Differential metabolic and endocrine adaptations in llamas, sheep, and goats fed high- and low-protein grass-based diets

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\textbf{Abstract}

This study aimed to elucidate whether distinct endocrine and metabolic adaptations provide llamas superior ability to adapt to low protein content grass-based diets as compared with the true ruminants. Eighteen adult, nonpregnant females (6 llamas, 6 goats, and 6 sheep) were fed either green grass hay with (HP) or grass seed straw (LP) in a cross-over design experiment over 2 periods of 21 d. Blood samples were taken on day 21 in each period at \(0, 30, 60, 150,\) and 240 min after feeding the morning meal and analyzed for plasma contents of glucose, triglyceride, nonesterified fatty acids, \(\beta\)-hydroxy butyrate (BOHB), urea, creatinine, insulin, and leptin. Results showed that llamas vs sheep and goats had higher plasma concentrations of glucose (7.1 vs 3.5 and 3.6 \(\pm 0.18\) mmol/L), creatinine (209 vs 110 and 103 \(\pm 10\) mmol/L), and urea (6.7 vs 5.6 and 4.9 \(\pm 0.5\) mmol/L) but lower leptin (0.33 vs 1.49 and 1.05 \(\pm 0.1\) ng/mL) and BOHB (0.05 vs 0.26 and 0.12 \(\pm 0.02\) mmol/L), respectively. BOHB in llamas was extremely low for a ruminating animal. Llamas showed that hyperglycemia coexisted with hyperinsulinemia (in general on the HP diet; post-prandially on the LP diet). Llamas were clearly hypercreatinemic compared with the true ruminants, which became further exacerbated on the LP diet, where they also sustained plasma urea at markedly higher concentrations. However, llamas had markedly lower leptin concentrations than the true ruminants. In conclusion, llamas appear to have an intrinsic insulin resistant phenotype. Augmentation of creatinine and sustenance of elevated plasma urea concentrations in llamas when fed the LP diet must reflect distinct metabolic adaptations of intermediary protein and/or nitrogen metabolism, not observed in the true ruminants. These features can contribute to explain lower metabolic rates in llamas compared with the true ruminants, which must improve the chances of survival on low protein content diets.

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\textbf{1. Introduction}

Llamas (\textit{Lama glama}) are herbivorous ruminating animals with an expanded foregut and substantial microbial fermentation like other ruminant animals and with similar end products of fermentation \cite{1}. Despite the similarities with ruminants, the digestive tract in llamas differs from that of true ruminants anatomically with less complete separation of individual segments of the expanded foregut and there are functional differences as well \cite{1,2}. Llamas have been shown to have higher liquid passage rate but lower passage rate of solid material \cite{3}. We \cite{4} and others \cite{5} have previously shown that llamas in addition have lower energy expenditure than ruminants. Furthermore, llamas...
have shown a superior ability to reduce energy requirements for maintenance on low protein content, grass-based diets compared with true ruminants [4].

The functional differences between ruminants and llamas have allegedly provided llamas with a superior ability to digest and survive on diets with poor digestibility and low protein contents.

It is reasonable to believe that this apparent superiority of llamas to small ruminants to survive on poor-quality diets must be based on other evolutionary traits not only in the function of the digestive tract but also in their intermediary metabolism. Llamas have been reported to have a greater protein requirement per unit of energy compared with other ruminants [6], although we have shown that they are capable of compensating for inadequate dietary nitrogen supply by reducing urinary nitrogen excretion [4]. A few studies have previously looked into metabolic characteristics of llamas compared with other true ruminants showing that cameldads had high concentrations of blood glucose [7]. It has also been suggested that llamas may be able to recycle urea (ie, transferring urea from blood to the digestive tract via saliva) more efficiently presumably due to a lower rate of kidney urea excretion and/or greater urease activity compared with the ruminants [8]. The objective of this study was to test the hypothesis that distinct endocrine and metabolic adaptations in llamas have provided them superior ability as compared with the true ruminants to adapt to low protein content diets. To test this hypothesis, a comparative cross-over experiment was conducted, where metabolic and endocrine profiles were studied in llamas, sheep, and goats when fed either artificially dried grass hay with 14.8% crude protein (HP) or grass seed straw with 6.2% crude protein (LP).

2. Materials and methods

2.1. Experimental animals

The experiment was conducted at the experimental facility for large animals, Rørrendegård, Faculty of Health and Medical Sciences, University of Copenhagen, Højega-Taastrup, Denmark. All experimental procedures were approved by the National Committee on Animal Experimentation, Denmark. Details regarding the experimental design, feeding, feed analyses, nutrient intakes and digestibilities, as well as quantitative data of energy and protein metabolism have previously been published by Nielsen et al [4]. Briefly, 18 mature and nonpregnant animals including 6 llamas, 6 Danish Landrace goats, and 6 Shropshire sheep were used in a cross-over design experiment. Llamas, sheep, and goats had 135 ± 30, 75 ± 6, and 45 ± 5 kg live weight (mean ± standard deviation), respectively. Before the onset of the experiment, all animals were given an anthelmintic treatment (Ivomec Vet. Injection; Merial, Skovlunde, Denmark). The experiment consisted of 2 consecutive periods. Each period lasted for 21 d; animals were fed ad libitum to obtain 10% residuals. The daily ration was divided into 2 equally sized meals given at 7 30 AM and 3 30 PM. During each period, half of the animals within each species were fed either artificially dried green grass hay with 14.8% crude protein (HP) and the other half were fed grass seed straw with 6.5% crude protein content (LP). Daily dry matter, crude protein, and energy intakes of llamas, sheep, and goats on both HP and LP diets is shown in Table 1.

2.2. Blood sampling and analysis

Blood samples were collected on day 21 in each experimental period at – 30, 60, 150, and 240 min related to the morning feeding time. The day before blood samplings, one jugular vein was catheterized under local anesthesia (Lidokain, AstraZeneca, Albertslund, Denmark) with a temporary catheter (Portextruslurent PVC, 0.63 ID, 1.40 OD, Smiths SIMS Portex limited, UK). Catheters were flushed with heparinized saline (100 IU/mL) after each blood sampling. Volume of each blood sample was about 10 mL, and the first mL of collected blood was always discarded. Blood tubes were centrifuged at 2,100g at 4°C for 15 min not later than 20 min after the blood was sampled. Plasma samples were subsequently transferred to cryotubes and stored at –20°C pending analyses. The analysis plasma glucose, urea, creatinine, β-hydroxy-butyrate (BOHB), nonesterified fatty acids (NEFAs), and triglycerides (TGs) were performed by using an autoanalyzer, ADVIA 1650 Chemistry System (Bayer Corporation, Tarrytown, NY, USA) at the Faculty of Science and Technology, Aarhus University, Denmark. All blood samples were analyzed in duplicate. Blood plasma glucose and creatinine concentrations were determined according to standard procedures (Siemens Diagnostics Clinical Methods for ADVIA 1650). NEFAs were determined using the Wako enzymatic method (NEFA, ACS-ACOD assay method, Wako Chemicals, Richmond, VA, USA). BOHB was determined by a spectrophotometric method [9]. The production of NADH was measured as an increase in the absorbance at 340 nm in the presence of BOHB dehydrogenase in a slightly alkaline pH. Blank samples were included, and oxamic acid was included in the media to inhibit lactate dehydrogenase. Leptin analyses were performed at the University of Western Australia, Perth, by a species-specific Radioimmunoassay (RIA) using ovine leptin raised against bovine leptin [10]. The interassay and intra-assay coefficient of variations for the leptin assay were below 5% and 10%, respectively. Insulin was analyzed with commercially available kits from Merodia (Merodia AB Sylveniusgatan 8A, SE-754 50 Uppsala, Sweden). Ovine insulin enzyme-linked immunsorbent assay (ELISA) was used for the sheep and the goats, whereas bovine insulin ELISA was used for the llamas. Samples for a given species were analyzed with one of the kits and no comparisons were made between the ovine and the bovine kit. The intra-assay and interassay coefficient of variations for the specific type of kit were less than 7.5% for both the insulin kits.

2.3. Statistical analysis

The data were statistically analyzed using SAS/STAT software, version 9.2 of the SAS system for windows, copyright 2008 SAS Institute Inc, Cary, NC, USA [11]. Normality of residuals was tested using Shapiro–Wilk test,