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Summary of the cumulative Dissertation

Host genetic and rumen microbial determinants of nitrogen (N) utilisation and N excretion in lactating Holsteins

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Enhancing the nitrogen (N) utilisation efficiency (NUE) of dairy cows by breeding selection was considered relevant for environmental footprints, feeding costs and animal health. Due to the difficult data obtainment of NUE, the cow-individual milk urea (MU) value was suggested as an indicator trait for breeding purposes. However, the complexity of N metabolism in ruminants has prevented a uniform understanding of MU's accuracy to picture NUE yet. It became evident that a deeper knowledge on the underlying principles of cow-individual N utilisation and N excretion is indispensable before MU can be seriously considered for breeding strategies on enhanced NUE.

Considering the key function of the rumen microbiome in the N metabolism it was hypothesised that cow-individual NUE is determined by the entire "holobiont", thus by the host genome and the rumen microbiome. In this context four study approaches were conducted, which focused on the host genome–trait, the host genome–rumen microbiota and the entire host genome–rumen microbiota–trait axis, aiming for a holistic exploration of cow-individual N utilisation and N excretion as a pre-step for future breeding strategies.

The studies were based on data material of two different cow populations. The 1st and 4th studies were conducted on data of 371 genotyped, lactating Holsteins, which were sampled once each for rumen fluid, milk, urine and faeces on a practice- operating dairy farm. For 358 cows, a genomic breeding value for MU (GBVMU) was estimated. The 2nd and 3rd studies were based on a population of 20 non-pregnant lactating Holsteins, which were grouped due to their predisposition for high or low MU values (HMUg *vs.* LMUg). The cows were housed on an experimental farm and fed with either a normal or a low CP diet (NP *vs.* LP), in a 4x5 balanced design (NPxHMUg, NPxLMUg, LPxHMUg, LPxLMUg; n= 5 cows each group). The 20 cows were slaughtered after two weeks and sampled for rumen tissue and rumen fluids directly after slaughter.

Initially, the host genome–trait axis was explored by a genome-wide association study (GWAS) for nine N traits in milk and urine. Potential candidate genes were identified, namely *GJA1*, *RXFP1*, *FRY1* (MU), *SH3D19* (MU yield), *RCAN2, CLIC5, ENPP4*, *ENPP5* (urinary urea concentration (UU)), *ELF2* and *SLC7A11* (minor N fractions in milk), and *ITPR2*, *MYBPC1*, *STIM2*, *SGCD*, *SLC6A2*, *TMCC2* and *MFSD4A* (specific non-urea-N metabolites in urine).

The 2nd and 3rd study focused on the host genome–rumen microbiota axis. The analysis of 16S rRNA amplicon sequencing data (microbial data) and holistic transcriptome profiling (host- gene expression) of the rumen epithelia identified significantly differential abundances of 10 microbial genera (DAG) and 28 gene transcripts (DEG) which distinguished HMUg and LMUg cows' rumen profiles. Greater abundances of the ureolytic genus *Succinivibrionaceae_UCG-002* and *unclassified Ruminococcaceae* were identified in LMUg animals, whereas HMUg cows had enhanced occurrences of the *Butyrivibrio* genus. Differential

expression analysis revealed genes of the bovine Major Histocompatibility Complex (*BOLA* genes) as well as *MX1*, *ISG15* and *PRSS2* displaying candidates of MU predisposition that were further attributed to enhanced immune system activity in LMUg cows. The analysis of a potential microbial–host interplay revealed 157 significantly correlated microbe–gene pairs and two pronounced microbe–transcript clusters, which jointly distinguished the rumen profiles of HMUg and LMUg cows. Strikingly positive correlations of *BOLA-DRA* transcripts with abundances of *Roseburia* and the *Lachnospiraceae* family might constitute particularly prominent microbial–host interplays of MU predisposition. The reduction of feed N was followed by 18 DAG in HMUg and 19 DAG in LMUg, depicting pronounced interest on *Shuttleworthia*, which displayed controversial adaption in HMUg and LMUg cows. Lowering feed N further elicited massive downregulation of immune response and energy metabolism pathways in LMUg cows.

The 4th study focused on the exploration of the entire host genome–rumen microbiota–trait axis. Therefore, the large cow population was grouped for GBVMU into two extreme groups (GBV_{HMU}, GBV_{LMU}, n=59) and one medium group (GBV_{MED} n=299). The analysis of 16SrRNA microbial data by contrasting the extreme groups identified a microbial signature of 24 genera, which potentially inferred from GBVMU selection. In accordance with the results of the 2nd and 3rd study, the microbial signature uncovered higher abundance of the ureolytic *Succinivibrionaceae_UCG-002* in GBV_{LMU} cows, whereas GBV_{HMU} hosted higher abundance of hyper ammonia producing bacteria. The abundances of the microbial signature were further investigated for correlations to proxies of NUE in milk, urine and faeces in the entire cow population (n=358). Three genera of the *Lachnospiraceae* family revealed significant correlations by their ruminal abundances to MU values, proposing them as considerable players in the host genome– rumen microbiota–MU axis. The significant correlations of *Prevotellaceae UCG-003, Anaerovibrio, Blautia* and *Butyrivibrio* abundances with MU, milk nitrogen and the N concentration in faeces (FaecN) suggested their contribution to genetically determined N utilisation in Holstein cows. Moreover, noticeable positive correlations between *WCHB1-41 ge*, *CAG-352* and *Bacteroidales BS11 gut group* abundances to FeacN suggested a contribution of these genera to genetically determined N losses due to non-assimilated N.

The study results were further discussed in regard of MU as a potential indicator trait for NUE. Significantly lower MU and UU values in GBV_{LMU} compared to GBV_{HMU} cows, similarities of DAG identified in both cow populations and a possible correction of MU records by cow-individual N intake data in the near future let assume potential for MU data being utilised for future breeding programs.