuren auf soziopositives und agonistisches Verhalten von weiblichen Meerschweinchen, sowie auf Hormonkonzentrationen während des Östruszyklus, bevor und während die Weibchen zusammen mit Männchen gehalten wurden, untersucht. In letzterer Phase wurde zusätzlich das Sexualverhalten beobachtet. Weibliche Meerschweinchen wurden in drei Diätgruppen unterteilt (Kontrolle, UFA, SFA) und mit Männchen verpaart. Die Tiere wurden täglich mit Futterpellets gefüttert. Die Pellets der UFA- und SFA Gruppe wurden zusätzlich mit Fettsäuren im Verhältnis mit 10 % Öl vermischt. Walnussöl wurde als Zusatz für die UFA Gruppe herangezogen, die Pellets der SFA Gruppe wurden mit Kokosfett vermischt. Die Kontrollgruppe wurde mit unbehandelten Pellets gefüttert. Verhalten, Körpergewicht und Hormonkonzentrationen wurden vor der Paarungsphase, als Weibchen getrennt von Männchen gehalten wurden und während der Paarungsphase, sowie während des Östrus und Diöstrus beobachtet. Bei SFA Weibchen wurden positive Effekte auf das Paarungsverhalten beobachtet. Die Weibchen verhielten sich weniger aggressiv und übertrafen die anderen Gruppen im Fortpflanzungserfolg. Negative Effekte wurden hingegen bei der UFA Gruppe festgestellt. In der SFA- und Kontrollgruppe wurden jeweils sieben Weibchen trächtig, in der UFA Gruppe hingegen

nur zwei. UFA Weibchen verhielten sich aggressiver gegenüber Männchen und hatten höhere Cortisolwerte während der Paarungsphase. Die Diäten hatten keine Auswirkungen auf Progesteron- und Östradiolsekretion, sowie das Verhalten zwischen den Weibchen. Alle Weibchen interagierten mehr untereinander, bevor sie mit Männchen gehalten wurden. Zwischen den Männchen wurden eindeutige Dominanzverhältnisse festgestellt und dominante Männchen zeigten deutlich mehr Sexualverhalten zeigte. Schlussfolgernd wird vermutet, dass SFA Weibchen einen besseren Fortpflanzungserfolg aufwiesen, weil sie durch höhere Fettaufnahme mehr Energiereserven zur Verfügung hatten als die Kontrollgruppe. UFA Weibchen waren vermutlich durch die Anwesenheit der Männchen gestresst, die genauen Ursachen für die geringe Reproduktionsrate der UFA Gruppe bleiben allerdings ungeklärt.

Meerschweinchen, Reproduktion, Fettsäuren, Progesteron, Östradiol, Cortisol