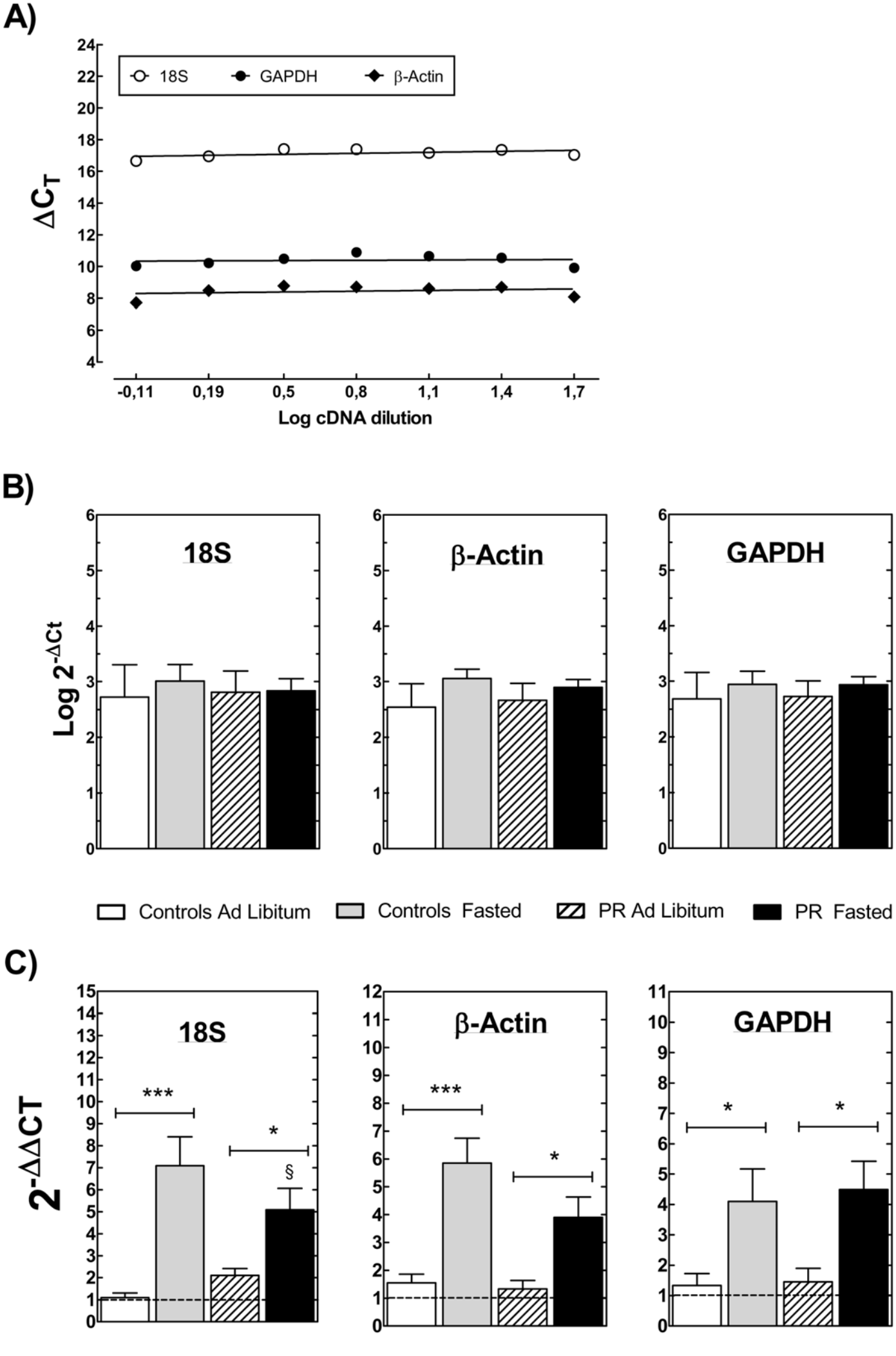
da Silva Aragão et al., Supplemental Figure 1



**Supplemental figure 1** Validation of the use of 18S as housekeeping gene for the quantitative analysis of gene expression in control and protein-restricted rats. A) Amplification efficiency of UCP3 in relation to 18S, β-actin, and GAPDH. Serial dilutions of cDNA synthesised from extracted RNA of a control rat were amplified by real-time PCR using the reaction conditions described in the Material and Methods section and the following set of primers: β-Actin forward: TCCTGGGTATGGAATCCTGTGG; β-Actin reverse: TCTCCTTCTGCATCCTGTCAGC; GAPDH forward: CGGCAAGTTCAACGGCACAG; GAPDH reverse: TCCACGACATACTCAGCACCA. The primers for 18S are described in Table 1. The threshold cycle ($C_T$), corresponding to the amplification cycle at which the fluorescence exceeds 10 times the standard deviation of the baseline, was determined for all the genes. The relative amount of UCP3 normalised to the endogenous expression of each one of the housekeeping genes was then calculated by the formula $2^{-\Delta C}{}_T$ where $\Delta C_T = C_T$ UCP3 – $C_T$ housekeeping gene. B) mRNA expression levels for 18S, β-actin, and GAPDH in the liver of adult offspring born to dams fed a standard or a protein-restricted diet during pregnancy and lactation. Histograms illustrate the variations in gene expression in relation to those observed under *ad libitum* feeding conditions using the expression in control fed animals for each one of the groups as calibrator. The relative amount of each housekeeping gene was calculated using the $2^{-\Delta C}{}_T$ equation where $\Delta C_T = C_T$ fasting – $C_T$ Ad libitum. C) Relative abundance of UCP3 in the liver of control and protein-restricted rats. Histograms illustrate the mRNA expression levels of UCP3 in relation to those of the endogenous 18S, □-actin or GAPDH amplified within the same RNA samples and under the same experimental conditions. Data correspond to the variations in gene expression in relation to those observed under *ad libitum* feeding conditions using the expression in fed animals of the control group as calibrator. The relative expression levels were determined using the equation 2-DDCt where DDCt = $[(C_T$ UCP3 - $C_T$ housekeeping gene$)_{Fasting}]$ – $[(C_T$ UCP3–$C_T$ housekeeping

gene)$_{\text{Ad libitum}]}$. Each bar in (B) and (C) represents the mean ± S.E.M. of 6 different mRNA samples. All determinations were performed in triplicate and there was less than 5% variation among them. *$p<0.05$; **$p< 0.01$; $^{\S}p < 0.05$ compared to *ad libitum* fed control animals. Note the identical variations in gene expression independently of the used housekeeping gene.