**Individual phenotyping of feed efficiency in lambs fed stable isotopes through maize silage**

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Abstract

Recording feed intake and thus feed efficiency in ruminants is challenging. Hence, there is a need to establish indicator traits that can capture the individual variation in feed efficiency, without requiring individual feed intake recordings. This study aimed to explore whether the methodology established in salmon using individual indicator traits for feed efficiency based on stable isotope profiling could be transferred to lambs since the product for both species is meat. We used a total mixed ration based on maize silage to record the individual indicator traits for feed efficiency. The experiment was done with twelve weaned female lambs of the Norwegian White Sheep grouped equally in three, one group fed a total mixed ration enriched with 13C for 42 days, the second group grazed for 21 days and then fed the total mixed ration for 21 days, and the third group grazed for 42 days. The latter group was included to establish a baseline value for the stable isotope percentage in the back and thigh muscle tissues. Recording of 13C in the back muscle of the animals in the first two groups, resulted in a phenotypic correlation as large as 0.92 between individual feed conversion ratio and the individual indicator traits for feed efficiency based on stable isotope profiling. An assessment through individual indicator traits would be independent of the recording of individual feed intake.

Keywords: stable isotopes, carbon metabolism, feed conversion ratio indicator trait, sheep

# 1. Introduction

Selective breeding of feed efficiency in ruminants is a challenge due to difficulties with recording of individual feed intake in large-scale production systems, in which forages fed *ad libitum* often constitute over 50% of ruminant diets (Harstad, 2011). Hence, selection has in large been based on indirect selection for production traits. Selection for an increased growth rate, e.g., relevant for lambs, reduces the number of feed days for a given weight and thus improves feed efficiency. Biologically there exist losses between gross energy and metabolizable energy available for the animal and one of the causes is digestibility which has been shown with genetic variation (Dvergedal et al., 2019a). From metabolizable energy to net energy, the genetic variation has been shown to be captured by using stable isotope profiling of muscle tissue (Dvergedal et al., 2019b). Hence, it would be important to develop indicator traits from stable isotopes that can capture the individual variation in feed efficiency, without requiring recording of individual feed intake. Dvergedal et al. (2019b) have proposed indicator traits for feed efficiency in Atlantic salmon parr based on the use of the stable isotopes, 13C and 15N. The indicator traits have been validated against the observed feed efficiency in family tanks (23 families in duplicate tanks), with a genetic correlation to feed conversion ratio (FCR = feed intake (kg) / weight gain (kg)) on a tank-level approaching unity. However, feed efficiency is not necessarily the same trait in a ruminant relative to a carnivore species such as salmon. Moreover, it would be a requirement for being practically applicable that the stable isotope can be fed through natural feed containing 13C. The financial benefit of improved feed efficiency for the agricultural industry is significant. The genetic improvement of feed efficiency implies a reduction in the amount of feed used per unit produced, with consequences for sustainability. We aimed to explore whether the methodology established in salmon could be transferred to ruminants, through a pilot study with lambs since the product for both species is meat. Thus, we hypothesized that recording individual FCR in ruminants can be done by the use of these indicator traits for feed efficiency by examining the phenotypic relationship between the individual indicator traits for feed efficiency and individual FCR. We used a feed based on maize silage and studied the phenotypic relationship between the individual indicator traits for feed efficiency and individual FCR, using female lambs as a model. Maize, being a C4 photosynthetic plant, has a higher level of 13C than grass which is a C3 plant (Bahar et al., 2005). Thus, maize creates a contrast in 13C that makes it possible to trace the allocation of nutrients to different tissues from the feed.

2. Materials and methods

2.1 Animals and facilities

Animals were handled according to laws and regulations for experiments with live animals in EU (Directive 2010/637EU) and Norway (FOR-2015-06-18-761). The experiment was carried out over 42 days (September to October 2019) at the Center for Livestock Production, Norwegian University of Life Sciences (NMBU), Aas, Norway. Twelve weaned female lambs of the Norwegian White Sheep with average initial body weight (±SD) of 43.1 ± 1.4 kg (recorded at day 0 of the experiment: ) were randomly allocated to three feeding regimes, after balancing for initial body weight, with four individuals per regime. The first group (G1) was fed a total mixed ration (TMR) enriched with 13C (largely composed of maize silage) for 42 days, the second group (G2) grazed for 21 days and then fed the TMR for 21 days, and the third group (G3) grazed for 42 days to establish a baseline value for the stable isotope percentage in the tissues. Individuals in all groups were weighed at day 21, which we denoted mid weight When feeding the TMR, lambs were kept in individual pens to allow individual registration of feed intake. All lambs were healthy at the start of the experiment, however, one lamb in the second group died on day 27 of the experiment for unknown reasons, as stated in the autopsy report.

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## 2.2 Feeds, feeding and sampling

Lambs at the pasture had free access to a paddock for the whole experimental period. For lambs kept indoor, a TMR largely composed of maize silage was prepared (feed composition, Table 1) in one batch, allotted into daily portions, and frozen at -20 . The daily portions were thawed at room temperature before feeding. Feed supply allows for approximately 10% feed refusal. The daily feed allowances were offered in two portions: 40% of the DM in the morning (08:00-09:00 am) and the remaining 60% in the afternoon (2:00-3:00 pm). Feed refuses were collected and weighed every day before offering the fresh feed. All lambs had free access to clean drinking water. To describe the chemical composition of the pasture, pasture samples were collected at four time-points, one just prior to the experiment and three within the experimental period. For the TMR one representative sample was taken *a priori* to freezing the daily portions.

2.3 Chemical analysis

The diets were freeze-dried, ground, and analyses were performed in duplicates. Diets were analyzed for dry matter by drying to constant weight at 104 , ash by combustion at 550 , crude protein by Kjeldahl nitrogen x 6.25 according to European Commission Regulation (EC) No 152/2009 and starch as described in McCleary et al. (1994). Lipid was analyzed after extraction with petroleum ether and acetone (70/30) in an Accelerated Solvent Extractor (ASE 200) (Dionex Corp, Sunnyvale, CA, USA). Gross energy was determined with PARR 1281 Adiabatic Bomb calorimeter (Parr Instruments, Moline, IL, USA) according to ISO 9831. Neutral detergent fiber (aNDF) was analyzed using α-amylase and sodium sulphite as described by Mertens (2002) in an Ankom200 Fiber Analyzer (ANKOM Technology, Macedon, New York, USA), and the results are expressed exclusive of residual ash (aNDFom). The chemical composition of the diets is given in Table 1.

2.4 Tissue sampling

On the last day of the experiment, the final weight was recorded for all the lambs. Then, all lambs were euthanized with a captive bolt pistol, followed by exsanguination. Tissue samples from back and thigh muscles were collected into a cryotube, snap-frozen in liquid nitrogen, and stored at -20 for stable isotope analysis. Tissue sampling was standardized; muscle from the back was from the sirloin taken after the last rib (*Thoracic* and *cervical spinalis* and *semispinalis*) and muscle from the thigh was always taken from the inside of the left thigh (*Gluteobiceps*).

2.5 Stable isotope analysis

Tissue samples were freeze-dried and homogenized and approximately 1 mg per sample was weighed into small tin capsules (8x5 mm, Elemental Microanalysis, Devon, UK). Samples were analyzed for C-isotope compositions using a Nu Horizon isotope-ratio mass spectrometer (IRMS) (Nu Instruments, Wrexham, UK) coupled to a Eurovector element analyzer (EA) 3028 (Eurovector S.p.A, Redavalle, Italy) at the Institute for Energy Technology (Kjeller, Norway). Analyzed contents of 13C in the two diets are given in Table 1 as values, while atom percentage was calculated as follows (Fry, 2006):

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where and are the proportions of 13C divided by the proportion of 12C, in the sample and in the reference standard (Vienna Pee Dee Belemnite for carbon, VPDB), respectively; = 0.0112372 (Craig, 1957). After feeding with enriched feed, the atom % 13C in excess (*APE*) is proportional to the fraction of newly deposited nutrients in the tissue, resulting from both tissue growth and replacement of previously deposited carbon, denoted as metabolism. *APE* is the total *Atom % 13C* in the sample adjusted for the initial isotope percentage in the sample (*IA %*). The *IA %* was assessed by using the four individuals in G3 (grazed for 42 days). The 13C average and standard deviations in the thigh and back muscles were: 1.081 ± 0.0001 and 1.081 ± 0.0004, respectively.

Calibration of 13C was performed against international certified reference materials and internal standards, and the results of 13C analyses were plotted on a two-point calibration line calculated from analysis of the USGS-24 standard (-16.05‰) from the United States Geological Survey and an in-house (Institute for Energy Technology) graphite standard (-31.56‰) from Spectrapure. The internal IFE Trout standard was prepared by Soxhlet extraction with CH2Cl2: 7% CH3OH for approximately two hours, cleansed with 2N HCl, and rinsed with distilled water to a neutral pH. The 13C composition of IFE trout was calibrated against the USGS-24 standard. The average 13C from six analyses of the IFE trout was -20.05‰, with a standard deviation of 0.11.

2.6 Calculations and statistics

The initial , mid and final weights were recorded for each lamb *i*. From these variables, individual weight gain and relative weight gain were calculated as follows:

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For each lamb in G1 and G2 the feed intake (, g dry matter over 42 and 21 days, respectively) was recorded, and individual feed conversion ratio ) for these animals were calculated as:

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From the tissue samples, the following individual variables were available: Atom % 13C in thigh muscle and Atom % 13C in back muscle . Individual isotope-based indicator traits for feed conversion ratio , from both and , were derived as described by Dvergedal et al. (2019b). They were defined as follows (taking as an example):

where = , with equal to 1.081 for 13C in both thigh and back muscles. For a more detailed description of these variables, see Dvergedal et al. (2019b).

Differences between G1 and G2 for IW, MW, FW, WG, RG, FI, FCR, and IFCR variables were tested with a one-way analysis of variance by use of PROC GLM in SAS®. Pearson correlations between FCR and IFCR variables were calculated by the use of SAS®, V.9.4 (SAS, Inst. Inc., Cary, NC).

3. Results and discussion

The pasture had a δ13C level of -30.5, while the δ13C level in TMR was -13.1 (Table 1). This shows that we were able to create a distinct isotopic contrast of δ13C of 17.4. The chemical analyses of the diets (Table 1), shows that the major differences between the two diets were in the contents of aNDFom, starch, and ash. The pasture had high levels of aNDFom, which is due to an increase in cell wall material such as hemicellulose, cellulose, and lignin throughout the pasture season (Harstad, 2011). However, both diets had comparable levels of gross energy (Table 1).

The carbohydrate fraction in the TMR was mainly starch and aNDFom (Table 1), and these nutrients are being hydrolyzed and converted to volatile fatty acids (VFAs) i.e., propionate, acetate, and butyrate, which is the main source of energy available to the host ruminant (Sjaastad et al., 2016). The VFAs are absorbed and transported to the liver and other tissues, where they are metabolized to glucose and energy, or stored as glycogen and fatty acids (acetate).

Descriptive statistics of the data are given in Table 2. Average weight gains for the three groups over the 42 days were 7.0 (G1), 7.3 (G2), and 5.5 kg (G3), respectively. The relative weight gain was numerically largest for G2 over both 42 and 21 days, however, the differences between groups were not significant. Correspondingly, G2 had the largest FI over 21 days (*p* = 0.02, result not shown), but this did not result in a significant FCR difference between G1 and G2. Since G1 was fed the TMR longer than G2, G1 had significantly larger ATC and ABC values than G2 (*p =* 0.0027 and *p =* 0.0004, respectively, results not shown). Finally, the IFCR variables were numerically larger in G1 than G2, however, significant differences could not be found.

In Figures 1 and 2 IFCR\_ABC and IFCR\_ATC, respectively, (both containing RG) and FCR are plotted for lambs fed the TMR for 21 and 42 days (G2 and G1, respectively). In both groups, a positive association was observed, meaning that high FCR was followed by high IFCR value and vice versa. The three individuals in G2 had in main numerically lower IFCR and FCR values than those for the four animals in G1, which can be due to chance, from the restricted number of animals or compensatory growth (for G2 grazing 21 days longer than G1 animals). Irrespective of the cause, Figures 1 and 2 indicate a similar regression pattern in both groups. Since FCR and IFCR are ratios (and made independent of the length of the sampling period) and the 13C values increase close to linear over time (comparing G1 and G2 in Table 2), a joint correlation analysis can be motivated across G1 and G2. The results for the correlation analysis between FCR, IFCR\_ATC, and IFCR\_ABC are shown in Table 3. A highly significant phenotypic correlation (*p* < 0.05) between IFCR\_ABC and FCR (r = 0.92) was found, and a non-significant correlation of 0.74 (*p* < 0.10) was obtained to IFCR\_ATC. Moreover, the two IFCR variables were estimated with an internal correlation of 0.91 (Table 3). This suggests a potential for phenotyping feed efficiency in growing ruminants, by feeding a diet of maize silage, with an enhanced level of 13C and subsequently recording the level of 13C in the central tissues, i.e., muscle tissues. This would mean an individual feed efficiency assessment that does not require individual feed intake recordings.

The logic behind the use of the IFCR variables is that individuals with a high maintenance requirement will deposit more 13C, and this relates to FCR. This can be inferred from the energy metabolism pathways generating energy for metabolic activity, which is important to obtain the structure and function of the different organs and tissues in the body. The sum of energy used to cover for the energy losses associated with the minimum level of catabolism and anabolism and heat increment of feeding is defined as maintenance costs (Baldwin et al., 1980). Also, a lowered activity has been associated with enhanced feed efficiency in livestock (Knap, 2009; Luiting, 1990), which can be related to reduced maintenance costs in less active animals (Braastad & Katle, 1989). The individual variation in maintenance costs in ruminants (McBride & Kelly, 1990; Seal & Reynolds, 1993; Waghorn & Hegarty, 2011) might explain why some individuals having a higher feed intake grow less, which again leads to a variation in feed efficiency between animals. Increased maintenance costs will increase the nitrogen and carbon metabolism in the muscle tissues, and this variation is captured by the IFCR variables, measuring the amount of newly deposited body nutrients, as measured by atom % 13C, to total body growth, over a given time period (Dvergedal et al., 2019b). Thus, individuals with a high maintenance requirement will deposit more 13C, implying that the amount of deposited nutrients for an individual becomes proportional to , and will correspondingly become proportional to FCR.

Albeit a limited number of animals included in the experiment, the results indicate that there can be a potential to improve the selection for feed efficiency in growing ruminants by basing selection on variables derived from carbon metabolism in muscle tissues, measured as the fraction of deposited new nutrients. However, the use of the indicator traits on the selection candidate itself or its relatives requires that the animals are sacrificed, and it would be advantageous to take a muscle biopsy on live animals. Although we have established a phenotypic relationship between FCR and IFCR, a genetic application would require estimation of a genetic correlation between the two variables through a family material, or to establish a phenotypic difference between groups of animals that are truly genetically different with respect to feed efficiency.

# 4. Conclusions

The study suggests a potential for individual phenotyping of feed conversion ratio in growing lambs through indicator ratio traits for individual feed efficiency, based on recording 13C in muscle tissues from feeding a TMR with an enrichment of 13C based on maize silage. Such an assessment would not require individual feed intake recordings.

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Authors’ contributions

All authors contributed to the conceptualization of the experiment. H.D. and A.K. were responsible for feed production and A.K. was responsible for the experiment. All authors contributed to data curation. H.D. prepared samples for formal analysis. H.D. and G.K. conducted the statistical analysis. H.D. wrote the original draft and the manuscript was reviewed and edited by A.K., G.K., L.T.M., M.Ø., and H.F.O. All authors have approved the final manuscript. ​

Declaration of interest

The authors declare no competing interests.

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Figures

**Figure 1.** A positive linear relationship between the individual isotope-based indicator of feed conversion ratio, from Atom % 13C in the back muscle (IFCR\_ABC), and the individual feed conversion ratio (FCR), for lambs in the two groups, fed the enriched 13C diet for 42 and 21 days (Group 1 and 2), respectively.

**Figure 2.** A positive linear relationship between the individual isotope-based indicator of feed conversion ratio, from Atom % 13C in the thigh muscle (IFCR\_ATC), and the individual feed conversion ratio (FCR), for lambs in the two groups, fed the enriched 13C diet for 42 and 21 days (Group 1 and 2), respectively.

Tables

**Table 1**

Feed composition (g/kg DM) and the analyzed chemical composition (g/kg DM if not stated otherwise) of experimental diets.

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | Diets | |
|  |  | TMR† | Pasture |
| *Feed composition* | |  |  |
| Corn silage | | 847.3 |  |
| Crushed corn grain | | 118.2 |  |
| Urea (feed grade) enriched with Na2SO4 | | 14.8 |  |
| VitaMineral® Normal Sau | | 19.7 |  |
|  | |  |  |
| *Nutrients analyzed*‡ | |  |  |
| aNDFom§ | | 298.9 | 533.0 |
| Starch | | 328.9 | 1.6 |
| Crude protein | | 131.1 | 149.0 |
| Lipid | | 38.6 | 28.7 |
| Ash | | 44.0 | 94.0 |
| Gross energy, MJ/kg DM | | 18.9 | 18.8 |
|  | |  |  |
| *Analyzed content*‡*, ‰* | |  |  |
| δ13C | | -13.1 ± 0.10 | -30.5 ± 1.23 |

†Total-mixed ration.

‡Values of the TMR are a mean of duplicate analyses, and for pasture, an average of duplicate analyzes of four samples (one collected just prior to the experiment and three within the experimental period).

§Neutral detergent fiber assayed with heat-stable amylase and expressed exclusive of residual ash.

**Table 2**

Descriptive statistics of the recorded phenotypes.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Phenotypes** | **Abbreviation** | ***N*** | **Mean** | **Min** | **Max** |
| Initial weightG1 (kg) | IWG1 | 4 | 43.4 | 42.0 | 44.4 |
| Initial weightG2 (kg) | IWG2 | 3 | 44.0 | 43.3 | 44.5 |
| Initial weightG3 (kg) | IWG3 | 4 | 42.3 | 40.0 | 43.9 |
| Mid weightG1 (kg) | MWG1 | 4 | 45.7 | 43.7 | 46.6 |
| Mid weightG2 (kg) | MWG2 | 3 | 45.7 | 44.0 | 47.8 |
| Mid weightG3 (kg) | MWG3 | 4 | 44.9 | 43.6 | 45.6 |
| Final weightG1 (kg) | FWG1 | 4 | 50.4 | 49.6 | 51.3 |
| Final weightG2 (kg) | FWG2 | 3 | 51.3 | 50.0 | 52.3 |
| Final weightG3 (kg) | FWG3 | 4 | 47.8 | 46.3 | 50.4 |
| Weight gainG1**\_**0†: (FWG1 - IWG1) (kg) | WGG1**\_**0 | 4 | 7.0 | 6.4 | 7.6 |
| Weight gainG1**\_**21‡: (FWG1 - MWG1) (kg) | WGG1**\_**21 | 4 | 4.7 | 3.9 | 5.9 |
| Weight gainG2**\_**0: (FWG2 - IWG2) kg) | WGG2**\_**0 | 3 | 7.3 | 6.7 | 7.8 |
| Weight gainG2**\_**21: (FWG2 - MWG2) kg) | WGG2**\_**21 | 3 | 5.5 | 4.5 | 6.1 |
| Weight gainG3**\_**0: (FWG3 - IWG3) kg) | WGG3**\_**0 | 4 | 5.5 | 4.6 | 6.5 |
| Weight gainG3**\_**21: (FWG3 - MWG3) (kg) | WGG3**\_**21 | 4 | 2.9 | 1.5 | 4.8 |
| Relative weight gainG1**\_**0: (((FWG1 - IWG1)/FWG1) x 100) (%) | RGG1**\_**0 | 4 | 13.8 | 12.7 | 15.3 |
| Relative weight gainG1**\_**21: (((FWG1 - MWG1)/FWG1) x 100) (%) | RGG1**\_**21 | 4 | 9.3 | 7.7 | 11.9 |
| Relative weight gainG2**\_**0: (((FWG2 - IWG2)/FWG2) x 100) (%) | RGG2**\_**0 | 3 | 14.2 | 13.4 | 14.9 |
| Relative weight gainG2**\_**21: (((FWG2 - MWG2)/FWG2) x 100) (%) | RGG2**\_**21 | 3 | 10.8 | 8.6 | 12.0 |
| Relative weight gainG3**\_**0: (((FWG3 - IWG3)/FWG3) x 100) (%) | RGG3**\_**0 | 4 | 11.6 | 9.6 | 13.6 |
| Relative weight gainG3**\_**21: (((FWG3 - MWG3)/FWG3) x 100) (%) | RGG3**\_**21 | 4 | 6.0 | 3.2 | 9.5 |
| Feed intakeG1**\_**0 (kg dry matter over 42 days) | FIG1**\_**0 | 4 | 53.7 | 50.8 | 55.2 |
| Feed intakeG1**\_**21 (kg dry matter over 21 days) | FIG1**\_**21 | 4 | 28.2 | 27.1 | 29.5 |
| Feed intakeG2 (kg dry matter over 21 days) | FIG2 | 3 | 31.3 | 30.1 | 33.0 |
| Feed conversion ratioG1**\_**0: (FIG1 / FWG1 - IWG1) | FCRG1**\_**0 | 4 | 7.8 | 6.7 | 8.6 |
| Feed conversion ratioG1**\_**21: (FIG1**\_**21 / FWG1 - MWG1) | FCRG1**\_**21 | 4 | 6.2 | 4.7 | 7.3 |
| Feed conversion ratioG2: (FIG2 / FWG2 - MWG2) | FCRG2 | 3 | 5.7 | 5.0 | 6.7 |
| Atom % 13C in thigh muscleG1 (%) | ATCG1 | 4 | 1.089 | 1.088 | 1.089 |
| Atom % 13C in thigh muscleG2 (%) | ATCG2 | 3 | 1.086 | 1.085 | 1.087 |
| Atom % 13C in thigh muscleG3 (%) | ATCG3 | 4 | 1.081 | 1.081 | 1.081 |
| Atom % 13C in back muscleG1 (%) | ABCG1 | 4 | 1.088 | 1.087 | 1.088 |
| Atom % 13C in back muscleG2 (%) | ABCG2 | 3 | 1.086 | 1.085 | 1.086 |
| Atom % 13C in back muscleG3 (%) | ABCG3 | 4 | 1.081 | 1.080 | 1.081 |
| Indicator feed conversion ratio from thigh muscleG1: (FWG1 x APE§ / FWG1 - IWG1) | IFCR\_ATCG1 | 4 | 0.54 | 0.50 | 0.62 |
| Indicator feed conversion ratio from thigh muscleG2: (FWG2 x APE / FWG2 - MWG2) | IFCR\_ATCG2 | 3 | 0.48 | 0.36 | 0.63 |
| Indicator feed conversion ratio from back muscleG1: (FWG1 x APE / FWG1 - IWG1) | IFCR\_ABCG1 | 4 | 0.52 | 0.49 | 0.57 |
| Indicator feed conversion ratio from back muscleG2: (FWG2 x APE / FWG2 - MWG2) | IFCR\_ABCG2 | 3 | 0.44 | 0.39 | 0.51 |

†Over 42 days in group 1 (G1)

‡Over 21 days in group 1 (G1)

§APE = ATC - IA %, with IA % = 1.081. Correspondingly for ABC.

**Table 3**

Pearson correlation coefficients between feed conversion ratio (FCR), and individual isotope-based indicator of feed conversion ratio, from Atom % 13C in the thigh and back muscles (IFCR\_ATC and IFCR\_ABC, respectively) †, based on seven animals.

|  |  |  |  |
| --- | --- | --- | --- |
|  | FCR | IFCR\_ATC | IFCR\_ABC |
| FCR |  |  |  |
| IFCR\_ATC | 0.74\* |  |  |
| IFCR\_ABC | **0.92** | **0.91** |  |

†Significance levels: Bold = *p* ≤ 0.05, \* = *p* < 0.10