621 Fatty acid composition in rumen bacteria isolated from ruminal and duodenal digesta. B. Vlaeminck[1], R. J. Dewhurst[2], and V. Fievez*[1], [1]*Laboratory for Animal Nutrition and Animal Product Quality, Ghent University*, *Ghent, Belgium*, [2]*Institute of Grassland and Environmental Research*, *Aberystwyth, UK*.

The objective of this study was to compare the fatty acid (FA) composition in rumen bacteria isolated from the liquid (LAB) and solid (SAB) phase of the rumen and duodenal digesta (DB) from dairy cows fed diets varying in forage:concentrate (F:C). In a 4 × 4 Latin square experiment, 4 dairy cows were fed ryegrass silage and a standard dairy concentrate with F:C of 35:65, 50:50, 65:35 and 80:20. Differential centrifugation was used to separate SAB and LAB from rumen contents, collected four hours after the morning feeding and duodenal bacteria from a reconstituted duodenal sample, collected over 24h. Bacterial samples were freeze-dried prior to fatty acid analysis by GLC. Results were tested by ANOVA with orthogonal contrasts and the significance of linear effects is given. Total FA content of SAB (124 mg/g) was higher compared with LAB (58 mg/g) and decreased with increasing F:C (104, 90, 89, 80 mg/g DM; SEM=4.6; P=0.016). Diet showed no effect on proportions of the two major FA, C16:0 and C18:0. The latter was enriched in SAB (55.7 vs. 34.2% of total FA; SEM=0.94; P<0.001), whereas C16:0 was more abundant in LAB (25.9 vs. 18.0%, SEM=0.26, P<0.001). Increasing F:C decreased bacterial content of trans-10 C18:1 (1.74, 1.12, 0.62, 0.47%; SEM= 0.196; P<0.001), C18:2 (n-6) (3.31, 3.28, 2.53, 2.12%; SEM= 0.264; P=0.015) and cis-9,trans-11 C18:2 (0.36, 0.31, 0.24, 0.22%; SEM= 0.037; P=0.045). In contrast, C18:3 (n-3) increased with increasing F:C (0.82, 0.87, 1.06, 1.27%; SEM= 0.088; P=0.011) whereas trans-11 C18:1 remained constant (3.96%). No differences were found between SAB and LAB in C18:3 (n-3) (1.00%) whereas LAB were enriched in trans-10 C18:1 (1.19% vs. 0.78%, SEM=0.139, P=0.074) and C18:2 (n-6) (3.53% vs. 2.09%, SEM=0.187, P=0.004) and SAB in trans-11 C18:1 (4.93% vs. 2.98%, SEM=0.108, P<0.001) and cis-9,trans-11 C18:2 (0.43% vs. 0.14%, SEM=0.026, P=0.026). Dietary effects on the fatty acid content and composition of DB were generally in agreement with effects observed in LAB and SAB.

Key Words: Rumen Bacteria, Fatty Acid, Dairy Cow

622 Proportions of solid- (SAB) and liquid-associated (LAB) rumen bacteria in duodenal content as estimated by bacterial odd and branched-chain fatty acids. B. Vlaeminck[1], R. J. Dewhurst[2], and V. Fievez*[1], [1]*Laboratory for Animal Nutrition and Animal Product Quality, Ghent University*, *Ghent, Belgium*, [2]*Institute of Grassland and Environmental Research*, *Aberystwyth, UK*.

The objective of this study was to estimate the proportions of LAB and SAB in duodenal content and their contribution to duodenal flow of N and fatty acids (FA). In a 4 × 4 Latin square experiment, 4 dairy cows were fed ryegrass silage and a standard dairy concentrate with forage:concentrate (F:C) ratios of 35:65, 50:50, 65:35 and 80:20. Differential centrifugation was used to separate SAB and LAB from rumen contents, collected four hours after the morning feeding and duodenal bacteria from a reconstituted duodenal sample, collected over 24h. Bacterial samples and duodenal contents were freeze-dried prior to FA analysis by GLC. LAB were enriched in iso C15:0 (1.37 vs. 1.12 mg/g DM; SEM=0.020; P=0.007), anteiso C15:0 (4.22 vs. 1.87 mg/g DM; SEM=0.108; P=0.001) and C15:0 (2.55 vs. 2.30 mg/g DM; SEM=0.069; P=0.026) whereas SAB contained higher proportions of iso C17:0 (0.413 vs 0.331 mg/g DM; SEM=0.018; P=0.059) and C17:0 (0.795 vs. 0.551 mg/g DM; SEM=0.018; P=0.001). Variation in odd and branched-chain fatty acids (OBCFA) between SAB and LAB were used to estimate their relative proportions in duodenal bacteria by means of linear programming. Proportions of SAB increased with increasing F:C (64.7, 70.7, 73.8 and 74.8%; SEM = 1.78; P = 0.026) which might reflect the increased attachment to forage particles or a decreased growth rate of LAB. The latter was illustrated by a decrease in LAB of cytosine:N - a proxy for growth rate in bacteria (0.071, 0.060, 0.057 and 0.055 g/g; SEM=0.003; P=0.020) with increasing F:C. Proportion of duodenal N from LAB and SAB increased with increasing F:C (21.5, 18.0, 23.8 and 25.1%; SEM=1.58; P=0.059 and 32.0, 40.3, 38.4 and 40.6; SEM=2.18; P=0.050 for LAB and SAB, respectively) whereas no dietary effects were observed on contribution to duodenal FA of LAB (11.5%) and SAB (52.5%). In conclusion, variation in OBCFA can be used to estimate proportions of SAB and LAB in duodenal contents and their contribution to duodenal flow of nutrients.

Key Words: Odd and Branched Chain Fatty Acids, Rumen Bacteria, Dairy Cow

623 Development of an in vitro method to estimate fat digestibility in the small intestine of ruminants. T. Glindemann[1], K.-H. Suedekum*[1,2], and E. Wisker[1], [1]*University of Kiel*, *Kiel, Germany*, [2]*University of Bonn*, *Bonn, Germany*.

Supplementation of dairy cow diets with rumen-protected fat (RPF) has consistently increased yields of milk and milk fat, and may also improve reproductive performance of ruminants. Although one important feature of the effects of RPF is digestibility in the small intestine, this variable is only very infrequently reported. The objective of the current study was therefore to develop an in vitro method to estimate the digestibility of fats in the small intestine of ruminants by closely mimicking the chemical and physical conditions within the small intestine. First, the fat is blended with an aqueous solution of gum arabic as emulsifier and thickener. The blend is intensively stirred in a mixer under continuous cooling to prevent segregation between the aqueous solution and the fat. Second, taurocholic acid (bile salt) and lecithin (phospholipid) are added and brought to 37°C under continuous stirring to further support development of an emulsion. Calcium as a co-factor for lipase action is added as $CaCl_2$ and the pH is adjusted at 8.8. Third, pancreatin is added, which contains lipase and colipase. The pH is maintained at 8.8 by immediate titration of free fatty acids (FA) with NaOH (0.1 *M*) and digestion is usually stopped after 75 min. Release of FA from triglycerides is calculated from the amount of NaOH that was titrated during digestion. Because lipase can cleave only the two external ester bonds of a triglyceride and monoglycerides also can be absorbed from the small intestine, digestibility of fat was considered to be 100%, when two thirds of the FA were released from the fat. In in vitro digestibility experiments using the above procedure, conventional fat sources (olive oil, coconut fat, cod-liver oil, and butter fat) had very high fat digestibilities of almost 100%, whereas a crystalline vegetable RPF product (Bergafat T-300) had a digestibility of only 80%. The coefficient of variation of four repeated digestibility estimates on each single fat source was smaller than 5% in all cases. This indicates that the method can produce repeatable and robust estimates of fat digestibility in the small intestine.

Key Words: Fat Digestion, Ruminant, Small Intestine

624 Conversion of oleic acid to 10-hydroxy and 10-keto stearic acids in vitro and their accumulation in milk of cows fed added fat. T. C. Jenkins*, A. A. AbuGhazaleh, E. J. Thies, and M. B. Riley, *Clemson University*, *Clemson, SC*.

Previously, 10-hydroxy stearic acid (HSA) and 10-keto stearic acid (KSA) were present in continuous cultures of mixed ruminal microorganisms at 6-12% of total fatty acids, and were shown to arise primarily from oleic acid based on tracer studies using a pulse dose of ^{13}C-labelled oleic acid. Additional in vitro and in vivo studies were conducted aimed at two objectives; 1) to determine if HSA and KSA originated directly from oleic acid or if they originated from an intermediate of oleic acid biohydrogenation and 2) to determine if HSA and KSA could be detected in rumen and milk samples. When 20, 30, or 40 mg of oleic acid were added to batch cultures of ruminal microorganisms, there were linear ($P \leq 0.05$) net gains in KSA (0.40, 0.60, and 0.75 mg/flask) and HSA (0.43, 0.53, and 0.97 mg/flask) over 24 h of incubation. When the same amounts

of a *trans* monoene were added to cultures, no increases in HSA or KSA were detected. Batch cultures were then run with 17 mg 12-HSA added per flask. After 24 h of incubation, HSA concentration dropped 4.2 mg with a 5.9 mg net increase in KSA. The yields of stearic acid and *trans* monoene did not change. Four Holstein cows were then fed 0, 1, 2, or 3% free fatty acids (65.5% linoleic, 19.5% oleic, and 10.5% linolenic acid) in a 4 x 4 Latin square with 2-week periods. The HSA concentrations in ruminal samples increased ($P \leq 0.01$) linearly from 0.70 to 1.98 % of total fatty acids as fatty acids in the diet increased from 0 to 3%. In milk, the HSA concentration increased ($P \leq 0.05$) linearly (0.01, 0.22, 0.46, and 0.65% of total fatty acids) for diets containing 0, 1, 2, and 3% added fatty acids, respectively. These results show that HSA in ruminal contents originates directly from oleic acid and is subsequently converted to KSA. The HSA concentration in ruminal contents and milk are linearly related to fatty acid intake by cows.

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Key Words: Biohydrogenation, Rumen, Oleic Acid

625 Effect of feeding supplemental palmitic acid (C 16:0) on performance of lactating dairy cows under summer heat. J. L. Warntjes*[1], P. H. Robinson[1], E. Galo[2], E. J. DePeters[1], and D. Howes[3], [1]*University of California, Davis*, [2]*Dairy Consulting Services of California, Inc., Madera, CA*, [3]*Howes Consulting Inc., Nampa, ID*.

The objective was to determine performance changes of early lactation high producing dairy cows under summer heat, by adding a supplemental fat (99% total lipid (DM); Energizer-RP10, IFFCO, Johor, Maylasia) which analyzed as 85.6% C 16:0 (of total fatty acids), to the total mixed ration (TMR). Early lactation multiparous Holstein cows on a commercial dairy near Madera (CA), in two pens of 99 and 115, were used in a 2 * 2 Latin Square design with 35 day periods. The study occurred from July through September (2004) when high and low temperatures averaged 34.3°C and 15.9°C, and there were no rain events. The TMR was 66.1% DM and contained 16.3% (DM) corn silage, 6.5% alfalfa haylage, 19.2% alfalfa hay and 58.0% premix which contained 1.7% (DM) Energizer-RP10, 38.2% corn grain, 1.3% molasses, 7.6% soybean hulls, 7.3% wheat middlings, 13.9% corn distillers dried grains, 6.6% canola meal, 15.6% whole upland cottonseed and 7.8% of a mineral/protein premix. The Control TMR was formulated for 17.6% CP (DM), 26.5% soluble CP (of CP), 27% NDF (DM), 6.7% total lipid (DM) and 1.74 Mcal/kg NEl (DM). The TMR was the same for each group, except that the vitamin/mineral/protein premix had no added RP10 (Control; C) or added RP10 at a level designed to deliver approximately 450 g/cow/d of Energizer-RP 10, if cows consumed 26.5 kg/d of DM. Parameters measured included yield (kg/d) of milk, fat and protein, as well as milk components (i.e., fat %, protein %, and somatic cell count (SCC)). Cows were scored for body condition (BCS) and locomotion (BLS) at the beginning and end of each period by two scorers. Milk fat % decreased with RP10 feeding (3.75 vs. 3.60; $P = 0.02$), but protein % and SCC were not affected. There was a tendency ($P = 0.10$) to an increase in milk yield (36.7 vs. 38.0 kg/d), as well as milk protein yield (1.08 vs. 1.13 kg/d; $P = 0.06$) with RP10. Milk fat yields was unaffected, as were BCS (mean = 2.95) and BLS (mean = 1.21), as well as changes in BCS and BLS. Supplemental palmitic acid changed milk composition, and tended to increase yield of milk and milk protein in dairy cows under summer heat.

Key Words: Palmitic Acid, Milk Production, Dairy Cows

626 Effect of different levels of nonfiber carbohydrates with and without spplemental fat on production and composition of Holstein dairy cows. M. Bashtani, A. A. Naserian*, and R. Valizadeh, *Ferdowsi University of Mashhad*, *Mashhad, Khorasan, Iran*.

The effect of inclusion of different levels of nonfiber carbohydrates (NFC) with and without supplemental fat on performance of Holstein dairy cows was investigated using a change over design with four treatments and four periods. Eight multiparous Holstein dairy cows were used with mean milk production and days in milking of 36 ± 5 kg and 46 ± 8 days.The experimental diets were 1) high nonfiber carbohydrates, no added fat; 2) high nonfibe carbohydrates, 2.5% fat; 3) low nonfiber carbohydrates, no added fat and 4) low nonfiber carbohydrates, 2.5% added fat. The DMI, milk production and composition were not affected by supplemental fat or nonfiber carbohydrates. The digestibility of fiber components were higher ($P < 0.05$) for diets with low nonfiber carbohydrates.Ruminal pH values were not affected by the experimental diets, but ruminal ammonia-N, BUN and MUN were significantly increased ($P < 0.05$) by decreasing the level of nonfiber carbohydrates.Glucose and cholestrol of plasma were significantly increased ($P < 0.05$) by increasing nonfiber carbohydrates and adding fat respectively. Activity chewing were not affected by nonfiber carbohydrates or fat. It appeared that response of dairy cows to adding fat was better in low fermentable carbohydrate diets.

Key Words: Fat, Nonfiber Carbohydrate, Dairy Cow

627 Milk fat trans-10 C18:1, trans-10 cis-12 CLA and trans-9 cis-11 CLA: association with fish oil-induced milk fat depression. M. A. S. Gama[1], J. M. Griinari[2], P. C. Garnsworthy[3], P. H. M. Rodrigues[4], P. R. Leme[4], L. W. O. Souza[4], and D. P. D. Lanna*[1], [1]*ESALQ-USP*, *Piracicaba, SP, Brazil*, [2]*University of Hensinki*, *Finland*, [3]*University of Nottingham*, *UK*, [4]*FZEA-USP*, *Pirassununga, Brazil*.

Trans-10, cis-12 CLA is currently the only conjugated linoleic acid (CLA) isomer unequivocally shown to inhibit milk fat synthesis. Increased formation of this isomer occurs when low fiber diets are fed to induce milk fat depression (MFD). Fish oil (FO)-induced MFD is a notable exception as many studies have observed minimal or no increase in milk fat trans-10, cis-12 CLA. In contrast, increased levels of milk fat trans-10 C18:1 have been observed in all types of diet-induced MFD. This study was conducted in two periods: 1) Baseline: cows (n=12) received high fiber diet (HF) without FO (baseline diet) for 12 d; 2) Supplementation: cows (n=4) received three treatments for 21 d: a) Low fiber diet (LF) b) HF+FO and c) LF+FO. NDF content for HF and LF were 40 and 25%, respectively. Roughage was corn silage and FO was included at 1.6% DM. Milk fat content and yield were reduced by FO treatments ($P<0.05$), but diet fiber level had no effect. Concentration of trans-10, cis-12 CLA in milk fat was lower (different superscripts, $P<0.05$) in LF diet than in HF+FO and LF+FO diets (0.002^{a}vs. $0.014^{b}$and 0.016^{b}% of total fatty acids, respectively). Values of trans-10 C18:1 and trans-9, cis-11 CLA in milk fat were 3.76^{a}, 2.57^{ab}, $0.65^{b}$and 0.05^{a}, 0.03^{ab}, 0.01^{b}, respectively for LF+FO, HF+FO and LF treatments. Concentrations of trans-10 C18:1 and trans-9, cis-11 CLA in milk fat were closely correlated (R^2= 0.97) and both fatty acids also showed independently a close inverse relationship with the degree of MFD (R^2= 0.68 and R^2= 0.55, respectively). In contrast, the association between trans-10, cis-12 CLA in milk fat and MFD was very poor (R^2= 0.02). Close correlation between trans-9, cis-11 CLA and MFD can be interpreted in two ways: trans-9, cis-11 CLA is an inhibitor of milk fat synthesis or it is a product associated with the formation of a yet unidentified inhibitor.

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Key Words: Milk Fatty Acids, Dairy Cow, Milk Fat Depression

628 Source and amount of pelleted cottonseed influences fat digestibility and milk fat composition through ruminal metabolism of fatty acids in lactating cows. C. Reveneau*, M. L. Eastridge, and J. L. Firkins, *The Ohio State University*, *Columbus*.

Pelleting cottonseed (CS) improves handling characteristics for dairy operations. Our objectives were to determine if increasing the particle size of the CS pellet or dilution of a smaller pellet with delinted CS would limit the rate of CS oil release to optimize digestibility of fatty acids (FA) and fiber while maintaining milk fat production. In Trial 1, dietary treatments were 1) whole CS (WCS) control, 2) larger pelleted fuzzy CS (LFZ), and a blend of 1/2 small fuzzy pellets

(SFZ) plus 1/2 partially delinted CS fed at 3) 100% (SFC100) or 4) 90% of WCS (SFC90). Sixty cows averaging 105 DIM were fed the WCS diet for 2 wk and then assigned to one of the 4 diets for 12 wk. In a 5 x 5 Latin square with 3-wk periods (Trial 2), 5 rumen-cannulated cows were fed: 1) control with CS hulls and CS meal plus tallow and Ca soaps of FA, 2) WCS, 3) SFZ, 4) LFZ, or 5) SFC100. Diets contained 39.6% concentrate, 14.4% CS, and 46% forage (40:60, alfalfa hay:corn silage) on a DM basis. Unless stated, $P \leq 0.05$. For Trial 1, milk production trended to progressively increase for SFC100 and SFC90 than WCS or LFZ (diet x time, $P=0.07$). Milk fat was lower for LFZ (2.74%) and SFC90 (2.85%) than WCS or SFC100 (3.07 and 3.08%). 3.5%FCM was lower for LFZ than WCS, SFC100, and SFC90 (31.2, 35.0, 37.3, and 34.5 kg/d). In trial 2, NDF digestibility was unaffected, but N digestibility was lowest for SFC. FA digestibility was higher for WCS, SFZ, and LFZ (81.1, 82.6, and 82.3%) than control or SFC treatments (78.8 and 75.3%). The 18:1 *trans*-11 in milk from cows fed SFZ and LFZ (7.10 and 6.73%) was greater than control, WCS, and SFC (1.65, 4.13, and 3.78%). The % 18:1 *trans*-10 in milk from control (.36), SFZ (.27), and LFZ (.36) were higher than those in WCS and SFC (.04, .05). Based on estimated passage rates from NRC, fat disappearance in situ was 27.8, 65.7, 52.6, and 44.7% for WCS, SFZ, LFZ, and SFC. Although having a lower FA digestibility, SFC100 appeared to minimize negative effects of free oil in the rumen from SFZ, explaining higher DMI and milk production than WCS or LFZ.

Key Words: Cottonseed, Fatty Acid, Lactating Dairy Cows

629 Effect of feeding whole fuzzy cottonseed with elevated concentrations of free fatty acids on production of lactating dairy cows. K. M. Cooke* and J. K. Bernard, *The University of Georgia*, *Tifton*.

Twenty-four lactating Holstein cows were used in an 8 wk randomized block trial to examine the effects of feeding whole cottonseed (WCS) with elevated concentrations of free fatty acids in the oil (FFA) on intake and performance. Treatments included a control WCS with normal concentrations of FFA (6.8%) and two lots of WCS with elevated FFA; HFFA1 (24.1%) or HFFA2 (22.3%). Compared with control and HFFA1, the HFFA2 contained slightly more moisture (9.4, 10.6, and 11.9 %, respectively) and less oil (18.4, 17.1, and 15.9 %, respectively) and were visibly discolored. There were no differences in concentrations of ADF, NDF, or minerals among WCS treatments. Cows were trained to eat behind Calan doors and individually fed once daily. The WCS composed 14% of the total DM of the ration. Dry matter intake was highest ($P = 0.06$) for cows fed HFFA2 (23.5 kg/d) compared with control and HFFA1 (21.6 and 22.0 kg/d, respectively). No differences in milk yield (average 34.7 kg/d) were observed among treatments. Milk fat percent was lower ($P = 0.007$) for HFFA1 (3.64%) and HFFA2 (3.58 %) compared with control (4.22%). Percentage of milk protein, lactose, and SNF was similar among treatments. No differences were observed in concentrations of MUN although values were numerically higher ($P = 0.15$) for diets containing WCS with elevated FFA. While molar proportions of butyrate and isobutyrate were higher for HFFA1 and HFFA2 compared with control ($P = 0.08$ and $P = 0.0004$, respectively), no differences were observed in concentrations of acetate or propionate. Results of this trial indicate that feeding WCS with high concentrations of FFA may slightly increase DMI and decreases milk fat percentage but does not alter milk yield. The decrease in milk fat percentage is apparently not due to changes in proportions of individual VFA.

Key Words: Cottonseed, Free Fatty Acids

Animal Behavior and Well-being: Dairy Cattle Housing, Management, and Stress

630 The use of animal-based measures to evaluate tie stall design on dairy farms in Ontario. K. Zurbrigg*[1], D. Kelton[2], N. Anderson[1], and S. Millman[2], [1]*Ontario Ministry of Agriculture and Food*, *Fergus, Ontario, Canada*, [2]*University of Guelph*, *Guelph, Ontario, Canada*.

Poor tie stall design can cause injury, lameness and mastitis. These problems affect cattle welfare and increase the probability of premature culling, lost production and negative public attitudes toward the dairy industry. The objective of this study was to describe the prevalence of animal-based measures of cow comfort and associations among measures of cow comfort, tie stall dimensions, milk production and milk quality.

All lactating cows on 317 randomly selected tie stall dairy farms across Ontario were included in this cross sectional study. Each cow was scored for the presence of the animal measures listed in Table 1. Using multivariate negative binomial regression techniques, these measures were analyzed for their associations with stall length, stall width, tie rail height, chain length and the presence of an electric trainer. The proportion of the herd affected with each problem and the farms tie stall dimensions were also analyzed for associations with the volume of milk shipped and bulk tank somatic cell count (BTSCC). The prevalence of open hock wounds was 36% greater for cows housed in stalls with electric trainers compared to stalls without (p=0.05). For each inch increase in tie chain length there is a 1.4% decrease in the prevalence of cows with dirty hind limbs (p=0.05). A 1% increase in the percent dirty cows was associated with an increase in BTSCC, of 1100 cells per ml (p=0.003).

Benchmarking the prevalence of lameness, cleanliness and injuries allows individual farms to assess their own herd scores and thereby to determine their farms strengths and weaknesses.

Table 1. The proportion of cows affected with each animal measure ranked from the lowest to highest scoring farms in each category.

Variable	Best 20% (%)	2nd Best 20% (%)	Middle 20% (%)	2nd Worst 20% (%)	Worst 20% (%)
Swollen hocks	0-4	5-9	10-15	16-26	27-61
Hock hair loss	0-15	16-27	28-42	43-53	54-81
Hock wounds	0-1	2-3	4-7	8-12	13-100
Neck lesions	0	0	0-1	2-4	5-48
Broken tails	0	0	0-1	2-5	6-50
Rotated hind claws	0-7	8-15	16-22	23-34	35-74
Arched backs	0	0-1	2-3	4-6	7-21
Dirty udders	0	0-1	2-3	4-7	8-48
Dirty hind limbs	0-3	4-9	10-18	19-36	37-94

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Key Words: Tie-Stall, Injuries, Dairy Cows

631 The comparison between cow behavior to free stall and straw bedding system. S. Ghasemi* and A. A. Naserian, *Ferdowsi University*, *Mashhad, Khorasan, Iran*.

Free stall systems are increasing on dairy farms in Iran as an alternative to straw bedding. The objective of this study was to compare cows' responses to free stall and straw bedding systems, in different seasons. This experiment was conducted during 2004, in a dairy farm in north of Iran near the Caspian sea, with humid and hot weather in summer, annual rain fall of 450mm and minimum