*animal* journal

**Carbohydrate and amino acid metabolism and oxidative status in Holstein heifers precision-fed diets with different forage-to-concentrate ratios**

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Short title: Forage:concentrate affects heifer metabolism

**Supplementary Material S1**

**Materials and methods**

*Statistical analysis*

The statistic codes used in R:

install.packages("lsmeans")

library("lsmeans")

setwd("c:/Users/desktop")

AN<-read.csv("metabolomic data.csv", head=T)

results<-rep(0,13)

for(i in 3:ncol(AN)){

 lm <- lm(AN[,i] ~ 1 + Group, data=AN)

 lsm<-lsmeans(lm, list(poly ~ Group, pairwise ~ Group))

 lsmp<-lsm$`polynomial contrasts of contrast`

 pvalue<-summary(lsmp)$p.value

 mean<-lsm$`lsmeans of Group`

 lsmean<-summary(mean)$lsmean

 pdiff<-lsm$`pairwise differences of contrast`

 pdif<-summary(pdiff)$p.value

 result<-c(lsmean,pdif,pvalue)

 results<-cbind(results,result)

}

results

write.table(results, file="p.txt", quote=FALSE, sep="\t")

**Table S1.** Basic information for the identified metabolites and significant metabolites between heifers fed diets with different forage-to-concentrate ratios. RT, retention time; VIP, variable importance projection; QC, quality control. The C20, C40, C60, and C80 diets contained 20%, 40%, 60%, and 80% of concentrate, respectively.

See ‘Table S1 basic information of metabolites and DEG-PEAK.xlsx’ file

**Figure S1.** High-resolution GC-TOF/MS total ion chromatograms (TIC) of plasma samples from different forage-to-concentrate ratio diets fed heifers. GC-TOF/MS TIC chromatograms of plasma samples for heifers fed 20% (A), 40% (B), 60% (C), and 80% (D) concentrate. GC-TOF/MS, gas chromatography time-of-ﬂight/mass spectrometry.

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**Figure S2.** Total principal component analysis plot of the plasma samples from different forage-to-concentrate ratio diets fed heifers. The C20 diet contained 20% of concentrate, while the C40, C60 and C80 diets contained 40%, 60% and 80% concentrate, respectively. PC, principal component; QC, quality control.



**Figure S3.** Corresponding validation plots derived from the GC-TOF/MS metabolite profiles of plasma samples between heifers fed diets with different forage-to-concentrate ratios. Corresponding validation plots (respectively) for: (A) the C80 group *vs.* C60 group; (B) the C80 group *vs.* C40 group; (C) the C80 group *vs.* C20 group; (D) the C60 group *vs*. C40 group; (E) the C60 group *vs*. C20 group; (F) the C40 group *vs.* C20 group. GC-TOF/MS, gas chromatography time-of-ﬂight/mass spectrometry; R2, the interpretability of this model; Q2, the predictability of this model. The C20 diet contained 20% of concentrate, while the C40, C60 and C80 diets contained 40%, 60%, and 80% concentrate, respectively.

