Emulsified gels: a refined vehicle for accurate and rapid oral administration of lipid based preparations to rats

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Abstract

Oro-gastric gavage is used to accurately administer nutritional substances or drugs to animals. However it induces stress and has a substantial risk of mishap. Incorporation into edible gels is difficult for lipid-based preparations. We report a new methodology for producing emulsified oil-enriched gels, their effectiveness in pilot studies and subsequent larger experimental studies. Emulsified fish oil-enriched gels were produced using non-polar starch. Multiple gel types were made incorporating 0.05ml or 1ml oil doses, oxidised or unoxidised oil and with or without raspberry flavouring. The palatability and safety were assessed with i) 8 gel types in female SD rats consuming a chow diet (40 treatments) and ii) 3 gel types in rats consuming a high fat diet (45 treatments). Subsequently, palatability and safety were further assessed in a large cohort of pregnant rats (n=155; 4,242 treatments). Across both studies, all gels were eaten completely, whether the rats consumed a chow or high-fat diet. There was a 5-day period of acclimatisation. Raspberry flavoured gels were consumed more quickly than unflavoured gels. Rats exhibited positive behaviour towards receiving the gels and there were no ill-health effects. In subsequent experimental studies 4,242 doses were given to pregnant rats and all were completely consumed. Oil-enriched emulsified gels represent an easily administered highly acceptable, reliable and safe method of lipid delivery to rats, that we propose is superior to oro-gastric gavage.

Keywords: refinement, stress, pregnancy, Animal Welfare, emulsified gels, lipid administration

Introduction

Administration of nutritional supplements or drugs to animals can be challenging and the method of administration may represent a potential experimental confounder. Commonly, oral administration of agents represents the most physiologically (and translationally) appropriate method. However, oral administration is associated with important challenges. Animals may not consume a substance placed in their cage or may do so slowly or incompletely. This can be solved by incorporating it into food or water but the volume of substance consumed will scale with appetite or thirst, so that it cannot be precisely controlled. Some substances have additional challenges. For example, polyunsaturated fatty acid rich oils such as marine oils are highly prone to oxidation[1,2] and oxidation is known to alter their effects on health.[1,3,4] Thus, incorporation into food, would risk unacceptable loss of quality that could mask important health effects, or cause inadvertent harm.

Oro-gastric gavage; passing a tube through the mouth into the stomach, is an appealing solution as it allows rapid administration of a carefully controlled dose. However in rats, even when expertly performed, gavage is invasive and involves restraint of the animal, causing stress.[5] Furthermore, mishap may lead to trauma, aspiration or even death[6-8] and the cumulative risk of a mishap occurring becomes much higher if gavage is repeated daily for the same animal, in one study reaching 56%.[9] Administration of viscous substances by gavage may magnify risks due to the potential for the substance to coat the outside of the tube and lead to aspiration and the need for larger bore

gavage tubes which may be more traumatic.[10] Viscous substances could stick within the syringe and tubing leading to incomplete delivery. Stress has immediate effects such as increasing heart rate and blood pressure,[11] increasing corticosterone secretion[12-14] and inducing insulin resistance[14] which can all act as confounders in cardiometabolic studies. Of note, it has been recommended that gavage is avoided in toxicity studies[13] as it may increase toxicity[8] and death due to mishap could be misattributed. In addition, stressful stimuli in pregnancy has been shown to lead to lower birthweight[15-17] which is a known risk factor for the development of cardiometabolic disorders in later life[18] which may be in part the consequence of changes in maternal care.[19] We planned a series of supplementation studies in rat pregnancy examining effects of unoxidised fish oil on offspring metabolism and of oxidised fish oil on toxicity. Thus prior to conducting these upcoming studies, we aimed to develop a refined method of administration to avoid the need for oro-gastric gavage but retain its benefits (controlled dose and rapid consumption).

Previous studies have incorporated drugs or nutritional substances into flavoured gels[20,21] which may be an enriching rather than aversive experience for the animals consuming them. Moreover, use of a gel vehicle has been recommended as a refined method of substance administration by a multidisciplinary, multiagency joint working group focussed on Animal Welfare.[22] However as gels are water based, lipid treatments are difficult to incorporate, especially when given at high doses. Thus we developed a method of emulsifying fish oil into gels and conducted a series of pilot studies in rats to ensure they were consumed quickly and completely, both in the context of a standard diet and a highly palatable high-fat diet. We also observed their effects on the health of the animal.

Methods

Animal Ethics

Ethical approval was granted by the Animal Ethics Committee at the University of Auckland (approval #R001936) and the studies were performed in accordance with all appropriate institutional and international guidelines and regulations of animal research.

Rats

Adult female Sprague-Dawley (SD) rats were sourced from the Vernon Jenson Unit at the University of Auckland. All animals were individually housed under standard conditions in an open top cage at 25°C with a 12-h light: 12-h dark cycle. Specific details around age, weight and experimental diets are detailed for each protocol below. Given our past experience in dietary studies using female SD rats and the tight regulation of caloric intake in these animals, an n = 5 per group was considered sufficient for the Pilot studies.

Production of flavoured edible oil-enriched gels

We devised a protocol to produce 5ml oil-enriched gels from 100ml of gel solution. A variety of different gel types were produced with varying quantities of fish oil and flavoured jelly crystals and their macronutrient content was calculated (Table 1). As fish oil and water are immiscible, we used a non-polar starch emulsifier; N-Creamer 46 (Ingredion ANZ Pty Ltd, Auckland, New Zealand) to produce an oil-in-water emulsion.

The final gel solution had a 4% concentration of non-polar starch. 4g of non-polar starch was dissolved in 50ml of water at 70°C using a magnetic stirrer over a period of 90 minutes. This solution was then poured into a blender and the fish oil was added dropwise and pulsed to facilitate emulsification. This was continued until all the oil was added, until a uniform emulsion was achieved. Then, 8.6g of gelatine (Ward McKenzie Pty Ltd, Victoria, Australia) and the raspberry flavoured jelly crystals (Cerebos Greggs Ltd, New Zealand) were mixed with 20ml of 50°C water (water at higher temperatures was not used due to the potential to cause the fish oil to oxidise). The oil and water emulsion and the gelatine/jelly mixture were both added to a volumetric flask which was mixed vigorously but with care to avoid foaming and then topped up with water to 100ml. The flask sat in a 50°C water bath to prevent the solution from setting. 5ml of mixture was transferred into individual moulds of ice trays, which were labelled, covered and refrigerated overnight (4°C). The following day the individual gels were removed and stored at 4°C. To aid separation of the gels, the ice tray was placed in a bath of warm water for 20 seconds, before the gels were scooped out with a small spatula.

Control gels were produced similarly, excluding the step of adding the oil, unflavoured gels were produced without adding the raspberry flavoured jelly powder to the mixture and double raspberry gels used twice the quantity of raspberry powder (Table 1).

To reduce the potential for oxidation and to prevent mould growth, we stored gels refrigerated in small sealed containers and in the dark, prior to use and created fresh gels every 3 days.

Pilot Study 1

Hypothesis

Adult female rats will consume oil-enriched gels completely and within 60 minutes, whether they contain 0.05ml (a comparable dose to human consumption[23]) or 1ml (used in a previous rat study[24]) of fish oil, the oil is oxidised or unoxidised and whether the gel is flavoured or unflavoured.

Gel Type	Pilot Study 1				Pilot Study 2		
	0.05ml oil	0.05ml oil + RJC	1ml oil	1ml oil + RJC	Control + RJC	0.05ml oil + RJC	0.05ml oil + 2x RJC
Components of 100ml of gel solution							
Nonpolar starch (g)	4	4	4	4	4	4	4
Gelatin (g)	8.6	8.6	8.6	8.6	8.6	8.6	8.6
Raspberry jelly crystals (g)	-	17	-	17	17	17	34
Study Oil* (ml)	1	1	20	20	-	1	1
Water** (ml)	85	70	70	50	70	70	50
Nutritional composition of 5ml gels							
Energy (kJ)	5.4	21.9	38.4	54.9	20.1	21.9	38.3
Protein (g)	0.19	0.25	0.19	0.25	0.25	0.25	0.32
Carbohydrate (g)	0.19	1.09	0.19	1.09	1.09	1.09	1.99
Fat (g)	0.046	0.046	0.92	0.92	0	0.046	0.046

Table 1. Components of 100 ml of gel solution and nutritional composition of 5ml gels RJC; raspberry flavoured jelly crystals.
* Fish oil as received from the manufacturer, or that had been intentionally oxidised.
** approximate water volume added, to produce a final volume of 100 ml in a volumetric flask

Study Design
Eight gel types with oxidised or unoxidised oil, with or without raspberry flavouring (17g in 100ml) and with 0.05ml or 1ml of oil were prepared as described above (Table 1). The oxidised fish oil was produced by bubbling oxygen through a large glass bottle of oil for 1 month under fluorescent lighting (peroxide value: 48.8 meq/kg, p-anisidine value: 4.5).[24] The peroxide and p-anisidine values were determined according to the European Pharmacopoeia 8.0 method.[25]

Five adult female SD rats (age: 106 ± 1 days) weighing 245 ± 2.8 g were used for the study (Figure 1) and individual rats were considered the experimental unit. Rats were fed a standard chow diet (2018 Teklad global 18% protein rodent diets, Envigo, USA) *ad libitum*. The food hopper was topped up to 100g daily. Gels were placed in the food hopper in the morning at the same time and position every day for 10 days. Each rat received each gel type once, in an order determined using a random sequence generator (www.random.org). Timers were set from the time the gels were placed with the animals and continuously observed for the first hour and then reviewed hourly until no gel was found in the cage. The timers were stopped when the gel was completely eaten by the rat and the time taken to eat the gel completely was recorded. After 24 hours, if the gel was incompletely eaten, the percentage of gel remaining was visually estimated within the following categories: <25%, ≥25% but <50%, ≥50% but <75%, ≥75%, untouched). Food consumption was recorded daily when the animals were weighed and inspected for wellbeing. Signs of stress or ill-health including abnormal respiration or motor postures, piloerection, fur loss, changes in the eyes, weight loss or fearful or aggressive behaviour towards the researcher were recorded as well as the Rat Grimace Scale score.[26] All observations were made by the same researcher.

Pilot Study 2

Hypothesis
Adult female rats that are fed a highly palatable high-fat diet (HFD) will consume fish oil-enriched gels completely and within 60 minutes of placement, independently of whether the gel has a standard content of raspberry flavouring or double flavouring.

Study Design
Three raspberry flavoured gel types were prepared: a control gel with no fish oil and standard raspberry jelly content (17g in 100ml) and 2 low dose unoxidised fish oil (0.05ml) gels, one containing standard raspberry jelly and one with double-strength raspberry jelly (34g in 100ml) (Table 1).

Five adult female SD rats (age: 84 ± 1 days) weighing 228.8 ± 7.9 g were individually housed as detailed above with individual rats considered the experimental unit. They were fed a commercially available HFD (D12451, Research Diets Inc., New Brunswick, NJ, USA) containing 45% kcal as fat *ad libitum* for three days before initiating with the control gel (gel with flavouring but no oil). Food was topped up to 100g daily.

In Pilot Study 1, the time to consume gels reduced after the first two days suggesting the need for a period of acclimatisation prior to starting the experiment. Hence in Pilot Study 2, the rats initially received a control gel for three days. Subsequently, the intervention began with each of the 3 gel types administered twice in a randomised order over 6 days (order determined by random sequence generation (www.random.org)). Timers were set to record the time taken to eat the gels. Each day the rats were weighed, inspected for wellbeing and food consumption was recorded (Figure 2).[26]

Figure 1. A female rat holding an oil-enriched gel in its paws.

Two major experimental studies utilising oil-enriched gels during rat pregnancy

Following the pilot studies, two major experimental studies were carried out as informed by the results of the pilot studies. The major purpose of these two experimental studies was to determine the effects of fish oil supplementation during pregnancy on the offspring (not reported here). However gel consumption and effects on wellbeing were assessed and are reported here.

In the first trial, 98 SD dams were allocated to a high-fat or a matched control diet prior to mating. Control gels were started 5 days before the first attempt at mating. Once mated, dams received a gel on each day of gestation, and the lactation period, while continuing on their allocated diet. These gels were raspberry flavoured and contained 0.05ml of unoxidised fish oil, or no oil (control) (Figure 1).

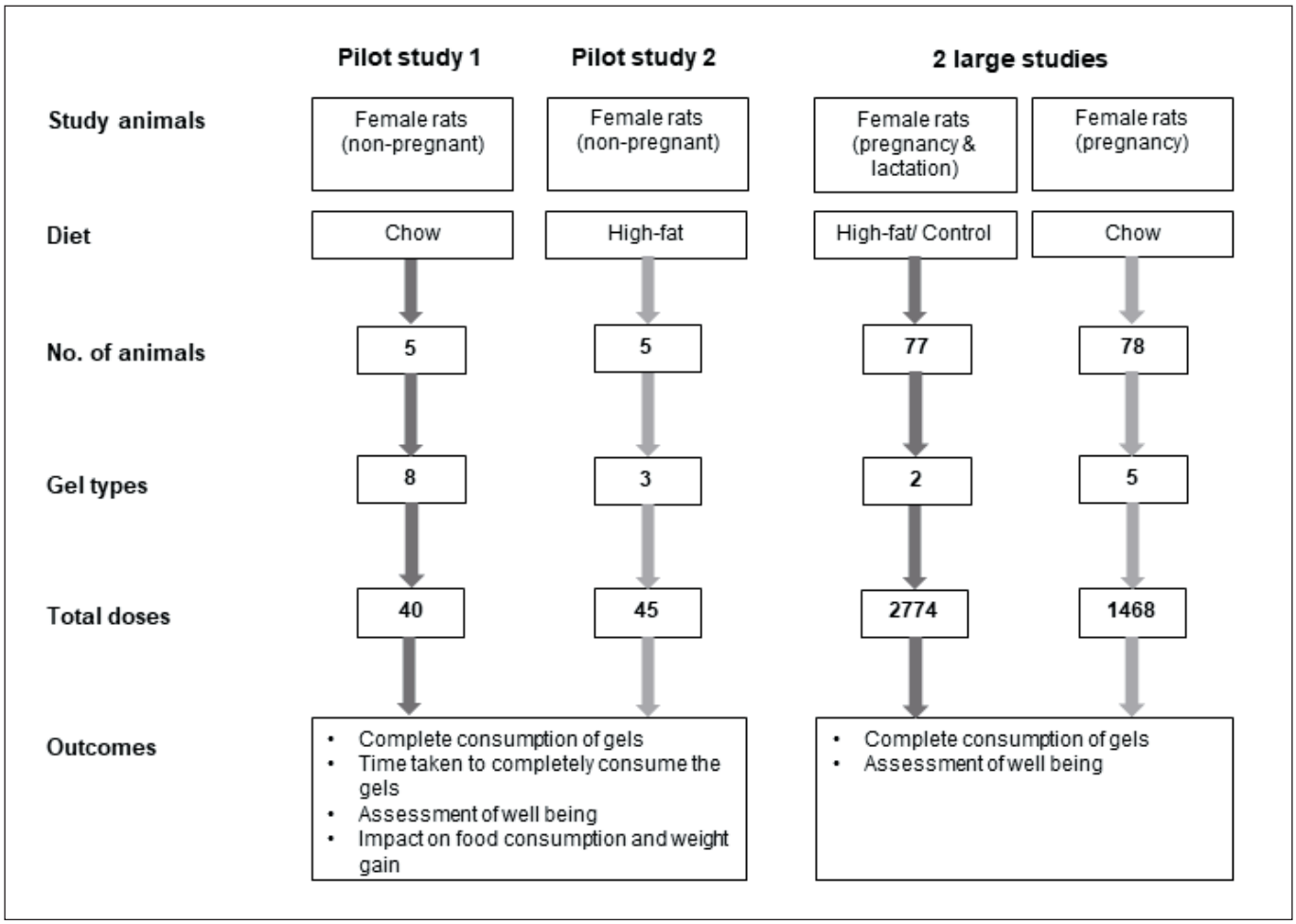

Figure 2. Flow diagram, representing the 4 studies used to assess the reliability of emulsified gel treatments.

In the second trial, 99 SD dams were fed the standard chow diet and control gels were started 5 days before the first attempt at mating. Once mated, dams received a gel on each day of pregnancy. These gels were raspberry flavoured and were either control gels (no oil) or contained 0.05ml of fish oil that was oxidised to various levels (peroxide value 5, 10, or 40 meq/kg), or 1ml of highly oxidised fish oil (peroxide value 40 meq/kg) (Figure 1).

On each day a gel was placed into the food hopper and the following day it was noted whether or not it had been completely eaten. The animals were observed daily for signs of stress and wellbeing.[26]

Statistical analyses

Across both studies, differences in the time taken to eat the gels completely, weight change and food consumption were compared using the Mann-Whitney test. The change in the time taken to eat the gels across the intervention period in Pilot Study 2 was analysed using Spearman's rank correlation coefficient. All statistical analyses were carried out in IBM SPSS Statistics v.26.0.0.0 (IBM Corp.). Data are presented as mean +/- standard error and significance was determined as $p<0.05$.

Results

Treatment	Time to consume (in min)	Food consumption (g/ 24hours)	Weight gain (g) in next 24 hours
Pilot Study 1			
Run in (no gel)		20.9 ± 1.1	4.1 ± 1.4
Unoxidised oil 0.05ml	47.5 ± 25.4†	20.2 ± 1.2	2.0 ± 1.7
Oxidised oil 0.05ml	23.3 ± 16.7	18.8 ± 1.1	5.6 ± 2.9
Unoxidised oil 1ml	85.5 ± 48.7	18.2 ± 1.1	2.2 ± 3.2
Oxidised oil 1ml	19.5 ± 29.5	16 ± 3.2††	(4.0) ± 2.6†††
Unoxidised oil 0.05ml + RJC	2.9 ± 0.4†	20 ± 1.4	5.3 ± 3.4
Oxidised oil 0.05ml + RJC	4.6 ± 1.1	21.5 ± 0.7	5.3 ± 3.2
Unoxidised oil 1ml + RJC	10.1 ± 6.4	15.8 ± 2.4††	1.0 ± 3.8
Oxidised oil 1ml + RJC	13.2 ± 7.3	17.8 ± 1.6	1.0 ± 3.3
Grouped Analyses:			
All gels with RJC	7.7 ± 2.5$^{¥}$	18.5 ± 1.0	3.1 ± 1.7
All gels with no RJC	51.1 ± 15.7$^{¥}$	17.8 ± 1.1	1.7 ± 1.4
All gels with Oxidised oil	22.4 ± 8.8	18.8 ± 0.9	2.2 ± 1.7
All gels with Unoxidised oil	36.5 ± 14.8	18.4 ± 0.9	2.5 ± 1.5
All gels with 1ml oil	39.3 ± 15.0	16.9 ± 0.9$^{¥¥}$	0.22 ± 1.6
All gels with 0.05ml oil	19.6 ± 8.1	20.1 ± 0.6$^{¥¥}$	4.5 ± 1.3
Pilot Study 2			
Run in (no gel)		16.3 ± 0.6	1.9 ± 1.3
Control (no oil + RJC)	44.8 ± 16.9	14.1 ± 4.9**	3.2 ± 1.5
Unoxidised oil 0.05ml + RJC	44.2 ± 33.2	14.6 ± 2.7	2.3 ± 1.6
Unoxidised oil 0.05ml + 2X RJC	42.4 ± 17.3	13.8 ± 1.9*	5.2 ± 3.6

Table 2. Time to completely consume gels, and food consumption and weight gain in the following 24 hours in rats fed either a chow diet (Pilot Study 1) or a high fat diet (Pilot Study 2). Data are shown as mean ± standard error, n=5 per group. Data in parentheses are negative numbers. †indicates a difference between the unoxidised 0.05ml flavoured gel and the unoxidised 0.05ml unflavoured gel p=0.001. *p=0.03 **p=0.01 for comparison with the run in (no gel) period. ††p=0.06 ††† p=0.02 for comparison with the run in (no gel) period. $^{¥}$p=0.0003 for comparison between all gels with RJC and all gels with no RJC. $^{¥¥}$p=0.009 for comparison between all gels with 1 ml and all gels with 0.05 ml of oil. RJC; raspberry flavoured jelly crystals.

Pilot Study 1

There was a 2-day period of acclimatisation, as across all groups the time to fully consume the gel was substantially greater in the first 2 days (381.0 ± 177.8 min vs 29.4 ± 8.5 min, p<0.0001). This was identified as the study was being carried out and it was recognised that this did not reflect individual gel types but the novelty of receiving any gel. To prevent introducing a bias towards greater times for gels delivered on those days, the first 2 days were excluded from the analysis and repeated at the end of the study to make up the full 8 days of treatment. The analysis presented represents the 8 days after acclimatisation.

All gel types were eaten completely and the mean time for complete consumption was <90 minutes for all gel types (Table 2). No animals showed any signs of stress or ill-health. Amongst the gel types containing 0.05ml of unoxidised fish oil, the time taken to fully consume them was lower if the gel was raspberry flavoured (2.9 ± 0.4 vs 47.5 ± 25.4 minutes; p=0.001). There were no other significant differences between the individual gel types as regards pattern of consumption.

To increase statistical power, gel types were grouped. Overall, gel types containing raspberry flavouring were consumed more quickly than those without (7.7 ± 2.5 vs 51.1 ± 15.7 minutes; p=0.0003). There was no difference in the time to fully consume gel types containing 1ml and 0.05ml of fish oil, or between gel types containing oxidised and unoxidised fish oil.

Gel type	Baseline food intake (g)	Food intake post gel (g)
Pilot Study 1: Rats consuming a chow diet		
0.05ml oil	20.9 ± 1.1	20.0 ± 1.0
0.05ml oil + RJC	20.9 ± 1.1	20.0 ± 0.8
1ml oil	20.9 ± 1.1	16.9 ± 1.3*
1ml oil + RJC	20.9 ± 1.1	18.0 ± 1.3*
Pilot Study 2: Rats consuming a high fat diet		
Control (no oil + RJC)	16.3 ± 0.6	14.1 ± 4.9*
0.05ml oil + RJC	16.3 ± 0.6	14.6 ± 2.7
0.05ml oil + 2x RJC	16.3 ± 0.6	13.8 ± 1.9*

Table 3. Effect of gel consumption on daily food intake in rats. Data are shown as means ± standard error, n=5 per group. Note that for Pilot Study 1 oxidised and unoxidised gel types have been grouped together as oxidised and unoxidised oil have identical macronutrient content and did not have different effects on food intake. RJC; raspberry flavoured jelly crystals.

*p<0.05 for the difference between food consumption or nutritional intake after consumption of the gel compared with baseline.

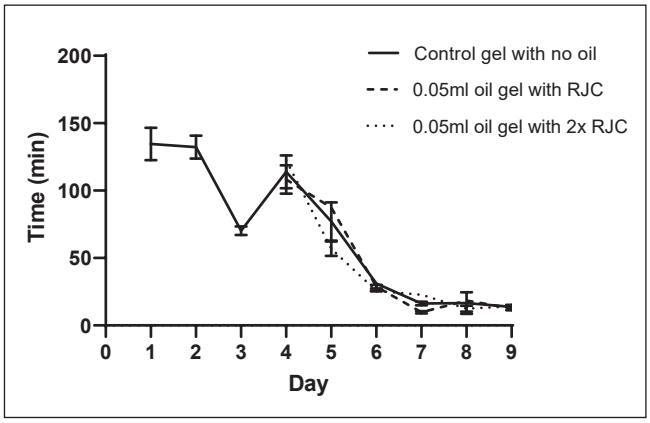

Figure 3. Time taken to completely consume the gels across the period of Pilot Study 2. Data are shown as means ± standard error, n=5 per group. RJC; raspberry flavoured jelly crystals.

Following consumption of the gels, there were no between-gel type differences in food consumption or 24-hour weight gain. Further, grouped analyses showed that the presence or absence of raspberry flavouring or whether the oil was oxidised or unoxidised did not affect food consumption. However, food consumption was reduced after addition of the gel, compared to the lead-in period when the rats did not receive a gel (20.9 ± 1.1 g/day). This reached significance for gels containing 1ml of fish oil (with raspberry 18.0 ± 1.3 g/day; p=0.03, without raspberry 16.9 ± 1.3 g/day; p=0.03), but not for those containing 0.05ml of fish oil (with raspberry 20.0 ± 0.8 g/day; p=0.24, without raspberry 20.0 ± 1.0 g/day; p=0.38) (Table 3). Food consumption was lower following consumption of a gel containing 1ml of oil, compared with gels with 0.05ml of oil (16.9 ± 0.9 vs 20.1 ± 0.6 g/day; p=0.009).

Pilot Study 2

In rats fed a high-fat diet, all gel types were consumed completely within 24 hours but regression analysis indicated a reduction in the time to consume the gels over the study period (control: no oil with standard raspberry; β=(-17.0); p=0.0004, 0.05ml fish oil with standard raspberry; β=(-20.1); p=0.002, 0.05ml fish oil with double raspberry; β=(-19.4); p=0.03). This occurred over the first 5 days, so that across all groups gels were eaten much faster in the final 4 days (104.3 ± 6.7 minutes vs 17.9 ± 1.6 minutes, p<0.0001) (Figure 3). There were no between gel-type differences in the time to consume the gels, weight gain or food intake (Table 2).

However, following consumption of gels there was a reduction of food intake from baseline (16.3 ± 0.6 g/

day) to 14.1 ± 4.9 g/day (p=0.01), 14.6 ± 2.7 g/day (p=0.17) and 13.8 ± 1.9 g/day (p=0.03) per day after consumption of control, raspberry and double raspberry gels respectively (Table 2).

Use of oil-enriched gels in 2 major experimental studies of rat pregnancy

Across the two major experimental studies 155 of the 197 dams were successfully mated and received gels throughout pregnancy with 57 dams also receiving gels throughout lactation, a much longer period than in the pilot studies. A total of 4,242 doses of gel were provided. The dams appeared to seek out the gel treatments and began to eat them almost immediately. They showed no signs of stress upon handling and appeared comfortable with researchers. All gel types were eaten completely across all groups irrespective of whether the dam consumed a control or high-fat diet and even in those dams that received gels containing a high dose of highly oxidised fish oil.

Discussion

We have reported a method for producing oil-enriched flavoured gels using an emulsifier and shown that they represent an acceptable, reproducible and efficient method of oral oil administration in female rats. All adult female rats consumed the gels whether they were flavoured or not, enriched with 1ml or 0.05ml of oil and whether the oil was highly oxidised or not. Even when fed a highly palatable high-fat diet, the gels were still completely and quickly consumed. As there was no difference in the time taken to consume gels between those made with a standard concentration of raspberry flavouring and those with double-concentrated flavouring, the standard concentration (which has a lower sugar content) should be preferred. Importantly, the rats remained in good health and showed no visible signs of stress associated with provision of gels.

Both pilot studies indicated that rats take time to accept and rapidly consume the novel gel treatment. In rats fed a chow diet, the time taken to fully consume the gels reduced after 2 days but this took 5 days when they were fed the highly palatable high-fat diet. In studies where it is critical that the gel is eaten quickly, such as when it contains a chemically unstable constituent such as fish oil, a lead-in period using control gels of at least 5 days is appropriate.

We have demonstrated that gels of relatively large volume (5ml) can be completely and reliably consumed by an individual rat. Such large volume gels were necessary in order to incorporate a proportionally large amount of lipid (1ml). However, it is likely that most future studies utilising emulsified gels, could use smaller sized gels e.g. 0.5ml or 1ml and that these would be completely consumed even more rapidly.

It is critical to consider the nutritional impact of any oral intervention as vehicles such as gels could affect macronutrient and total energy intake. Importantly, the gels containing a low dose of fish oil (0.05ml), comparable to human consumption had a minimal effect on food intake.[23] However, when rats consumed the gels containing a high dose (1ml) of fish oil they reduced their food consumption which altered their overall macronutrient intake (data not shown). It is likely that the nutritional impact would be substantially reduced with smaller gels, so that where the volume of the supplement or drug is low, a smaller gel should be utilised (e.g 1ml).

It is important to consider each of the gel components and whether they were necessary. The starch emulsifier was essential in order to incorporate the lipid into the gels. However, in studies without a lipid component this would not be required. Using gelatine in addition to the raspberry jelly powder was also necessary as without it the lipid enriched gels liquefied at room temperature. Using the proportions reported we were able to produce relatively firm, resilient gels that did not melt when brought into the animal laboratory which is key given the ambient temperatures of most small animal research facilities. The raspberry jelly flavouring was not essential, in that gels were completely eaten when unflavoured. However it led to much faster consumption which is advantageous when a gel component is chemically unstable (such as fish oil[1,3]). We observed no advantage of using double-strength raspberry jelly, even in the context of the high fat diet and we calculated that it substantially increased the carbohydrate content of the gel (data not shown).

Welfare is a critical ethical consideration in any study involving animals. We are obligated to work towards the 3Rs i.e. *replace* or *reduce* the use of animals where possible and where animal use is essential *refine* methodology to improve welfare.[27] The use of oil-enriched gels we report represents an important refinement to study design that directly improves Animal Welfare. Oro-gastric gavage requires a highly skilled operator and even when expertly performed is a stressful procedure with a high risk of mishap[8] which can harm the animal and potentially confound the results of a study.[13] It is unphysiological as it bypasses the oral phase of digestion and it has been recommended that oro-gastric gavage should not be used in toxicity studies[13] In contrast, the production of gels and provision to the animals described is simple and solves the same problems that gavage does (controlled dose, and rapid administration) but instead of risking mishap and inducing stress, appeared to be an enriching experience for the rats.

In the pilot studies, animals were single-caged. This was because the future studies for which the gels were developed were to include pregnant dams that would be single-caged. Rats are social animals and should be allowed to exhibit their normal social behaviours. Although being housed within sight and smell of each other can ameliorate some of the stresses associated with singleton housing, such caging should only be used when necessary. We believe the gel treatments we have developed could be used for co-housed rats. Where two animals are caged together, placement of the gels in opposite ends of the cage may be sufficient to ensure each animal is dosed. Where more precise control is necessary, temporary cage dividers could be used after gel placement until the gels are consumed.

In principle, replacement of daily gavage with provision of a gel, would be expected to reduce stress. In support of this, across all the presented studies, animals did not present with visible signs of stress and appeared comfortable with researchers, which contrasts with a previous study of daily gavage in pregnancy where the animals did appear stressed.[24] However, we did not directly compare these methods of administration and did not measure physiological markers of stress such as heart rate, blood pressure, and corticosterone concentration. This could be addressed in future research.

The major strength of this series of studies was the very large number of total gel doses administered, all of which were completely consumed with no signs of ill-health. The large cohort of rats included pregnant, lactating and non-pregnant females. Moreover, the gels were eaten even when they contained oxidised oil or the animals were fed a highly palatable diet. However this study has limitations. Only female rats were studied and we did not study other small animals commonly used in biomedical research. There is no reason to expect that males would be less likely to fully consume the gels but it is important that reliability of consumption is assessed in other target animals such as mice. The gels were always provided in the morning, if future studies planned administration at other times of the day, when the animals were more or less active, it would be wise to conduct a brief pilot to ensure rapid consumption. Lastly, following the principle of *Reduction*, it was important to use a small number of animals in the pilot studies.[27] While this limited statistical power to detect small differences between gel types, the sample sizes were sufficient to demonstrate the presence of an acclimatisation period and that raspberry flavoured gels were more quickly consumed than unflavoured gels. Importantly, the critical finding, that every gel was completely consumed, was corroborated by the large experimental studies.

In summary, we report a methodology for oil to be incorporated into small gels with the use of an emulsifier. Such gels are highly acceptable to rats and enable a complete dose to be administered quickly. This represents an important refinement over oro-gastric gavage in rats, replacing a stressful and risky experience with an enriching one. This could be of great importance in many studies including those with behavioural outcomes or investigating the developmental origins of health and disease.[18] Future animal studies examining the effects of oral nutritional supplementation or drug administration should consider the use of gels as the vehicle.

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Authors' contributions

The gel treatment was conceived by MHV and BBA and developed by BBA and VVS. The pilot and experimental trials were conceived by MHV, BBA, and VVS carried out by BBA, VVS and PEB. Data was analysed by BBA and VVS with input from MHV, and interpreted by MHV, BBA, VVS and WSC. The draft was written by VVS and all authors contributed to and approved the final manuscript.

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Conflicts of Interest

Nothing to declare

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