COMMENTARY



# Chow diet in mouse aging studies: nothing regular about it

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Abstract Chow diet is used in the majority of rodent studies and, although assumed to be standardized for dietary source and nutritional contents, it varies widely across commercial formulations. Similarly, current approaches to study aging in rodents involve a single-diet formulation across the lifespan and overlook age-specific nutritional requirements, which may have long-term effects on aging processes. Together, these nutrition-based disparities represent major gaps in geroscience research, affecting the interpretation and reproducibility of the studies. This perspective aims to raise awareness on the importance of rodent diet formulation and proposes that geroscientists

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include detailed descriptions of all experimental diets and feeding protocols. Detailed reporting of diets will enhance rigor and reproducibility of aging rodent studies and lead to more translational outcomes in geroscience research.

Keywords Chow diet · Dietary source · Aging

# Introduction

Rodent models of aging are critical for advancing the field of geroscience but biological heterogeneity has long hampered the reproducibility of aging rodent studies [1, 2]. It is known that diet has a major impact on aging and that dietary intake of macronutrients modulates the aging process [3, 4]. Furthermore, subtle variations in dietary composition can have substantial effects leading to variable rodent phenotypes that impact metabolism and aging-associated outcomes [2-4]. Despite its importance, the role of diet heterogeneity is particularly underappreciated when interpreting results of aging studies. We propose that enhancing awareness regarding the importance of diet among geroscience investigators and requiring more rigorous descriptions of diets in funding applications and in peer-reviewed publications will improve reproducibility in aging rodent studies. While acknowledging that the same principles apply to all animal models of aging, this review will be focused on rodent models.

### There is no such thing as a "regular chow diet"

Most peer-reviewed publications using rodent models of aging include little, if any, description of the diet used in the study design. Indeed, the majority contain terms such as "food and water" or "chow." The assumption behind this lack of detail on the diet is that all "standard" chow diets are the same. However, careful comparison of the nutritional values of two commonly used diets such as LabDiet® 5K0G and Teklad 2018 (Table 1, Supplemental Data) reveals major differences between the two. For example, gross energy (kcal/g) is 4.18 in LabDiet® 5K0G versus 3.1 in Teklad 2018, while levels of vitamin A and vitamin D<sub>3</sub> are, respectively, 4.44- and 117.3fold greater in the former compared to the latter. By contrast, levels of vitamin E and iodine are, respectively, 1.64- and 2.8-fold greater in Teklad 2018 compared to LabDiet® 5K0G. Notably, nine dietary constituents found in LabDiet® 5K0G are absent or not reported in Teklad 2018 (taurine, arachidonic acid, omega-3 fatty acids, carotene, cholesterol, sulfur, fluoride, cobalt, and chromium; Table 1), all of which have known bioactive properties that may impact metabolism. These differences may contribute to diverse rodent metabolic phenotypes and aging outcomes, lack of reproducibility, and erroneous conclusions even in highly controlled experimental setups involving genetically identical inbred rodents.

It has been long appreciated within the nutrition field that standardization of rodent research diets is critical for reproducibility of results across all disciplines [5]. Nutritional guidelines can reduce variability among rodent studies, and thus prevent "wasted effort and money caused by failure to duplicate research findings and faulty conclusions in nutritional, toxicological, behavioral, and cancer studies" [6]. While commercially produced chow diets have consistently supported growth in rodent models, their historically uncontrolled variability in minerals and vitamins has sometimes resulted in nutritional inadequacies [5]. This prompted the American Institute of Nutrition (AIN), now known as the American Society of Nutrition (ASN), to develop formula rodent diets in 1976, with clearly stated percentages and/or quantities of ingredients that met established rodent nutrient requirements at that time. This initial formulation did provide a critical framework to develop standardized diets for rodents but was not without challenges. In fact, the diet was designed to meet nutritional needs for all rodents but was found to induce ectopic calcification of the kidneys in female Sprague Dawley rats [7].

The AIN diet was revised in 1993 with a focus on weight gain over 3-4 months. The final adopted formulation(s), named AIN-93, resulted in a 13% weight increase in Swiss-Webster mice but not in Sprague–Dawley rats [8]. Of particular relevance to this commentary and the field of geroscience is the fact that these diets and their efficacy were designed to maximize bodyweight during growth of relatively young mice. Subsequent studies, which further examined the effects of micronutrient manipulation in mice, were performed on mature mice but not specifically aged mice. For example, dietary copper requirements were determined in 20-week-old male mice fed AIN-93 diets with variable levels of copper [9]. Thus, our understanding of nutrient requirements remains limited within the context of aging.

The current AIN-93 diet is assumed by many to be a "regular" chow diet. However, AIN-93 is a defined diet formulated from purified ingredients, while "chow" generally comprise a variety of grain or cereal-based diets, including soybean meal, corn, fish meal, and animal byproducts in either openly disclosed (open) or proprietary (closed) formulations (Table 1). This lack of dietary information poses issues when trying to reproduce and/or compare results among rodent studies. In addition, batch effects, which are impacted by factors including season-dependent nutritional quality of the crops, may result in distinct compositions of the diets within and across commercial manufacturers. In the absence of a direct analysis of the ingredients for each batch of diet, substantial variability in the formulation can be inadvertently introduced over the course of an aging rodent study. Diet variability can confound the results of studies that compare phenotypes of interest at different points in the lifespan, thus, heavily impacting the interpretation of research outcomes.

The different compositions of chow diets can induce a shift in macronutrient ratios that can in turn impact research results and create challenges in reproducibility across studies. For example, the carbohydrate component is 63% (10% sucrose and approximately 53% cornstarch) in the AIN-93G formula, while is 62% (cornstarch only) in the AIN-93 M formula (5). Importantly, the AIN-93G is formulated for

growth, pregnancy, and lactation, whereas the AIN-93 M is formulated for maintenance. The Open Formula NIH-31 diet (62% carbohydrates from ground corn and corn gluten meal), which is the standard rodent reference diet for the National Institutes of Health (NIH), is formulated for maintenance in addition to growth, reproduction, and lactation in rodents. No diet has been developed for the specific purpose of aging, yet every nutritional component in rodent diets may have distinct biological effects that impact aging processes.

Chow diet variability extends beyond that of absolute macro- and micronutrient concentrations. In fact, although autoclaving and irradiation are intended to sterilize diets to meet animal barrier facility regulations, heat treatment (heat, pressure, steam) conditions are variable and directly affect nutrient bioavailability [10]. For example, the autoclaving process is known to alter the levels of heat-labile vitamins such as vitamin K [11], and the bioavailability of soy protein [10]. As a way to circumvent this issue, the NIH-31 Open Formula Autoclavable diet contains additional amounts of vitamins to compensate for losses upon steam sterilization (Table 1).

# What is the appropriate diet for aging rodent studies?

In the absence of systematic long-term studies on the dietary requirements of aging rodents, it has been assumed that chow diets developed for the maintenance of younger animals are also suitable for studies on aging. However, the effects of such diets on aging in mice remain unclear, largely because it is hard to discern physiological changes associated with aging (mitochondrial dysfunction, cellular senescence, and neurodegeneration) from diet-induced effects.

Long-term studies, such as The Study of Longitudinal Aging in Mice (SLAM), will ultimately provide unique insight into normative aging (1). Here, 8-week-old inbred C57BL/6 J and outbred UM-HET3 mice of both sexes were purchased from The Jackson Laboratory (Bar Harbor, ME) and fed LabDiet 5KOG (22% protein, 16% fat, and 62% carbohydrates). During 1 month of acclimatization, mice were fed Envigo 2018SX (24% protein, 18% fat, and 58% carbohydrate) before being transitioned to the open source NIH-31 formula (24% protein, 14% fat, and 62% carbohydrate) (Envigo, Table 1) for the duration of the study. In addition, fiber content varied between 4.2% in LabDiet® 5K0G, 3.5% in 2018SX, and 4% in NIH-31 (Table 1). Fiber content impacts gut microbiome function and host metabolism [12]. Although it is likely that differences in fiber levels across diets will impact aging processes [12], it is still unknown whether these multiple modifications in macronutrient ratios (and their source) during the first 3 months of life will have an impact later in life. Regardless, we commend the SLAM investigators for publishing data on the diet manufacturers and formulations as well as timelines for transition of diets, as these data will be critical when comparing aging rodent studies that use different diet formulations.

### **Caloric restriction**

Caloric restriction (CR), through a reduction in total diet intake, has been demonstrated to extend longevity in rodents [13, 14]. In a unique rodent study comparing longevity between ad libitum fed and dietaryrestricted rats (31% less overall energy intake), Duffy et al. reported that survival rates for the ad libitum fed were lower than those of the dietary-restricted group, consistent with findings from other studies [14, 15]. Both groups of rats were fed a purified AIN-93 M diet (casein as source of protein) that had the same content of vitamins and mineral per gram of diet, such that the calorie-restricted animals had a commensurate reduction in micronutrient intake. The consequences of inadequate vitamin and mineral intake in dietary restricted rats were not analyzed in this work. The authors then compared their results to a prior rodent study in which rats were fed the cereal-based NIH-31 Open Formula, either ad libitum or at 25% or 40% dietary restriction [14]. In contrast to the AIN-93 M diet regimen which did not adjust for vitamins and minerals, the rats fed the NIH-31 Open Formula were fed a formulation that contained 1.67×additional vitamin mix. While survival was no different between the rats that were fed either diet ad libitum, the rats fed the AIN-93 M diets at 31% dietary restriction had lower survival compared to those fed the NIH-31 formula at both 25% and 40% dietary restrictions. Although there were no differences in survival when compared to NIH-31-fed rats, ad libitum consumption of AIN-93 M by Sprague Dawley

Table 1 Co	mpari	son or the	nutitiona	li values o	i commo	iny useu	ulets							
Diet Diet Name	Nutrient rannes	1 5K0G	2 5K52	3 Formulab Diet 5008	4 NIH-31 Open Formula	5 7012 (LM-485 Mouse/Rat	6 2018 (Teklad Global 18%	7 2016 (Teklad Global 16%	8 8604 (Teklad Rodent	9 5053 (PicoLab® Rodent Diet	10 PicoLab Diet 5058	11 SAFE® A04	12 AIN-03M	13 AIN-93G
		LabDietD	LabDiet9	LabOlet®	Envigo	Sterilizable Diet) Envigo	protein rodent diet) Envigo	protein rodent diet) Envigo	Diet) Envigo	20; irradiated) LabDiet0 PicoLab®	(irradiated) LabOwt® PicoLab®	(Europe) Scientific Diets	BioServ	BioServ
Compart Protein (% from kcal Fat (% from kcal Carbohydrate (% from kcal	12.6 - 26.849 3.1 - 21.635 40.2 - 67.5	15.908 61.852	16.618 61.294	16.71 56.441	14 62	17 58	18 58	12 68	4.7	13.205 62.144	21.635 55.176	3.1 60.4	4.1 67.5	7.1 59.3
Fiber (Crude, % Gross Energy, kcal)	2.2-4.8	4.2 4.18	4.1 4.21	3.8 4.15	4	4.6 3.1	3.5 3.1	3.3 3	4	4.7 4.07	2.2 4.8	3.9 3.339	4.8 3.58	4.8 3.74
Physiological Fuel Value, kcally Metabolizable Energy, kcally	3.07 - 3.56	3.46	3.5 3.14	3.3		MACE	RONUTRIENTS			3.41 3.07	3.56			
Arginine (%	0.45-1.44	1.09	1.08	1.44	1	AV 1.2	INO ACIDS	0.8	1.5	1.22	1.15	60	0.45	0.64
Cysterne (% Glycine (% Histidine (%	0.45-1.44 0.22-0.4 0.3-1.3 0.34-0.6 0.67-1.2 1.02-1.9	0.36	0.35	1.23	0.3 1 0.4	0.5	0.3	0.7	1.3	0.96	0.93	0.81	0.3	0.43 0.48
boleucine (% Leucine (%	0.67-1.2	0.79	0.77	1.2 1.87	0.8 1.4	0.8	0.8 1.8	0.7	1.9	0.97 1.56	1.02		0.67	0.98
Lysine (% Methionine (% Phenylatenine (%	0.72-1.4 0.28-0.7 0.55-1.1	0.97	0.63	1.4 0.43 1.08	0.8	0.4	0.9	0.8	1.4 0.4	0.7	0.67	0.72	0.91	1.3 0.45 0.78
Tyrosine (% Threenine (%	0.5-1	0.58	0.57	88.0 0.9	0.7	0.8	0.8	0.5	0.9	0.59	0.64 0.79		0.7	1 0.77
Tryptophan (% Valine (%	0.14-0.3 0.8-1.19 0.7-1.6 0.79-2.13 2.49-4.77 0.32-1.4 1.26-1.8 0.02-0.05	0.23	0.22	0.28	0.2 0.8	0.3	0.2	0.2	0.3	0.28	0.25	0.19	0.14	0.2
Serine (% Aspartic acid (% Glutamic acid (%	0.79-2.13 2.49-4.77	1.88	1.84	1.2 2.6 4.77	1.5 3.2	1.3 1.8 2.8	1.1 1.4 3.4	1 3.3	2.3 4.1	2.19 4.34	2.13 4.47		0.79 2.49	1.12 3.58
Alanina (% Prolina (%	0.32-1.4 1.26-1.8	1.16 1.42	1.17	1.59	1.1 1.5	1	1.1 1.6	0.9	1.4 1.6	1.15 1.47	1.34		0.32	0.46
Tautrie (% Fiber (Crude, %		4.2	4.1	3.8	4	CAR	BOHYDRATES	3.5	4	6.02	2.2	3.0	48	48
Cellulos Neutral detergent fiber (%	None 10.8-16.4	15.5	15.3	11.3	13.6	13.7	14.7	15.2	12.4	16.4	10.8			
Acid detergent fiber (% Nitrogen-free extract (%	6-Mar 49.4-60.4	52 538	5.1 53.6 35.0	4 49.4 24.0						6 52.9	3 51.8	60.4		
Sucrose (% Glucose (%	33.9-43.5 0.69-3.18 0.12-0.22	0.69	0.79	2.57 0.22						3.18 0.19	0.71 0.16	Sugars in general 3.2%		
Fructose (% Lactose (%	0.12-0.22 0.16-0.24 0-1.34	0.17		0.24 0.39						0.23	0.16 0.78	3.2%		
Linoleic acid (C18.2; % Linolenic acid (C18.3; %		2.66	2.87	1.37	1.9	2.6 0.3	3.1 0.3	2	1.9 0.2	2.19 0.26	2.32	1.5 0.12	2.04 0.27	3.57 0.48
Arachidonic acid (% Omega-3 fatty acida (%	<0.01-2 0.29-0.63 0.6-2.72	0.62	0.62	0.01						<0.01 0.33	2 0.32			
Total saturated fatty acids (% Palmitic acid (% Stearic acid (%	0.5-0.7	1.14	1.17	251	0.9	0.6	0.9	0.5	0.7	0.93	2.12	0.59	0.63	1.1
Total monounsaturated faity acids (% Oteic acid (%	0.06-0.2 0.7-2.88 0.48-1.3	1.27	1.42	2.32	12	1.3 1.3	1.3 1.2	0.7	1.1	0.99	2.88	0.48	0.91	1.59
Total polyursiaturated faity acids (% Choleaterol (ppm	2.1-4.04 50-280	243	247	285	2.1 50	2.9 No value	3.4 No value CONUTRIENTS	2.1 No value	2.1 50	141	200		2.31	4.04
Carotene (ppm	trace-4 2.5-80	15	1.5	4			WTAMINS			1.5	Trace			
Vitamin K3 (menadione) (ppm Thiamin (B1) (ppm	2.5-80 5.0-95 5.0-15	20 79.4	20 80	3.2 16	22 78	80 95	50 17	50 17 16	40 27 8	33	3.1 15	25 5 85	0.82 (K1; phylloquinone) 5.3 P	0.88 (K1; phylloquinone) 6 P
Riboflavin (B2) (ppm Niacin (nicotinic acid; B3) (ppm Pantothenic acid (B5) (ppm	30-109	87 37	2 86 37	0 109 15	7 87 39	14 100 87	15 70 33	15 75 33	6 63 21	8 90 17	8 90 21	0.5 70 10	6 30 14.7	6 30 14.7
Choline (ppm Folic acid (89) (ppm Parietories (85) (nom	10.0-87 1028-2530 0.35-7	2,001	2000 1.9	2000	1890 2 10.9	2200	1200	1030 4 19	2530 3	2000 3	2200 2.9	1600 0.35	1028	1028
Pyridoxine (B6) (ppm Biotin (ppm Vitamin B <sub>12</sub> (ppm	0.35-7 3.0-18 0.08-0.77 0.01-51	10 0.3 51	10 0.3 51	6 0.2 20	0.3	0.77	18 0.4 0.08	18 0.4 0.08	13 0.38 0.05	9.5 0.3 0.051	0.3 0.051	3 0.08 0.01	0.2 0.025	0.2 0.25
Vitamin A (ppm Vitamin D <sub>2</sub> (ppm	1.23-20 0.025-4.4 20.1-100.5	20 4.4	20 4.4	15 3.3	7.28	9 0.06	4.5 0.0375	4.5 0.0375	3.78	4.5	4.5 0.0825	2.25 0.025	1.23 0.025	1.242 0.025
Vitamin E (ppm or IU/kg Ascorbic acid (mg/gm	20.1-100.5 not reported	45 0	45 0	55 not reported	27.47	100.5	73.7	73.7	80.4	68.33 not reported	38.19 not reported	20.1	53.533 not reported	55.945 not reported
Ash (% Calcium (%	0.5-1.4	6.5 1.32	6.5 1.32	6.8 1	6.2 1.1	6.1 1	5.3	4.9	7.4	6.1 0.81	5 0.81	4.8	22	2.2 0.51
Phosphorus, non-phytale (% Phosphorus (%	0.33-0.95	0.71	0.95	0.42	0.7 1	0.4	0.4	0.4	0.7	0.33	0.53	0.55	0.28	0.28
Potassium (% Magnasium (% Sultur (%	0.36-1.1 0.051-0.3 0.0301-0.34	0.89 0.23 0.3	0.86 0.22 0.28	1.1 0.2 0.24	0.8	0.2	0.6	0.8	0.3	0.22	0.7 0.16 0.27	0.8	0.051 0.0301	0.38 0.051 0.0301
Sodium (% Chlorine (%	0.103-0.3 0.4-0.51	0.29 0.49	0.28 0.49	0.28 0.48	0.3	0.3	0.2 0.4	0.2 0.4	0.3 0.5	0.3 0.51	0.25 0.42	0.25	0.1032	0.103
Chloride (gkg Fluoride (ppm Iron (pom	n/a 1.0-36 36.6-360	35.4 359	36 360	19 230	270	240	200	200	300	10 220	12 200	270	1.6 1 36.6	1.6 1 37.2
Zinc (ppm Manganese (ppm		84 158	82 160	73 71	47 155	63 93	70 100	70 100	80 100	87 85	120 120	55 70	35.4 10.5	37.7 10.5
Copper (ppm Cobalt (ppm	10.5-160 6.0-25 0.4-0.82	11 0.82 2.14	10 0.82 2.2	13 0.4	13	23	15	15	25	13 0.71 0.97	17 0.55	16	6	6
Chromium (ppm	0.21-6 0.01-1.4 0.16-0.39	0.01	0.01	14			-	-	-	0.81	0.58		1	1
Seanum (ppm	0.16-0.39	0.39	0.39	0.23	0.3	0.16	0.23	0.23	0.34	0.3	0.3		0.17	0.17
Seenum (ppm	0.16-0.39	0.39 Whole wheat, ground corn, ground cets, wheat middlings,	0.39 Ground wheat, ground corn, ground cata, wheat middlings,	0.23 Ground corn, dehulled soybean meal, ground wheat, fish meal,	0.3 Ground wheat, ground com, ground cells, wheat		0.23 Cover a cover a c	0.23 Ground wheat, ground com, wheat middlings.	0.34 Dehulled soybean meal, wheat middings, fieled	0.3 Ground Corn, Dehulled Scybean Meal, Wheat	0.3 Ground wheat, ground corn, dehulied soybean meal, wheat	Barley, wheat, maize, soybean	0.17 Com syrup solids, destrose, com starch.	0.17 Com starch, casain, maltodestrin, sucrose,
Seenum (ppm	0.16-0.39	0.39 Whole wheat, ground com, ground cets, wheat middlings, dehulied soybean meal, fish meal, soybean oil, dehydrated defote meal new holes meal	0.59 Ground wheat, ground corn, ground oats, wheat middings, fish meal, dehulled soybean meal, soybean oil, dehydrated offelin meal one o hdon mend	0.23 Cround corn, dehulled soybean meal, ground wheat, fish meal, wheat middings, porcine animal fat preserved with BHA, care protected teamson drived used	0.3 Ground wheat, ground com, ground oats, wheat middings, fish meal, dehulied soybean meal, dehulied soybean meal, and	Ground corn, dehulled soybean meal, ground cabs, wheat middlings, dehudrated alfalfa meal	Ground wheat, ground com, wheat middlings, dehulled	Ground wheat, ground corn, wheat middlings, corn gluten meal, calcium	0.34 Detuiled soybean meal, wheat middlings, faloed corn, ground com, fish meal, care molesses, executed wheet drive where	0.3 Ground Corn, Dehulled Soybean Meal, Wheat Middlings, Ground Wheat, Fish Meal, Dried Plain Beet Pulp, Crean Michaese, Without Crean	0.3 Ground wheat, ground corn, dehulied soybean meal, wheat germ, fish meal, brewers dried yeasis, corn gluten meal, previne meal, or meal, brewers and	Barley, wheat, maize, soybean meal, wheat bran, hydrolyzed fah	0.17 Com syrup solids, destrose, com starch, casein, sucrose, soybean oll, cellulicse, minaral mix, utbeain mir celetine	0.17 Com starch, casein, małodextrin, sucrose, soybean ol, cellufose, mineral mic, vtiarnin mic, l. cettorian shelmin,
nqq mumere e	6.16-0.39	meal, soybean oil, dehydrated affalfa meal, com okiten meal	0.59 Ground wheat, ground corn, ground cats, wheat middlings, fish meat, dehufiled soybean meat, scoybean oil, dehyfrated affalfa meat, corn gluten meat, dicaticum pitosphate, brewens dried yeast, acticum citrbonate,	0.23 Cround corn, dehulied soybean meal, ground wheat, fish meal, wheat middings, porcine animal fat preserved with BHA, carne molesses, brewers dried yeast, porcine meat meal, wheat germ, ground oats, dried beet pulp.	dehulied soybean meal, dehvdrated alfalfa meal, com	Ground corn, dehulled soybean meal, ground cabs, wheat middlings, dehudrated alfalfa meal	Ground wheat, ground com, wheat middlings, dehulled	Ground wheat, ground corn, wheat middings, corn gluten meat, calcium carbonate, dicalcium phosohate, scybean oil.	0.34 Dehulled scybean meal, wheat middlings, faked corn, ground com, faih meal, care moleases, ground wheat, dried whey, scybean oil, brewers dried yeast, dicakcium	0.3 Ground Corn, Dehulled Soybean Meal, Wheat Middings, Ground Wheat, Fish Meal, Dried Plain Beet Puly, Cane Molasses, Wheat Germ, Brewen Dried Yasak, Ground Cets, Dehydrated Allaffa Meal,	0.3 Ground wheat, ground corn, dehulied soybean meal, wheat gens, fish meal, preveni dised yeast, corn gluten meal, porcine animal fat preserved with BHA, soybean oil, caloium carbonate, salt, dicaloium	Barley, wheat, mail, soybean meal, wheat bran, hydrolyzed fah proteins, dicakium phosphate, pre- mixture of	0,17 Com syrup solids, destrose, com starch, casein, sucrose, soybaan el, cellulose, mineral mix, vitamin mix, calcium silicata, magnesium staarate, cholme bitartriste	0.17 Com starch, casein, meltodextrin, sucrose, soybean ol, cellulose, minacal mix, vitarrin mix, L-cysteine, choline bitartrate, tBHQ
nqq mumpm	0.16-0.39	meal, soybean oil, dehydrated affalfa meal, com okiten meal	0.39 Ground wheat, ground corn, ground outs, wheat middlings, fish meat, deshulled soybean meats, acobasis oid, dehydnate dicatcium phosphate, hrewers, dicatcium phosphate, hrewers, dicatcium phosphate, hrewers, bisalfate (vitamin K), ast, DL- bisalfate (vitamin K), ast, DL-	fat preserved with BHA, care molesses, brewers dried yeast, porcine meat meal, wheat germ, ground oats, dried beet pulp, dehydrated atfatfa meal,	dehulled soybean meal, dehydrated alfalfa meal, com gluten meal, soybean oll, dicalcium phosphate, brewers dried yeast, calcium	Ground corn, dehulled soybean meal, ground cabs, wheat middings, dehydrated affaffa meal, soybean oil, corn gluten meal, calcium carbonate, dicalcium carbonate, dicalcium carbonate, briwwrs dried ywast, iodizae aat, choline chloride, kaolin	Ground wheat, ground com, wheat middlings, dehulled	Ground wheat, ground corn, wheat middings, corn gluten meat, calcium carbonate, dicalcium phosohate, scybean oil.	meal, cane molasses, ground wheat, dried whey, soybean oil, brewers dried yeast, dicatcium phosphate, calcium cerhorsate indiced salt	0.3 Ground Corn, Dahullad Soybaan Maal, Wheat Middings, Ground Wheat, Fash Maal, Dried Plain Beat Pulp, Cane Molassae, Wheat Garm, Brewers Dried Yasak, Ground Oals, Dahydsatid Alfalfa Meal, Soybaan Ol, Dried Whay, Caticum Catoonata, Sak, DL-	yeast, com gluten meal, porcine animal fat preserved with BHA, soybean oil, calcium carbonate, salt, dicalcium phosphate, monocalcium	hydrolyzed fish proteins, dicalcium phosphate, pre- mixture of minerals, calcium carbonate, pre-	0.17 Corn syrup solids, destrose, corn starch, casteri, succose, aoghean oil, callutiose, mineral mix, vitamin mix, calcium silicate, misgensium stearate, choline bitartrate L-cysteine	mineral mix, vitamin mix,
seenun (ph	0.16-0.39	meat, soybean oli, dehydrated aflefa mead, com gbiten meat, dicalcium phosphate, brewers dried yeast, calcium carbonate, menadione, dimethytghrinidinol bisutifite (source of Vitamin K), salt, BL-methionine, choline chloride, magnesium coide,	meai, soybean oli, dehydrated aflafa meak, com gluten meai, dicalcium phosphate, brewers dried yeast, calcium carbonate, meanadices dimethydynimidinol bisuffate (vitamin K), sait, DL- methionine, choline chloride, magnesium code, thiamine	fat preserved with BHA, care molasses, brevers dried yeast, prorine meat meat wheat germ, pround outs, dried beet pulp, dehydrated alfalfa meat, calcium carbonata, dried whey, sait, menedione dimethylgyrimidinot bisulfite, cholme chorisk, cholecaloferol,	dehydrated alfalfa meal, com gluten meal, soybaen oil, dicalcium phosphate, breweni dried yasad, caloium carbonate, iodized salt, choline chloride, magnesium oode, kaolin, ferroua sulfate,	Ground corn, dehalled soybean meal, ground cabi, wheat middlings, datydpatad alfalfa meal, asoybean oil, com gluten meal, calcium carbonate, dicatoium phosphate, brevers dried yeast, iodioud salt, cholme chloride, kaolin megnesium code. L-lysine, DL-methionine, femous	Ground wheat, ground com, wheat middings, dehulad soybean meal, corn gluten meal, soybean oil, caloium calocnate, Glacikum phosphate, brewer, dried yeast, iodiced salt, L-lysine, chioride, kaolin, magnesium colde, vitamin E acottao, menadione sodium bisulfe complex (soucce oi vitamin K	Ground wheat, ground corn, wheat middlings, corn gluter meal, calcium carbonate, dicalcium phosphate, scybean oil, berewrat dried yeast, iodized sait, I-lyaine, DLL- methiceine, choline chloride, magnesium oside, vitamin E acetate, manudices acetum bisuifite	meal, cane moleases, ground wheat, dried whey, soybean oil, brewers dried yeast, dicalcium phosphate, calcium cholme chioride, keolin, megnesium oxide, ferrous	Meal, Dried Plain Beet Pulp, Cane Molasses, What Germ, Brewers Dried Yeast, Ground Oats, Dahydrated Alfalfa Meal, Scybaan OB, Dried Whey, Calcium Carbonate, Salt, DL- Methicnine, Menacione Diresthydwinnidirol Bioliffe	yeast, com gluten meat, porcine animal fat preserved with BHA, soybean oil, calcium carbonate, salt, dicalcium phosphate, monocalcium phosphate, choline chicride, menadiose dimethylysimidinol bisulfite, DL-methionine,	Barley, wheat, maila, soybean hydrolyzad fah proteins, dicakium phosphate, pre- misture of minerala, cakium carbonake, pre- misture of vitamina.	0.17 Com syrup solida, destrose, com starch, casieri, sucrose, soybean oll, otalucos, misrará mis, vitarnin mis, calcium slicatat, magnesium slicatat, magnesium slicatat, magnesium biarstate L-cysteine	mineral mix, vitamin mix,
Sientum (pm	0.16-0.39	meat, soybean oli, dehydrated aflefa mead, com gbiten meat, dicalcium phosphate, brewers dried yeast, calcium carbonate, menadione, dimethytghrinidinol bisutifite (source of Vitamin K), salt, BL-methionine, choline chloride, magnesium coide,	meai, soybean oli, dehydrated afalfa mead, com gluten meai, dicalcium phosphate, brewers dried yeast, calcium carbonate, meanadices dimethydynimidinol bisuffate (vitamin K), sait, DL- methionine, choline chloride, magnesium code, thiamine	fat preserved with BHA, care molisses, brevers dide yeast, protine meat meat, wheat germ, ground oath, dried beet pulp, calchurn carbonate, dried whey, sait, meandone dimethytyprimidinol bisuiffer, holline chroinide, cholecaloiferol, vblamin A acatate, pyridoxine Indrochine, d-alabha	dehydrated alfalfa meal, com gluten meal, soybaen oil, dicalcium phosphate, breweni dried yasad, caloium carbonate, iodized salt, choline chloride, magnesium oode, kaolin, ferroua sulfate,	Ground corn, dehalled soybean meal, ground cabi, wheat middlings, datydpatad alfalfa meal, asoybean oil, com gluten meal, calcium carbonate, dicatoium phosphate, brevers dried yeast, iodioud salt, cholme chloride, kaolin megnesium code. L-lysine, DL-methionine, femous	Ground wheat, ground com, wheat middings, dehulad soybean meal, com glutan meal, soybean oil, calcium phosphate, dicalcium phosphate, breven dried yeast, iodized salt, L. hysine, DL-methionine, choline chloride, kaolin, magneaixm coide, vitamin E anotake, menadione sodium bisulfae complex (source oi vitamin 6) activity), mangenoxu oxide, ferroza suffaet, zino coide	Ground wheat, ground corn, wheat middlings, corn gluter meal, calcium carbonate, dicalcium phosphate, scybean oil, berewrat dried yeast, iodized sait, I-lyaine, DLL- methiceine, choline chloride, magnesium oside, vitamin E acetate, manudices acetum bisuifite	meal, cane moleases, ground wheat, dried whey, soybean oil, brewers dried yeast, dicalcium phosphate, calcium cholme chioride, keolin, megnesium oxide, ferrous	Meal, Dried Plain Beet Pulp, Cane Molasses, What Germ, Brewers Dried Yeast, Ground Oats, Dahydrated Alfalfa Meal, Scybaan OB, Dried Whey, Calcium Carbonate, Salt, DL- Methicnine, Menacione Diresthydwinnidirol Bioliffe	yeast, com gluten meat, porcine animal fat preserved with BHA, soybean oil, calcium carbonate, salt, dicalcium phosphate, monocalcium phosphate, choline chicride, menadiose dimethylysimidinol bisulfite, DL-methionine,	hydrolyzed fish proteins, dicalcium phosphate, pre- mixture of minerals, calcium carbonate, pre-	0.12 Corn syrup solids, destrose, con starch, casein, sucrose, soybean (), oubulcas, minerar mix, vitamin mix, calcium silicate, megnesium stearate, choline blattrate L-cysteine	mineral mix, vitamin mix,
ALL INGREDIENTS	6.16-0.39	meat, soybean oli, dehydrated aflefa mead, com gbiten meat, dicalcium phosphate, brewers dried yeast, calcium carbonate, menadione, dimethytghrinidinol bisutifite (source of Vitamin K), salt, BL-methionine, choline chloride, magnesium coide,	meai, soybean oli, dehydrated afalfa mead, com gluten meai, dicalcium phosphate, brewers dried yeast, calcium carbonate, meanadices dimethydynimidinol bisuffate (vitamin K), sait, DL- methionine, choline chloride, magnesium code, thiamine	fat preserved with BHA, care molisses, brevers dide yeast, protine meat meat, wheat germ, ground oath, dried beet pulp, calchurn carbonate, dried whey, sait, meandone dimethytyprimidinol bisuiffer, holline chroinide, cholecaloiferol, vblamin A acatate, pyridoxine Indrochine, d-alabha	dehydrated alfalfa meal, com gluten meal, soybaen oil, dicalcium phosphate, breweni dried yasad, caloium carbonate, iodized salt, choline chloride, magnesium oode, kaolin, ferroua sulfate,	Ground corn, dehalled soybean meal, ground cabi, wheat middlings, datydpatad alfalfa meal, asoybean oil, com gluten meal, calcium carbonate, dicatoium phosphate, brevers dried yeast, iodioud salt, cholme chloride, kaolin megnesium code. L-lysine, DL-methionine, femous	Ground wheat, ground com, wheat middings, dehulad soybean meal, com glutan meal, soybean oil, calcium phosphate, dicalcium phosphate, breven dried yeast, iodized salt, L. hysine, DL-methionine, choline chloride, kaolin, magneaixm coide, vitamin E anotake, menadione sodium bisulfae complex (source oi vitamin 6) activity), mangenoxu oxide, ferroza suffaet, zino coide	Ground wheat, ground corn, wheat middlings, corn gluter meal, calcium carbonate, dicalcium phosphate, scybean oil, berewrat dried yeast, iodized sait, I-lyaine, DLL- methiceine, choline chloride, magnesium oside, vitamin E acetate, manudices acetum bisuifite	meal, cane moleases, ground wheat, dried whey, soybean oil, brewers dried yeast, dicalcium phosphate, calcium cholme chioride, keolin, megnesium oxide, ferrous	Meal, Dried Plain Beet Pulp, Cane Molasses, What Germ, Brewers Dried Yeast, Ground Oats, Dahydrated Alfalfa Meal, Scybaan OB, Dried Whey, Calcium Carbonate, Salt, DL- Methicnine, Menacione Diresthydwinnidirol Bioliffe	yeast, com gluten meat, porcine animal fat preserved with BHA, soybean oil, calcium carbonate, salt, dicalcium phosphate, monocalcium phosphate, choline chicride, menadiose dimethylysimidinol bisulfite, DL-methionine,	hydrolyzed fish proteins, dicalcium phosphate, pre- mixture of minerals, calcium carbonate, pre-	0.12 Com syrup solds, destrose, com starch, casein, success, soybean di, calukase, mineral misr, vitamin mite, calcium siteante, megenaium stearate, choline bitatrate L-cysteline	mineral mix, vitamin mix,
ALL INGREDIENTS	6.16-0.39	mai, sopbar ol, dérydrated alfafa mai, com glater mai, dicol pump locophate, trevers menudicos, direntrybytrindlich biadfae (source of Vlatmin K), aut, DL-methytpytrindlich biadfae (source of Vlatmin K), aut, DL-methytpytrindlich biadfae (source), and the protection bytechlorida, protection bytechlorida, biadfae, magnatou author biadfae, magnatou author author, famous author, calcium iodate, DL-alpha tocophanela author (source)	maal, scybean OI, datrydrated affallar mad, corr gidsan mad, dicalcian phosphala, brewens dried ywaat, calcum carbonals, meandcore dimethylygrimidrol biselidfek (Waatmir K), sait, DL- marthionins, choline chlorids, magnesiam colds, thiurnin monostealas, pyridoana hydrochlorids, cholacacilitetti (vitarin D3), vitarin A acutate, mangareosa colds, calcum partothemah, ferrora saffals, calcum colds, calcum teoria acid, vitarini BLS), feccilia acid, vitarini BLS),	fat preserved with BHA, care moliasisa, breven died yeast, porcine maat meal, whaat germ, gorund oats, dried beet puby, dehydrated alfalls meal, catakam carbonata, dried whay, aut, meaedone diensthytyprinnidro blauffat, cholne chlords, cholecaldrent, Valenn A acatala, prividonie hydrochlorids, dia gha boopheny acatala, prividonie monomitale, folde acid, DC- muthicoma, incolonie acid, ribolawin, väemin B12 wanolecader monanona onie	dehüldei soybean maik, dehyhdisd aitik mail, corr glutan maik, soybean oli, dicalcium phosphale, berwens dried yeast, calcium carborako, olizade alat, cholme chioride, magnesiam adarbay, manganosa colia, sodiw, banin fexosiaka, risken, sodiwan paintahurak, nächra, calcium paintahurak, nächra, calcium paintahurak, nächra, beaten paintahurak, nächra, beaten paintahurak, nächra, beaten hödlavir, pyriöötese	Ground corr, dehulled sophean mail, ground cab, what middings, labydata dillata maul, aoybaan oli, com gluban mail, acidium achosta, dicaciom phosphate, acidiaciom phosphate, acidiaciom phosphate, acidiaciom phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, acidiaciona phosphate, acidiaciona phosphate, acidiaciona phosphate, acidiaciona phosphate, dicaciona pho	Ground wheat, ground com, wheat middling, dehulled soylean meal, corn gluten meal, soylean o, calcium calconate, dicaticum calconate, dicaticum basto, folkine aut. L-system, basto, folkine aut. L-system, dichorida, kador, aut. L-system, dichorida, kador, magnesium codie, vitamin E acottate, complex (source of vitamin % ferrus sulfate, zinc caida, marcia, calcium particificational, copper sulfate, protocime hydrochronia, rebolamin hydrochronia, rebolamin	Geoord wheat ground corn, wheat middings, corn gluten meat, calcium carbonate, clackium phosphate, soytean oll, indized ant, L-Iyarne, DLL- methiorine, choine chioride, magnesium oxide, vitarnie E acatale. Rive complex (bacres of vitarnie K activity), menganous oxide, Riesus, sublate, zino partorhenski, copper sublas, protoine, akiolim partorhenski, cabiori partorhenski, cabiori partorhenski, cabiori hismerin moscolitaka, misolitaki, filaren moscolitaka,	maia, care molassie, groond what, clead whey, aoybaan oil, brevers dirid yeast, cleaticiam phosphate, calciam phosphate, calciam carbonate, cloade at autota, vitamin E acattate, suifate, vitamin E acattate, isoane cloaded, famota menonitrata, vitamin E acattate, the activity), mangianous caide, racion, tharmin mononitrata, vitamin E a	Meal, Dried Plain Beet Pulp, Cane Molasses, What Germ, Brewers Dried Yeast, Ground Oats, Dahydrated Alfalfa Meal, Scybaan OB, Dried Whey, Calcium Carbonate, Salt, DL- Methicnine, Menacione Diresthydwinnidirol Bioliffe	yead, com gluter meal, prorine arimit fat preserved with BHA, soybean oli, calcium carbonate, sul, diairicium phosphate, monoactium phosphate, monoactium phosphate, choline chloride, vitarrin A acatate, biolacitalice, u-mitiorrine, vitarrin A acatate, bioline, diairia why, folic acatate, biolin, thamin monorihum, culcium pantofamate, leichin, kfolfawn, nicotine acid, casae	hydrolyzed fish proteins, dicalcium phosphate, pre- mixture of minerals, calcium carbonate, pre-	0.17 Cem syrup soldat, telesare, com storbian castrinationa, misezarination oli, catalucas, misezarination valaminimite, catolicam salicatat, misezariation stasarata, chicata batartata L-cyldaine	mineral mix, vitamin mix,
Section Loon	6.16-0.33	mai, sopbar ol, dérydrated alfafa mai, com glater mai, dicol pur piccephate, trevers menudicos, dirent fycymethio tennologi, magnasium oxid, thiarina entrophycinicitatic thiarina entrophycinicitatic protections hybrochecida, menocima hybrochecida, protections hybrochecida, biolin, marganous oxide, calcium iodate, Dcalpha tocophecia contate (otion of tocophecia contate (otion of tocophecia) contate (otion otion otion) contate (otion otion) contate (otion otion) contate (otion otion) contate (otion otion) contate (otion otion) contate (otion otion) contate (otion otion)	maal, scybean ol, datrydrated affallar mad, corr gidan maal, dicalcian phosphala, brewens dried ywaat, calcum carbonale, manufacea dinethytyprimidrol biselidae (vidanin K), sait, Dc marthonine, choline chords, magensaim colds, thiumni monosteate, pyridoane hydrochlands, cholacacitient hydrochlands, cholacacitient partothemah, ferroca saffala, calcum colds, calcum teor, and a cholas, D-alpha teocelia acid, vilamin BLS), menosmate inhyferian SLS, menosmate	fat preserved with BHA, care moliasisa, breven died yeast, porcine maat meal, whaat germ, gorund oats, dried beet puby, dehydrated alfalls meal, catakam carbonata, dried whay, aut, meaedone diensthytyprinnidro blauffat, cholne chlords, cholecaldrent, Valenn A acatala, prividonie hydrochlorids, dia gha boopheny acatala, prividonie monomitale, folde acid, DC- muthicoma, incolonie acid, ribolawin, väemin B12 wanolecader monanona onie	dehüldei soybean maik, dehyhdisd aitik mail, corr glutan maik, soybean oli, dicalcium phosphale, berwens dried yeast, calcium carborako, olizade alat, cholme chioride, magnesiam adarbay, manganosa colia, sodiw, banin fexosiaka, risken, sodiwan paintahurak, nächra, calcium paintahurak, nächra, calcium paintahurak, nächra, beaten paintahurak, nächra, beaten paintahurak, nächra, beaten hödlavir, pyriöötese	Ground corr, dehulled sophean mail, ground cab, what middings, labydata dillata maul, aoybaan oli, com gluban mail, acidium achosta, dicaciom phosphate, acidiaciom phosphate, acidiaciom phosphate, acidiaciom phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, acidiaciona phosphate, acidiaciona phosphate, acidiaciona phosphate, acidiaciona phosphate, dicaciona pho	Ground wheat, ground com, wheat middling, dehulled soylean meal, corn gluten meal, soylean o, calcium calconate, dicaticum calconate, dicaticum basto, folkine aut. L-system, basto, folkine aut. L-system, dichorida, kador, aut. L-system, dichorida, kador, magnesium codie, vitamin E acottate, complex (source of vitamin % ferrus sulfate, zinc caida, marcia, calcium particificational, copper sulfate, protocime hydrochronia, rebolamin hydrochronia, rebolamin	Geoord wheat ground corn, wheat middings, corn gluten meat, calcium carbonate, clackium phosphate, soytean oll, indized ant, L-Iyarne, DLL- methiorine, choine chioride, magnesium oxide, vitarnie E acatale. Rive complex (bacres of vitarnie K activity), menganous oxide, Riesus, sublate, zino partorhenski, copper sublas, protoine, akiolim partorhenski, cabiori partorhenski, cabiori partorhenski, cabiori hismerin moscolitaka, misolitaki, filaren moscolitaka,	maia, care molassie, groond what, clead whey, aoybaan oil, brevers dirid yeast, cleaticiam phosphate, calciam phosphate, calciam carbonate, cloade at autota, vitamin E acattate, suifate, vitamin E acattate, instructiona existing complex (source of vitamin monontrata)e, vitamin D 3 audite, vitamin D 3	Meal, Dried Plain Beet Pulp, Cane Molasses, What Germ, Brewers Dried Yeast, Ground Oats, Dahydrated Alfalfa Meal, Scybaan OB, Dried Whey, Calcium Carbonate, Salt, DL- Methicnine, Menacione Diresthydwinnidirol Bioliffe	yead, com gluter meal, prorine arimit fat preserved with BHA, soybean oli, calcium carbonate, sul, diairicium phosphate, monoactium phosphate, monoactium phosphate, choline chloride, vitarrin A acatate, biolacitalice, u-mitiorrine, vitarrin A acatate, bioline, diairia why, folic acatate, biolin, thamin monorihum, culcium pantofamate, leichin, kfolfawn, nicotine acid, casae	hydrolyzed fish proteins, dicalcium phosphate, pre- mixture of minerals, calcium carbonate, pre-	0.17 Cara 1949 solida, destinaya, corre alaroy, casain, succiosa, sopotano oli, caluticas, mismaral estas, adarum salicata, adarum salicata, adarum salicata, Logistama Logistama	mineral mix, vitamin mix,
Section (pr	6.16-5.35	mai, sopbar ol, dérydrated alfafa mai, com glater mai, dicol pur piccephate, trevers menudicos, dirent fycymethio tennologi, magnasium oxid, thiarina entrophycinicitatic thiarina entrophycinicitatic protections hybrochecida, menocima hybrochecida, protections hybrochecida, biolin, marganous oxide, calcium iodate, Dcalpha tocophecia contate (otion of tocophecia contate (otion of tocophecia) contate (otion otion otion) contate (otion otion) contate (otion otion) contate (otion otion) contate (otion otion) contate (otion otion) contate (otion otion) contate (otion otion)	meai, soybean oli, dehydrated aflafa meak, com gluten meai, dicalcium phosphate, brewers dried yeast, calcium carbonate, meanadices dimethydynimidinol bisuffate (vitamin K), sait, DL- methionine, choline chloride, magnesium code, thiamine	fat preserved with BHA, care moliasiss, breven died yeast, porcine maat meal, wheat germ, gorund oats, drieb beet pub, dehydrated alfalls meal, catalum carbonata, dried whey, aat, meaedone dimeth/spirmidino biauffat, cholne chlords, cholecald'erol, Valenin A acatala, prividoate hydrochlorids, daipha biochphrig acatala, prividoate biochphrig acatala, prividoate biochphrig acatala, prividoate menehana, faceblick, dai, menehana, faceblick, dai, methom werdbrande.	dehydrated alfalfa meal, com gluten meal, soybaen oil, dicalcium phosphate, breweni dried yasad, caloium carbonate, iodized salt, choline chloride, magnesium oode, kaolin, ferroua sulfate,	Ground corr, dehulled sophean mail, ground cab, what middings, labydata dillata maul, aoybaan oli, com gluban mail, acidium achosta, dicaciom phosphate, acidiaciom phosphate, acidiaciom phosphate, acidiaciom phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, dicaciona phosphate, acidiaciona phosphate, acidiaciona phosphate, acidiaciona phosphate, acidiaciona phosphate, dicaciona pho	Ground wheat, ground com, wheat middlen, chinaled soybean meat, corn gluten meat, soybean ci, calcium phosphale, brawers dide yeant, foldrea dail, L- javias, phosphale, brawers dide yeant, donce and a south bias different south and complex (source of viberin's dirichida, kadio misualitie complex (source of viberin's dirichida, photosita patibles, photosita	Geoord wheat ground corn, wheat middings, corn gluten meat, calcium carbonate, clackium phosphate, soytean oll, indized ant, L-Iyarne, DLL- methiorine, choine chioride, magnesium oxide, vitarnie E acatale. Rive complex (bacres of vitarnie K activity), menganous oxide, Riesus, sublate, zino partorhenski, copper sublas, protoine, akiolim partorhenski, cabiori partorhenski, cabiori partorhenski, cabiori hismerin moscolitaka, misolitaki, filaren moscolitaka,	maia, care molassie, groond what, clead whey, aoybaan oil, brevers dirid yeast, cleaticiam phosphate, calciam phosphate, calciam carbonate, cloade at autota, vitamin E acattate, suifate, vitamin E acattate, instructiona existing complex (source of vitamin monontrata)e, vitamin D 3 audite, vitamin D 3	Meal, Dried Plain Beet Pulp, Cane Molasses, What Germ, Brewers Dried Yeast, Ground Oats, Dahydrated Alfalfa Meal, Scybaan OB, Dried Whey, Calcium Carbonate, Salt, DL- Methicnine, Menacione Diresthydwinnidirol Bioliffe	yead, com gluter meal, prorine arimit fat preserved with BHA, soybean oli, calcium carbonate, sul, diairicium phosphate, monoactium phosphate, monoactium phosphate, choline chloride, vitarrin A acatate, biolacitalice, u-mitiorrine, vitarrin A acatate, bioline, diairia why, folic acatate, biolin, thamin monorihum, culcium pantofamate, leichin, kfolfawn, nicotine acid, casae	hydrolyzed fish proteins, dicalcium phosphate, pre- mixture of minerals, calcium carbonate, pre-	0.17     Carn 1996 solidi, destratage, corn starty, cares, naccess, sophere oli, califung, minaral mu oli califung, minaral mu silicata, solicitin statu Logisteine	mineral mix, vitamin mix,
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Table 1 Comparison of the nutritional values of commonly used diets

Green indicates that the dietary component is present in the diet. Black indicates that the dietary component is not found in the diet

rats resulted in approximately a 10% increase in bodyweight at 96 weeks of age as compared to ad libitum consumption of NIH-31 [16]. This study highlights the importance of documenting the exact composition

of diets when manipulating macronutrients to extend longevity in murine models. Of note, despite the importance of micronutrients (vitamins and minerals) in regulating metabolic processes and their likely impact on aging outcomes, alterations in their levels are generally not accounted for in CR rodent studies.

The team of LeCouteur and colleagues elegantly leveraged rodent aging studies that have complete diet information to demonstrate the importance of macronutrient ratios [3, 17]. These studies reported that female and male mice that were fed ad libitum with higher protein and lower carbohydrate diets had the greatest reproductive potential. In contrast, female and male mice fed ad libitum with lower protein and higher carbohydrate diets had longer lifespans, independent of their caloric intake. Several conclusions can be drawn from these studies. It is critical to better define and report macronutrient ratios and compositional subtypes (i.e., unsaturated versus saturated fats [18]) instead of simply reporting the calories and their degree of restriction in rodent studies. Perhaps more importantly, the fact that a protein-rich diet is beneficial during reproduction but detrimental to aging indicates that rodent diet formulations and their macronutrient ratios should be adjusted over the lifespan instead of being kept throughout, as in the current practice. Furthermore, it has been shown that circadian alignment impacts the effect of caloric restriction [19]. Therefore, it is important to include details on the exact time of feeding as related to the light cycle. Lastly, much more research is needed to determine whether it would be beneficial to adjust nutrient by age (age-specific diets).

### Looking forward

In order to improve scientific rigor and reproducibility, the geroscience research community needs to better define the composition of rodent diets used in aging studies, preferably across the lifespan. We propose two ways to enhance this awareness: (1) review process of grant applications submitted to the National Institute of Aging (NIA); and (2) review process of manuscripts submitted to geroscience journals.

NIH provides clear guidance on how to address rigor and reproducibility for all grant applications submitted, including a mandatory section entitled, "Consideration of relevant biological variables." Currently, biological variables are defined as sex, age, weight, and underlying health conditions. We propose that the NIA considers diet as an additional mandatory biological variable. Applicants should be required to describe the exact diet formulations and provide details of diet modifications to be made within the study design. This will raise awareness on the importance of rodent diet formulation in research studies and improve the assessment of rigor in prior research (20).

We propose that geroscience journals that publish rodent studies require a detailed description of both the control and experimental diets. When a diet composition is published for the first time in a journal, complete information on all the components, including any modifications to published diet compositions, should be presented in a table. Nutrition journals, such as *Journal of Nutrition*, have already implemented this requirement and it is our opinion that research progress in other fields would benefit from a standardization in the reporting of this important yet overlooked experimental variable.

In summary, detailed reporting of diets in aging rodent studies will enhance reproducibility and lead to more translational outcomes, thus improving the use and relevance of animal models in geroscience research.

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### Declarations

**Conflict of interest** The authors declare no competing interests.

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