29 Metabolism and development of bovine brown adipose tissue. S. B. Smith* and G. E. Carstens, *Texas A&M University, College Station, TX*.

Angus newborn calves have a greater ability to generate heat by nonshivering thermogenesis than Brahman newborn calves. We have worked to document the biochemical basis for this phenomenon. Brahman perirenal brown adipose tissue (BAT) contains two-to-three times as many -receptors as Angus BAT, although the dissociation constant is not different between breed types. Mitochondrial uncoupling protein (UCP1) mRNA concentration is greater in newborn Brahman BAT than in Angus BAT, whereas lipogenesis from acetate is greater in Angus BAT than in Brahman BAT. We obtained fetuses of each breed type at 96, 48, 24, 14, and 6 d before expected parturition, and at parturition. Glycerolipid synthesis from palmitate declined by 85% during the last trimester, but still contributed 98% to total lipid synthesis at birth. The concentration of UCP-1 mRNA tripled during gestation in both breed types. Uncoupling protein-1 mRNA initially was elevated in tailhead s.c. adipose tissue, but was barely detectable by birth, and tended to be higher overall in Angus than in Brahman s.c. adipose tissue. In a third experiment,

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30 Altered growing conditions can inhibit nisin production in lactic cultures by disrupting the signal transduction pathway. H. Li* and D. O'Sullivan, *University of Minnesota*.

A signal transduction pathway controls the production of the nisin bacteriocin by Lactococcus lactis. In this system, external nisin can signal the nisin genes to be switched on in a dose dependent fashion, involving the membrane bound kinase (NisK) and the intracellular regulator, NisR. Phosphorylated NisR initiates transcription of the genes involved in nisin production. However, we have found that nisin production can be switched off under certain conditions. These conditions are: 1) growth of L. lactis at it maximum growth temperature of 40(C;2) transfer of the nisin gene cluster into a dairy Enterococcus strain; 3) electroporation of plasmids into certain nisin producing L. lactis strains. In these three cases, Northern and RT-PCR analysis confirmed that the nisA gene was not expressed, but the immunity genes were. This suggested the lack of nisin production under these conditions was possibly due to a blockage in the signal transduction pathway. To address this hypothesis, gel shift experiments were conducted with a nisA promoter fragment using crude cell extracts from cultures growing under these conditions. A shift was observed for cell extracts from the positive control, L. lactis ATCC 11454 grown at 30°C, but not for crude extract from L. lactis ATCC 11454 grown at $40^{\circ}\mathrm{C}$ or the dairy Enterococcus strain containing the nisin gene cluster. Furthermore, a protein with the same size as NisR (26 kDa) was isolated by nisA competitive heparin-affinity column chromatography from crude extracts from L. lactis ATCC 11454 grown at 30°C, but not from cell extracts under the non-nisin producing conditions. This suggested the lack of an activated, phosphorylated NisR, under these conditions. In addition, RT-PCR and Northern hybridization confirmed the presence of the nisRK transcript, indicating that NisRK was most probably produced. Therefore, the nisin gene expression was likely blocked from the reduced inability of external nisin to initiate the signal transduction pathway during these conditions. This is the first evidence of a specific mechanism for inhibition of nisin production in lactic cultures. This novel finding should enable culture users to more reliably predict nisin production kinetics of cultures during specific culture uses.

31 Invasion of *Mycobacterium avium sub sp paratuberculosis*in bovine epithelial cells and bovine mammary epithelial cells. D. Patel^{*1}, L. Goddik¹, and L. Bermudez², ¹Food Science and Technology, Oregon State University, Corvallis, OR 97331-6602, ²Department of Biomedical sciences, College of Vet Med, Oregon state Univ, Corvallis OR 97331-4804.

Main objective of our experiment was to investigate invasion characteristics of Mycobacterium avium sub sp. paratuberculosis (MAP)against bovine epithelial cells and bovine mammary epithelial cells as targets. Johne's disease is a chronic infectious disease of ruminants caused by a bacterium Mycobacterium avium sub sp. paratuberculosis (MAP). It is estimated that about 30 % of dairy herds are infected with Johne's disease in the US. It is known that MAP infects the host by the oral route and young calves are infected at early age. Intestinal epithelial cells thus become primary site of infection. For current experiment we male Angus and Brahman calves were assigned to one of three groups; (1) newborn; (2) 48 h of warm exposure starting at 15 h of age; and (3) 48 h of cold exposure starting at 15 h of age. The calves in the newborn treatment were euthanized at 15 h of age. The warm-treatment calves (22C) and cold-treatment calves (4C) were maintained euthanized after 48 h at each temperature. Brahman BAT adipocytes were smaller than Angus BAT adipocytes initially and shrank with cold exposure, whereas Angus BAT adipocytes did not. The production of CO 2 from palmitate and acetate increased with cold exposure. Lipogenesis from acetate and palmitate was greater in BAT from calves held at warm or cold temperatures than in BAT from newborn calves. Also, BAT from Angus calves had greater rates of lipogenesis from palmitate than BAT from Brahman calves. The data indicate that BAT from Brahman calves mobilizes stored lipids more rapidly than BAT from Angus calves, but resynthesizes lipids more slowly. Thus, BAT from Brahman calves may be exhausted of lipid shortly after birth during times of cold exposure, leading to reduced thermogenesis during times of extended cold stress.

Key Words: Brown fat, Bovidae, Lipid metabolism

hypothesized : 1. Mammary epithelial cells can be a reservoir for MAP and therefore invasion could take place by apical or basolateral surface ; 2. MAP can enter intestinal epithelial cells. To test the above hypothesis we evaluated invasion employing immortalized epithelial cell lines, namely Bovine epithelial cell(MDBK purchased from ATCC) and Mammary epithelial cell (MACT, given by Dr. Sheffield, Univ of Wisconsin). MAP strain ATCC 19698 was used in our study. Invasion assay protocol was standardized in our lab. Based on the statistical analysis of data we found that MAP invades MAC-T and MDBK cells successfully, albeit poorly. MAP showed markedly higher rate of invasion in case of MDBK compared to MAC-T. Exposure of basolateral surface did not have marked influence on invasion in case of mammary epithelial cells, suggesting that the apical surface is the main route of entry however, exposure of basolateral surface of bovine epithelial cells significantly increased the uptake of bacteria., a puzzle that we cannot explain without further studies. MAP is a pathogen that is extremely resistant to wide spectrum of antibiotics. Its control lies in breaking its transmission cycle by inhibiting molecular interaction with epithelial cell surfaces. In this endeavor, in-vitro invasion assay described here can serve as a useful model in screening interesting MAP mutants with reduced or altered invasion efficiency. Main conclusion of our study is- MAP can successfully invade bovine epithelial cells and mammary epithelial cells. Exposure of basolateral surface significantly increases invasion rate in case of bovine epithelial cells.

Key Words: Mycobacterium avium sub sp paratuberculosis, Invasion assay, Bovine mammary epithelial cells

32 Epidemiology and ecology of *Listeria monocytogenes* at the pre-harvest food level. K. K. Nightingale*, E. D. Fortes, C. R. Nightingale, Z. Her, Y. H. Schukken, Y. T. Grohn, and M. Wiedmann, ¹*Cornell University*.

Listeria monocytogenes is a human foodborne pathogen and causes severe systemic infections in animals. L. monocytogenes is responsible for a significant portion of dairy product Class I recals. Raw milk may harbor L. monocytogenes and pasteurized dairy products may be contaminated if the pathogen become established in processing plant environments. A case-control study involving 22 case farms (13 dairy cattle, 1 beef cattle, 4 goat, and 2 sheep) and 22 pair-matched controls was conducted to probe the epidemiology and ecology of L. monocytogenes. A total of 1652 fecal (n=424), feed (n=420), and environmental samples (soil, n=397; water, n=411) were cultured for L. monocytogenes. While prevalence of L. monocytogenes was not significantly different $(p{=}0.1492)$ in bovine case (23.13%) and control (19.58%) farms, the pathogen was more common (p<0.0001) in small ruminant (caprine and ovine pooled) case farms (26.41%) than controls (4.40%). The prevalence of L. monocytogenes was not significantly different (p>0.05) in fecal, soil, feed, and water samples from bovine case and control farms. Small ruminant case farms showed a significantly higher prevalence (p < 0.05) of L. monocytogenes in all sampling categories than small ruminant controls. Molecular subtyping (EcoRI ribotyping) of clinical (n=15) and farm isolates (n=310) differentiated 49 unique ribotypes.

Ribotype DUP-1038B was associated with case farms and DUP-1045A was linked to control farms (p<0.05). Ribotype DUP-1038B was associated with feces while DUP-1045A was more common in the environment (p<0.05). L. monocytogenes subtypes isolated from clinical cases or fecal samples were more frequent in environmental than feed samples, indicting that case or carrier animals are important to L. monocytogenes dispersal. We determined that L. monocytogenes was abundant in preharvest food systems. Our data indicate that the epidemiology and ecology of L. monocytogenes differs between host species. While some L. monocytogenes subtypes may cause disease, others may protect against disease by stimulating host immunity or competitive exclusion. A complete understanding of the L. monocytogenes epidemiology and ecology is needed to implement effective control strategies and ultimately ensure food safety.

Key Words: Listeria monocytogenes, Molecular epidemiology, Preharvest food safety

33 The influence of sweet cream buttermilk on the compositional and rheological properties of a stirredcurd cheese. T. Lin^{*1}, J. Lucey¹, R. Govindasamy-Lucey², M. Johnson², and J. Jaeggi², ¹Department of Food Science, UW Madison, ²Wisconsin Center for Dairy Research.

Sweet cream buttermilk (SCB) is a by-product from the butter-making process and has been used as an ingredient for cheesemaking. The objective of this research was to determine how the addition of SCB might affect the cheese composition, melt, stretch, and free oil of cheese when used on a pizza. Three trials were done in which stirred-curd cheese was made from partially skimmed milk with the addition of 2, 4, and 6%(w/w) condensed SCB. Cheeses were assessed for composition, meltability using the UW Melt Profiler, free oil using modified Babcock test, and stretchability by the fork test, over a four-week ripening period. Cheese moisture content increased significantly (P<0.05) with increasing SCB levels, and ranged from 45% (w/w) for control cheese to 51%for the 6% SCB fortified cheese. Cheeses with 6% SCB had significantly (P<0.05) lower pH than control cheese during entire ripening period. Fat content in cheeses decreased from 23% for control to 20% for 6% SCB fortified cheese. Cheeses with no added SCB had the highest % recovery of its indigenous phospholipids (40%). Cheeses, made with 2, 4, and 6% SCB had total phospholipid recoveries of 32, 33 and 31%, respectively. The extent of flow, or cheese metability, was significantly higher (P < 0.05) for cheeses made with SCB than control cheese during the ripening period. This may be due to the high moisture and low pH of these cheeses. Free oil release was significantly reduced (P<0.05) for all the cheeses made with added SCB for the first week of ripening; however, there was no significant difference between treatments at the second and fourth week. Visually, no significant free oil formation was observed when cheeses were baked on pizza. Cheese stretchability was significantly reduced (P < 0.05) with increasing level of SCB. The stretchability of control cheeses increased during ripening, but decreased in cheeses with added SCB. Results showed the use of high level of SCB resulted in cheese with soft body, poor shredding, high extent of flow, low pH, and high moisture. Whether the physical and rheological changes in SCB added cheeses were due to the compositional changes in the cheese or to the disruption of SCB components in the cheese network is currently under investigation.

Key Words: Buttermilk, Pizza, Phospholipids

34 Characterization of proteolysis in Cheddar cheeses produced with isogenic, thermolytic starters expressing various cell envelope proteinases. S. Myaka, L. Metzger*, K. Baldwin, and L. McKay, *MN-SD Dairy Food Research Center, University of Minnesota, St. Paul, MN.*

If the use of thermolytic starter strains for accelerating Cheddar cheese ripening is coupled with the use of the extracellular starter proteinase with desired cleavage specificity a more controlled acceleration of ripening and improved flavor formation might be expected. The objective of this research was to investigate how the specificity of the cell envelope proteinase (CEP) in conjunction with the early released peptidases affects the accumulation of proteolytic products. In a previous study Cheddar cheese was manufactured with thermolytic isogenic lactococcal starters expressing CEPs from groups a, c, d, and g. Sensory evaluation revealed no flavor differences except for bitterness. In this study the peptide profiles of the cheeses were compared by RP-HPLC analysis of the 70%-ethanol soluble fractions and the most abundant peptides in the major chromatographic peaks were identified by Mass Spectrometry (MS). MS data were analyzed with Sequest[®] and peptides were matched against the casein sequences. The major peaks of the non-bitter cheeses eluted from the RP column in the first half of the chromatogram and from the bitter control cheese more towards the end of the run. The intermediate bitterness of treatment g coincided with an even spread of its major peaks from the beginning to the end of the elution time. Thus a correlation between bitterness and elution from a hydrophobic column was observed. Bitter peptides α S1(f1-13) and β (f193-209) accumulated to high levels in both bitter and non-bitter cheeses suggesting that these peptides are not the only compounds imparting bitterness. Peptides resulting from the specific CEP cleavage of $\alpha S1(f1-23)$ were found in all the cheeses but only in treatment g did they accumulate to higher levels and appear as separate peaks. Consequently the most abundant peptides in the major peaks are not necessarily traced back to the cleavage specificities of the different CEPs.

Key Words: Cell envelope proteinase, Proteolysis, Peptides

35 Identification of fecal/mothball flavor in Cheddar cheese. M. E. Carunchia Whetstine^{*1}, Y. Yoon¹, and M. A. Drake¹, ¹North Carolina State University.

Flavor of Cheddar cheese is a key parameter for consumer acceptance and marketing. The application of analytical sensory and instrumental methods to identify and characterize specific flavors and the chemicals that cause specific flavors enhances our understanding of cheese flavor chemistry. Some Cheddar cheeses have been found to exhibit a fecal/mothball (F/M) flavor. The objectives of this research were to identify and characterize aroma-active compounds that contribute to F/M flavor in Cheddar cheese. Blocks of Cheddar cheeses (6 to 15 months old) were collected and screened for F/M flavor by a descriptive sensory analysis panel (n=14). Two cheeses with F/M flavor and two cheeses of similar age without F/M flavor were selected and analyzed for volatile aroma compounds. Duplicate samples (300g) with internal standards (2-methyl pentanoic acid, 2-methyl-3-heptanone, and 1-pentanol) were extracted with diethyl ether, followed by isolation of volatile material by high vacuum distillation. Volatile extracts were analyzed by gas chromatography-olfactometry (GCO) and the most odor active compounds were determined using aroma extract dilution analysis (AEDA). Additionally, 5g of each cheese were frozen, grated, and subsequently analyzed using dynamic headspace purge and trap. Compounds were identified by comparison of retention indices, odor properties and GC-MS data against reference standards. Selected compounds were quantified by standard addition. Sensory analysis of model systems was used to confirm the relationship between selected compounds and specific flavors. Sensory analysis determined that F/M flavor was independent of cheese age; present in 6 month cheeses as well as 15 month cheeses. The 15 month cheeses had more intense brothy and sulfur notes while the 6 month cheeses had more whey, cooked, and milkfat/lactone flavors. Based on GC-O AEDA results, key volatile flavor compounds in both sets of cheeses were acetic acid (vinegar), hexanoic acid (sweaty), maltol (sweet), furaneol (burnt sugar), 3-methylindole (F/M), o-aminoacetophenone (grape), and g-dodecalactone (coconut). Increased concentrations of 3-methyl-indole and butyric acid were observed in both cheeses exhibiting F/M flavors compared to those cheeses without this flavor.

Key Words: Cheddar flavor, Fecal/mothball flavor, Aroma extract dilution analysis

36 Analysis of physico-chemical changes during early ripening of cheese utilizing FTIR Spectroscopy. P. Upreti* and L. E. Metzger, *MN-SD Dairy Foods Research Center*, *University of Minnesota, St. Paul, MN*.

The objective of this study was to evaluate the feasibility of Fourier Transform Infrared (FTIR) Spectroscopy for measurement of lactose and lactic acid in cheese/cheese curds and to monitor lactose fermentation in cheeses during the first few days of ripening. A Nicolet 560 FTIR Spectrometer with a ZnSe Attenuated Total Reflectance (ATR) crystal accessory was used, and a protocol for sampling cheese/cheese curds was developed. Cheese/cheese curds were ground to a paste and then mounted on the ATR crystal. Proper contact between the sample and the crystal was ensured using the pressure pad assembly. The sample

was left under these conditions for 5 min before the start of data collection. The spectrum was collected in the region between 4000 and 650 cm^{-1} at a resolution of 4 cm^{-1} , and a rate of 256 scans per sample. The crystal was cleaned between samples using distilled water, propanol, and distilled water, and then wiped to complete dryness. The spectrum obtained after subtraction of water spectrum from the sample spectrum was used to analyze for lactose and lactic acid. Cheeses spiked with lactose and lactic acid showed a shift in spectrum in the regions of 1200 to 1050 and 1700 to 1500 $\rm cm^{-1}$ respectively. In the next phase of the study, three replicates of cheeses with two different levels of residual lactose and calcium were manufactured. The levels of lactose and lactic acid were measured by HPLC and the FTIR spectra were collected for the cheese curds prior to salting, and cheese at day 1, 3, 5, 7, and 9 $\,$ during ripening. The level of residual lactose and calcium in the cheese at day 1 was significantly (p \leq .05) different for the two treatments (.73 and 1.93% for lactose; .85 and .66% for calcium), and the level of lactose decreased in both treatments during the first 9 days of ripening. Subsequently partial least squares and principal component analysis will be used to characterize changes in lactose fermentation to the shifts in the FTIR spectrum during initial cheese ripening.

37 Evaluation of salt whey as an ingredient in process cheese. R. Kapoor* and L. E. Metzger, *MN-SD Dairy Food Research Center, University of Minnesota, St. Paul, MN.*

Salt whey refers to the whey stream obtained during the salting and mellowing step of a cheese manufacturing process. Due to its high salinity level, it is underutilized and also leads to disposal costs. Consequently, alternative uses need to be pursued. The major components of salt whey (salt and water) are used as ingredients in process cheese. The objective of this research was to determine if salt whey, obtained from a traditional Cheddar cheese manufacture process, could be used as an ingredient in process cheese. Three replicates of Process Cheese (PC), Process Cheese Food (PCF) and Process Cheese Spread (PCS) with two treatments each were manufactured. Treatment 1 (C) used the control formula and treatment 2 (T) involved the modified formula using salt whey to replace salt and water. Salt whey was collected during the salting and pressing steps of the Cheddar cheese procedure at the University of Minnesota, followed by mixing and pasteurization. There were no significant differences $(p \ge .05)$ in process cheese composition between the treatments. Texture Profile Analysis (TPA) and Rapid Visco Analyzer (RVA)-melt analyses were performed on all the process cheeses. Schrieber melt test was performed on PC and PCF and the tube melt test on PCS. The mean TPA-hardness values obtained respectively for the C and T were 126 N and 115 N for PC, 61 N and 59 N for PCF, and 12 N and 12 N for PCS. The mean melt diameter obtained for C and T process cheeses were 48.5 mm and 49.4 mm for PC, and 61.6 mm and 63 mm for PCF. The tube-melt for PCS (C and T) was 75.1 mm and 79.8 mm respectively. There were no significant differences ($p \ge .05$) in the TPA-hardness and the RVA hot viscosity for PC, PCF and PCS between the treatments. The Schreiber melt of C and T for PC and PCF and the tube melt values for C and T in PCS also showed no significant differences ($p \ge .05$). The replacement of salt and water with salt whey in PC, PCF and PCS had no significant effect on their functionality.

Key Words: Process cheese, Salt whey

38 Strategies to improve stability and performance of calibration samples for infrared milk analyzers. K. E. Kaylegian* and D. M. Barbano, *Northeast Dairy Foods Research Center, Cornell University.*

Infrared milk analyzers are traditionally calibrated using sets of 10 to 12 raw milk samples from individual farms. Although taken from the local milk population, these sets of samples are limited by a short shelflife, a short and variable range in component concentration, nonuniform distribution of concentrations within the range, and correlation in concentration changes among components. An alternate approach using ultrafiltration (UF) to produce calibration samples provides a means to overcome these weaknesses and improve calibration performance. UF calibration samples were produced by gravity separating pasteurized milk, centrifugally separating the gravity skim to remove residual fat, and ultrafiltering the skim milk. The gravity cream (ca. 25% fat), UF retentate (2X). UF permeate, lactose α -monohydrate, and water were combined to make calibration sets designed to have a large range and incremental changes in each component and to uncouple the fat and protein correlation. The 12 sample UF calibration set had a range of 2.0-6.0% fat, $2.0\mathchar`-4.3\%$ true protein, and $4.0\mathchar`-5.3\%$ anhydrous lactose. Shelf life of preserved UF calibration samples was 4 wk compared to 2 wk for individual farm samples. Comparison of performance of individual farm and UF calibration sets was by standard deviation of the difference (SDD) between chemistry and infrared prediction, the stability of the instrument slope and bias with time, set to set variation in these values, and the frequency of high leverage samples within calibration sets. UF calibration sets had smaller SDD within sets and were more consistent among sets, indicating better calibration performance with respect to agreement with chemistry. The UF calibration samples exhibited a more stable slope and bias for each component and fewer high leverage samples than for farm milk calibration samples, both within calibration sets and among sets over several months of operation.

Key Words: Infrared milk analysis, Calibration, Ultrafiltration

39 WITHDRAWN , .

ADSA/ASAS Northeast Graduate Student Paper Competition

40 Effects of trans-8, cis-10 CLA and cis-11, trans-13 CLA on milk fat synthesis. J. W. Perfield II*1, A. Sæbo², and D. E. Bauman¹, ¹Cornell University, Ithaca, NY, ²Natural ASA, Hovdebygda, Norway.

Conjugated linoleic acid (CLA) supplements that cause a reduction in milk fat secretion in dairy cows have typically been comprised of 4 isomers (trans-8, cis-10; cis-9, trans-11; trans-10, cis-12; cis-11, trans-10, cis-12; cis-11, trans-11, tra 13 CLA). Abomasal infusion of pure isomers has shown that *trans-10*. cis-12 CLA is a potent inhibitor of milk fat synthesis, whereas cis-9, trans-11 CLA has no effect (Baumgard et al. 2000, Am. J. Physiol. 278:R179-84). However, there appear to be additional fatty acid intermediates that inhibit milk fat synthesis based on infusion of various CLA enrichments (Chouinard et al. 1999, J. Dairy Sci. 82:2737-45) and studies with rumen-protected CLA (Perfield et al. 2002, J. Dairy Sci. 85:2609-17). The objective of this study was to investigate the effects on milk fat synthesis of additional CLA isomers present in the rumenprotected supplements. Four rumen fistulated Holstein cows (141 \pm 8 DIM. mean \pm SE) were randomly assigned in a 4 X 4 Latin square experiment. Treatments were abomasal infusion of 1) skim milk (negative control), 2) trans-10, cis-12 CLA supplement (positive control), 3) trans-8, cis-10 CLA supplement, and 4) cis-11, trans-13 CLA supplement. Treatments 2 to 4 were targeted to provide 4 g/d of the CLA isomer of interest and the daily dose provided by infusion at 6 h intervals. Treatment periods were 5 d in length with 7 d washout periods. The trans-8, cis-10 CLA had no effect on milk fat yield whereas trans10, cis-12 CLA reduced milk fat yield by 35% (P < 0.01). The cis-11, trans-13 CLA supplement contained some trans-10, cis-12 CLA and when data were corrected to account for this, it was obvious that cis-11, trans-13 CLA also had no effect on milk fat synthesis. Milk fat content (g/100 g fatty acids) of specific CLA isomers was significantly elevated within respective treatment groups (trans-8, cis-10 CLA (0.27); trans-10, cis-12 CLA (0.18); cis-11, trans-13 CLA (0.23); P < 0.001). Milk yield (P < 0.37), DMI (P < 0.44) and milk protein yield (P < 0.22) were unaffected by treatment. Overall, abomasal infusion of trans-10, cis-12 CLA reduced milk fat synthesis, while the other major isomers present in rume-protected CLA supplements (trans-8, cis-10 CLA and cis-11, trans-13 CLA) had no effect.

Key Words: CLA, Milk fat depression, Dairy cow

41 Effect of prepartum dietary carbohydrate source and monensin on dry matter intake, milk production and blood metabolites of transition dairy cows. M. M. Pickett*, T. W. Cassidy, P. R. Tozer, and G. A. Varga, *The Pennsylvania State University, University Park, PA*.

Ninety-four multiparous Holstein cows $(3.39 \pm 0.05 \text{ BCS})$ were used in an complete randomized block design to evaluate the effects of carbohydrate source and monensin on dry matter intake, milk production and blood metabolites of transition cows. Two diets with (+) or without (-)