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Effects of extruded or pelleted diet on blood glucose, body weight, feed and water consumption in rats

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Introduction

Feed for animals can be processed in different ways, with pelleting and extrusion (or expansion) as two of the most common physical forms. The different forms have both advantages and disadvantages. Pellets are easy to produce and handle but may, depending on pressure used in the process, fall apart and form dust and wastage. Extruded feed is claimed to have a higher hygienic standard, palatability and digestibility, due to the high temperature (+100-150°C) used during the extrusion process *(Egana et al. 1991, Lewis et al. 1987)*. Even though the extruded feed will be sterilised during this phase, the feed is cooled and dried afterwards, with possibilities of recontamination of the surface and should not be regarded as a sterilised feed. Different studies have shown both increased and decreased digestibility after extrusion *(Dean & Edwards 1985, Delort-Laval & Mercier 1976, Stroucken & van der Poel 1996)*. Extrusion will also increase the price of the diet.

The advantages of higher digestibility has mainly been investigated in larger animals, but are used in marketing of laboratory animal feed as well. For animals with a high metabolism and a major feed intake during night, an increased digestibility might not be an advantage if it results in a high blood glucose peak shortly after intake and with a faster decrease of the level in the blood, especially not if the animals should be fasting.

The first aim of this study was to see if an extruded form of a diet would result in a rise in blood glucose levels and body weight and alter the feed and water consumption pattern in Sprague Dawley rats, compared to a pelleted form.

A second aim was to see if the extruded diet would cause alterations in blood glucose levels in fasted animals.

Materials and Methods

Animals

Twenty male Sprague-Dawley rats (Mol:SPRD (Hannover) from BU 150) from M&B AS, Møllegaard Breeding Centre, Ejby, Denmark were used. They were health monitored according to FELASA, except for *Clostridium piliforme*, *Leptospira* spp, *Klosiella* spp and *Trichosomoides crassicauda*. The analyses showed presence of other flagelates than *Giardia* and *Spironucleus* spp. On arrival the animals were weighed and housed singly in macrolon type IV cages, with feed and water *ad libitum*. The rats were acclimatised for 12 days before weighed again. The temperature in the animal room was +22 ±3°C, the relative humidity was 55±10% and the air changes 18/hour. Light was on from 06.00 hr to 18.00 hr, with ½-hour of dawn and dusk periods (06.00-06.30 hr and 18.00-18.30 hr). The rats were kept on wood chips (BeeKay Bedding, Norway) and the cages were changed twice a week. The animals were randomly assigned to a group of 10 rats, given either extruded or pelleted diet from arrival.

Feed

The recipe for pelleted R 70 (Lactamin, Sweden) was used and the dough was either extruded or pelleted. The processes are described in Figure 1-2. To assess if any significant changes had occurred during the process, a single sample of

each diet was analysed for a set of parameters prior to the study (Table 1) at AnalyCen Nordic AB, Lidköping, Sweden. Methods for analyses; Protein – determination of nitrogen according to Dumas no 202:1, 210:6; Fat – crude fat according to EU, AN 301, Tecator no 213:16,17; Moisture – SS 19 10 11 no 213:1, 2, 3; Calcium, Phosphor, Sodium, Potassium, Magnesium – NMKL 139:1991 no 208:201; Retinol – Roche 1988-270-98033, 2101 no 206:1; Salmonellae – NMKL 71:1991 4th Ed.; Fungal units – NMKL 98:1995 3rd ED.; Total bacteria – NMKL 86:1986 2nd Ed. (*Pehrson 1999*). The diets were stored in a rodent secured storage room with +16±3 °C and 35± 15% RH. The feed amount in the food hoppers was checked every day and additional feed given when needed.

Measurements

The animals were weighed at arrival. On day 12-15 the animals, as wll as feed and water were weighed at approx. 09.00 and 16.00 hr and on day 16 at 09.00 hr. The feed was withheld from 09.00 hr day 15 to 09.00 hr day 16.

On day 15 the rats were anaesthetised at 0 (09.00hr), 2, 5, 8, 12 and 24 hours with enflurane (Efrane®, Abbott Scandinavia AB, Kista, Sweden) 3-5% in O_2:N_2O (45:55) for 5-10 minutes and blood samples were taken from the tail vein. The anaesthesia was induced in a box and maintained with a mask. The vein was punctured under aseptic conditions and a drop of blood was placed on a test stick (BM-Test Glycemie 1-44, Boehringer Mannheim Scandinavia AB, Bromma, Sweden) and analysed in a Refrolux S (Boehringer Mannheim Scandinavia AB, Bromma, Sweden) one minute later. After the last blood sample the animals were then given feed again and left to recover until day 19.

From day 19-22 the animals, feed and water were weighed at approx. 09.00 and 16.00 hr and on day 23 at 09.00 hr. On day 22 blood samples were taken according to the schedule mentioned above, but the rats had access to feed during the sampling period this time. After the animals were weighed, anaesthetised and blood sampled on day 23 they were euthanased by exsanguination under anaesthesia. The intestines were cut distally to the stomach and proximally to the pelvis and removed in a standardised way. The whole intestine and the cecum with content were weighed.

Statistical analyses

The data are presented as mean value ± standard deviation (SD). Comparisons between the two groups were made at each timepoint regarding body weight, weight change, amount ingested feed and water and blood glucose levels. The values of blood glucose levels at hour zero on days 15 and 22 were compared to each other. Differences between mean values were evaluated using Student's unpaired *t*-test. Following significance levels were used: * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$.

Results

During the 23 days the rats were studied, both the groups receiving either pelleted or extruded feed had a similar weight gain except for day 15 during daytime (Table 2). The group fed pellets had a larger decrease in weight change, but there was no statistical difference in body weight. Both groups ingested similar amounts of feed (Fig. 3-4) and water (Fig. 5-6) except for day 12, where the group fed extruded feed had a significant decrease during daytime. The two groups had a similar feeding pattern, eating and drinking mainly during night (Fig. 3-6). This resulted in a weight gain during nighttime and a loss of weight during daytime (Table 2). There was no difference in the feeding pattern over time, as the two groups ingested similar amounts of feed and water day 12-16 and day 19-23 (Fig.3-6).

The body weight decreased during the 24 hours the animals were fasted (day 15-16) and blood sampled the first time (day 15), with a similar weight loss both during day and night. The decrease in weight during daytime in the group given pellets and during nighttime in both groups was statistically significantly different compared to previous days. When the rats were blood sampled the second time, day 22-23, the weight gain during nighttime was significantly decreased compared to the previous days, even though the animals had access to feed.

Blood glucose levels were within normal values 98(4,4-16,7 mmol/l) (*Loeb & Quimby* 1989, *Olfert* et al. 1993) throughout the measuring

Figure 1
The pelleting process

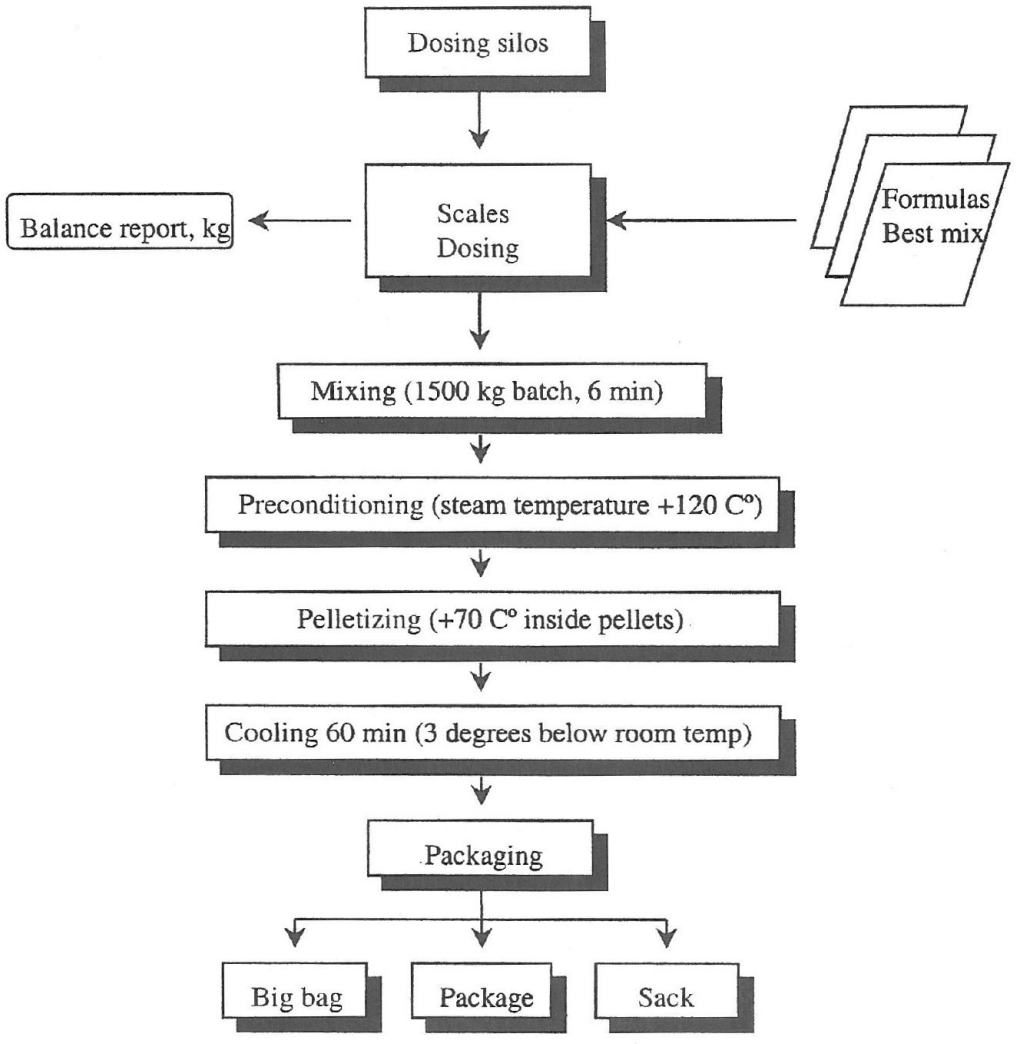


Figure 2
The extruding process

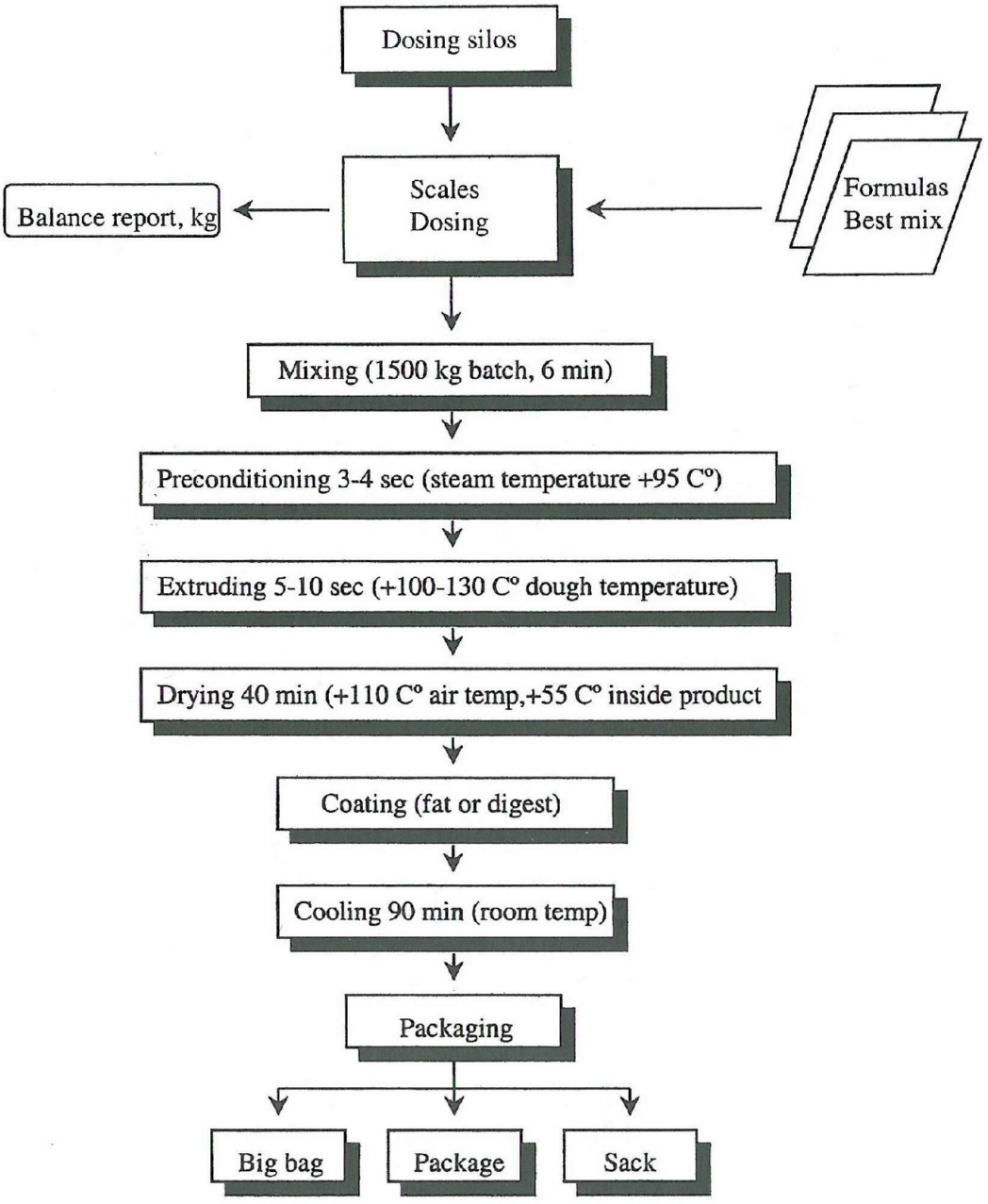


Table 1
Analyses of diets prior to study
NFE = nitrogen-free extract
NFE + crude fibre = carbohydrates

	Unit	Variation limits of test	Pelleted R 70	Expanded R 70
Protein	%	±1	15.3	14.2
Fat	%	±4	4.2	4.3
NFE	%		58.5	58,7
Crude fibre	%	±5	4,9	4,9
Moisture	%	±1	12.1	12.9
Calcium	%	±3-5	0.69	0.74
Phosphor	%	±3-5	0.58	0.58
Sodium	%	±3-5	0.18	0.25
Potassium	%	±3-5	0.76	0.68
Magnesium	%	±3-5	0.15	0.17
Retinol, vit. A	IU/kg	±5-50%	12.000	11.000
Digestible energy content	KJ/100g		1257	1246
Salmonellae	/25 gram		not detected	not detected
Fungal units	log cfu/g		2.0	<2
Total bacteria	log cfu/g		3.3	3.7

periods and there was no difference between the two groups (Fig. 7). The levels during the second measuring period seem to be a little higher but there was no significant difference when comparing the hour zero values.

The intestines from the rats fed pelleted diet weighed 25,4±2,5 g and the cecum weighed 5,5±0,6 g. The intestines from the animals fed extruded diet weighed 25,2±2,4 g and the cecum 5,4±0,8 g. There was no difference between the two groups.

In the analyses done prior to study, there was no major difference between the two forms of diet as the differences in the contents were within accepted variation of the tests (Table 1).

Discussion

The quality of laboratory animal feed has to be high, both in terms of nutritional values, hygiene and in reproduciability. To achieve this the diet is processed. The physical form has traditionally been a pelleted diet, but both pelleted and extruded diets are now available. The effect of the process is dependent on what type of ingredients the diet consists of. The extrusion process is performed in a higher temperature than pelleting and higher temperature will make the carbohydrates more digestible *(Delort-Laval & Mercier 1976, Gaeba et al. 1998, Lewis et al. 1985)*. Protein, on the other hand, might be more or less digestible *(Dean & Edwards 1985, Friesen et al. 1993, Ginste & DeSchrijver 1998, Serrano et al. 1998, Stroucken & van der Poel 1996)*, depending on both temperature and duration of the process. The extrusion process is also claimed to increase the palatability and decrease the feed wastage of the diet.

In our study, we found only minor differences between the two diets in terms of weight gain and in feed and water consumption. There was no difference in the weight of the intestines or cecum. This indicates that the physical form had no effect on palatability or digestibility. With both diets the wastage was minimal and comparable by a visual

Table 2
Body weight ± SD and weight change± SD during day 12-16 and day 19-23
a-f = pairwise comparisons (a-a etc) with *** significance

Day	0	12	12	13	13	14	14	15	15	16
Time	16.00	09.00	16.00	09.00	16.00	09.00	16.00	09.00	16.00	09.00
Pelleted R 70										
Body weight (g)	166±5	270 ± 6	263 ± 6	275 ± 6	269 ± 7	284 ± 8	277 ± 8	288 ± 8	276 ± 8	265 ± 8
Weight change (g)			-7±2	12±4	-6±3	15±3	-7±2 **a**	11±3 **b**	-12±2 **a,c**	-11±3 **b**
Extruded R 70										
Body weight (g)	164±3	274 ± 11	266 ± 11	275 ± 11	270 ± 13	284 ± 14	276 ± 14	288 ± 15	278 ± 14	267 ± 12
Weight change (g)			-8±2	9±6	-5±5	14±5	-8±3	12±2**d**	-10±1 **e**	-11± **d**
Day		19	19	20	20	21	21	22	22	23
Time		09.00	16.00	09.00	16.00	09.00	16.00	09.00	16.00	09.00
Pelleted R 70										
Body weight (g)		306 ± 11	299 ± 11	311 ± 12	307 ± 11	319 ± 13	312 ± 12	324 ± 13	316 ± 14	318 ± 14
Weight change (g)			-7±3	12±3	-4±5	12±5	-7±3	12±2 **e**	-8±3	2±4 **e**
Extruded R 70										
Body weight (g)		305 ± 16	299 ± 16	312 ± 17	306 ± 18	319 ± 17	313 ± 17	323 ± 20	317 ± 19	320 ± 19
Weight change (g)			-6±2	13±3	-6±3	13±5	-6±3	10±4 **f**	-6±3	3±1 **f**

Figure 3
Average feed consumption ±SD per rat during day 12 - 15

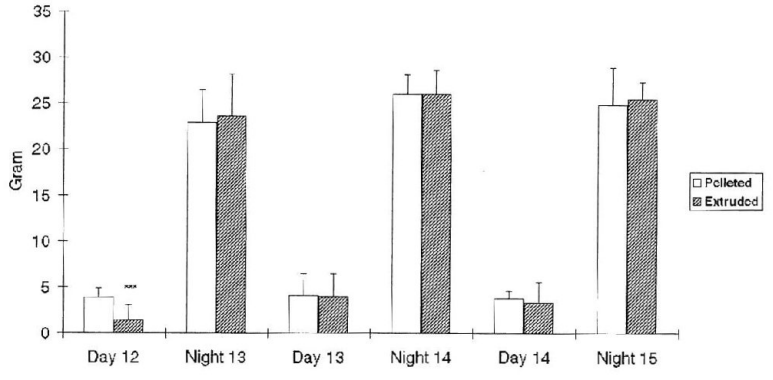


Figure 4
Average feed consumption ±SD per rat during day 19 - 23

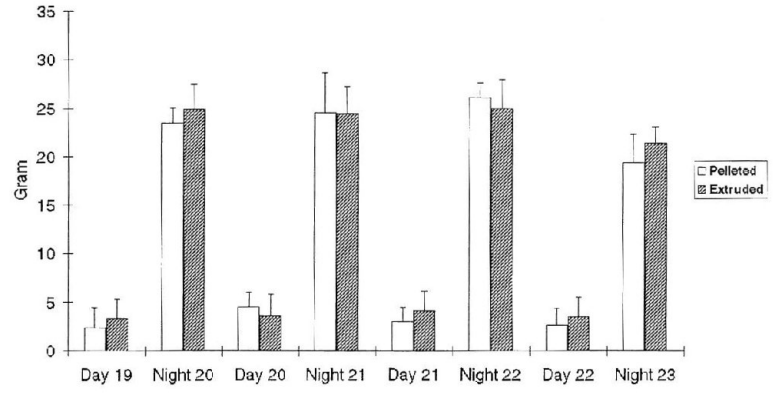


Figure 5
Average water consumption ±SD per rat during day 12 - 15

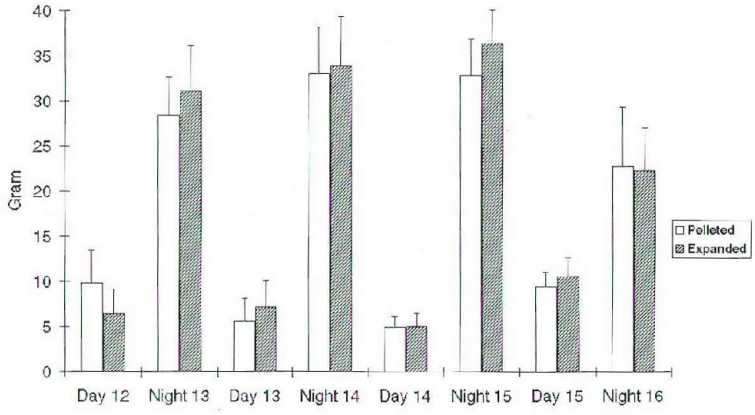


Figure 6
Average water consumption ±SD per rat during day 19 - 23

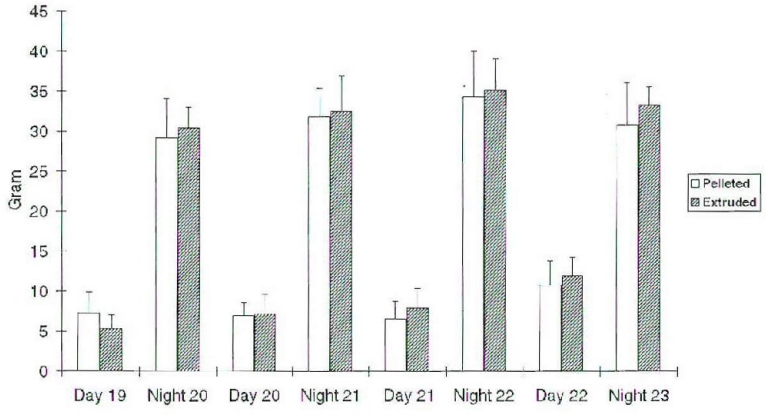
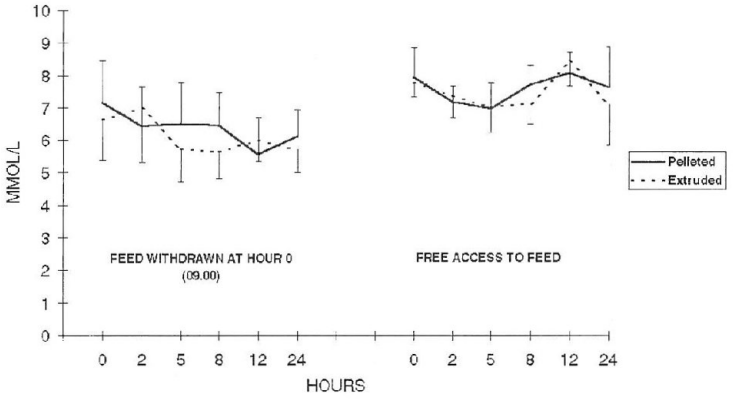


Figure 7
Blood glucose levels ±SD during day 15 - 16 (feed withdrawn between hour 0-24) and day 22 - 23 (free access to feed)



estimation.

We did expect that the extruded diet might affect the blood glucose level, with a more rapid increase after ingestion and a decrease of the level earlier, especially in the fasted rats. In the fed group given extruded diet, the increase of the blood glucose from hour 8 (17.00) to hour 12 (21.00) was faster and larger compared to the group with pelleted diet. The slope of the curves of the fasted rats were downwards for both diets, but with a sharper drop from hour 2 to hour 5 in the group with extruded diet. This is in line with our assumptions, but all differences are without statistical significance.

The anaesthesia might have altered the blood glucose level and thereby hidden effects of the diet, but there are studies showing both a retained and an elevated level after different forms of anaesthesia. Raised blood glucose levels in rats were found by Heath et al *(1978)* after halothane anaestesia and by Johansen et al *(1994)* after fentanyl-fluanison, but not after pentobarbitone sodium anaesthesia. When the rats were fasted Johansen et al *(1994)* did not see any elevation of the glucose level after the two different anaesthetic agents. Johansen et al *(1993)* also showed an increased blood glucose level after giving morfine to fed rats, but not to starved rats. Tabata et al *(1998)* found small or no difference in glucose levels after ether anaesthesia in fed rats. When Golda *(1998)* measured the blood glucose level after ether anaesthesia given to fed and starved rats and pentobarbitone sodium anaesthesia to starved rats, he recorded no increase. But after stressing the animals, the glucose level raised and more clearly in fed rats compared to starved.

In the present study the blood glucose levels in the groups with free access to feed, either extruded or pelleted, were a little higher than when the rats were starved, but all the values are within the normal range and the differences without stastistical significance.

Dean & Edwards *(1985)* did not find any apparent advantage of extruded diets over pelleted in terms of growth, food conversion efficiency and metabolizable energy in their study. There was a positive effect of processed feed (pelleted or extruded) compared to meal regarding protein

utilization.

The weight loss or reduced weight gain during the 24 hours the animals were blood sampled were most certainly an effect of fasting (day 15), handling stress and repeated anaesthesia. During the 24 hours the feed was withdrawn the animals seem to have been drinking more during daytime but less during nighttime, ending up with a slightly decreased water intake totally, compared to previous days. The difference was not significant, though.

The statistical difference in feed and water intake during day 12 is puzzling, without any known change at that time point. One contributing factor might be that the animals were weighed for the first time, after the initial weighing at delivery. The group given expanded feed were weighed after the group with pelleted feed and might have become more stressed by the procedure. The feed intake during daytime is small and a disturbance could result in a relatively large difference.

The higher temperature in the extrusion process does decrease the microbiological contamination more efficiently than the pelleting process, but will not result in a sterile product (*Dean & Edwards, 1985)*. As the extruded diet is relatively porous, a recontamination of the feed will probably take place during drying, cooling and packing in a higher degree than during the corresponding treatment of the pelleted feed. The analyses of the different diet forms in our study did not show any major difference (Table 1).

As long as pathogenic microorganisms are not found in diets and the handling before and after the heat treatment is conducted in a proper way, the relative reduction of microorganisms has little general importance.

The only practical benefit of an expanded diet that we could find, is that the weight per volume is decreased. An effect of this is that a cage cover loaded with expanded feed was lighter and easier to handle, compared to a cage cover loaded with pelleted feed. On the other hand you have to have a larger storage area or order feed more frequently, when using expanded diets.

Summary

Extruded diets are claimed to be more digestible, palatable and of a higher hygienic standard compared to pelleted diets. In a comparision of a pelleted and an extruded diet given to Sprague-Dawley rats, we found no difference in weight gain, feed and water consumption or in blood glucose level. In the microbiological analyses, there was no major difference either. When considering choice of diet the physical form seems to be of minor importance. The major factors should be the nutritional value, hygienic level (autoclavable or irradiated vs conventional), reproduciability, availability, confidence in producer and economical values.

Sammanfattning

Extruderade foder anses ha en högre smältbarhet, smaklighet och vara av en högre hygienisk standard jämfört med pelleterat foder. I en jämförelse mellan ett foder i pelleterad och extruderad form kunde vi inte se några skillnader i viktökning, foder- och vattenkonsumtion eller blodglukosvärden när de gavs till Sprague-Dawley råttor. Där var heller inga större skillnader vid den mikrobiologiska analysen. Vid övervägande av foderval verkar den fysikaliska formen vara av mindre betydelse. De tunga faktorerna bör vara näringsvärdet, den hygieniska nivån (autoklaverbart eller stråat vs konventionellt), reproducerbarheten, tillgängligheten, förtroende för producenten och ekonomiska värden.

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References

Dean J, DG Edwards: The nutrional value of rat diets of differing energy and protein levels when subjected to physical processing. Lab. Anim. 1985, *19*, 311-319.

Delort-Laval J, C Mercir: Evaluation of different technological treatments of cereals. 1. Choice of treatments and their effect on the carbohydrate fraction of wheat, barley and maize. Ann. de Zootech., 1976, *25* (1), 3-12.

Egana JI, A Lopez, C Quezada: The effect of extrusion on the acceptability and digestability of diets for growing dogs. Arch. Latinoam. Nutr. 1991, *41*(1), 111-120.

Friesen KG, JL Nelssen, RD Goodband, KC Behnke, LJ Kat: The effect of moist extrusion of soy products on growth performance and nutrient utilization in the early weaned pig. J. Anim. Sci. 1993, *71*(8), 2099-2109.

Gaebe RJ, DW Sanson, IG Rush, ML Riley, DL Hixon, SI Paisle: Effects of extruded corn or grain sorghum on intake, digestibility, weight gain and carcasses of finishing steers. J. Anim. Sci. *76*(8), 1998, 2001-2007.

Ginste JV, R DeSchrijver: Expansion and pelleting of starter, grower and finisher diets for pigs: effects on nitrogen retention, ileal and total tract digestibility of protein, phosphorus and calcium and in vitro protein quality. Animal Feed Sci. Technol., 1998; *72*(3-4), 303-314.

Golda V: The dermination of basal glycemia and glucose tolerance curve: the manner of blood sampling. Acta Medica, 1998, *41*(3), 121-123.

Heath DF, KN Frayn, JG Rose: Effects of halothane on glucose metabolism after injury in the rat. Br. J. An. 1978, *50* (9), 899-904.

Johansen O, T Tonnesen, T Jensen, PG Burhol, R Jorde, O Reikeras: Morphine and morphine/naloxon modification of glucose, glucagon and insulin levels in fasted and fed rats. Scand J Clin. Lab Inv. 1993, *53* (8), 805-809.

Johansen O, S Vaaler, R Jorde, O Reikeras: Incresed plasma glucose levels after Hypnorm anaesthesia, but not after Pentobarbital anaesthesia in rats. Lab Anim 1994, *28*(3), 244-248.

Lewis LD, ML Morris, MS Hand: Pet foods. In Small Animal Clinical Nutrition 1987, III. pp 2-23, Topeka: Mark Morris Associates.

Loeb WF & FW Quimby: (Ed) The clinical chemistry of laboratory animals; Appendix, pp 417-418, 1989 Pergamon Press, New York.

Olfert ED, BM Cross, AA McWilliam: (Ed) Guide to the care and use of experimental animals, 2nd Ed, Appendix V, Vol 1, p 176, Canadian Council on Animal Care 1993.

Pehrson S: (1999) AnalyCen Nordic AB, Lidköping, Sweden. Personal communication.

Serrano X, MD Baucells, AC Barroeta, F Puchal: Effects of extruded diet on the productive performance of weaning and post-weaned calves. Anim. Feed Sci. Technol. 1998, *70*(3), 275-279.

Stroucken WPJ, AFB van der Poel: Extruding vs pelleting of a feed mixture lowers apparent nitrogen digestibility in dogs. J. Sci. Food Agricult. 1996, *71*, 520-522.

Tabata H, T Kitamura, N Nagamatsu: Comparison of effects of restraint, cage transport, anaesthesia and repeated bleeding on plasma glucose levels between mice and rats. Lab Anim, 1998, *32*(2), 143-148. *129.*