Association of residual feed intake with growth and slaughtering performance, blood metabolism, and body composition in growing lambs

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The aim of this study was to determine the association of residual feed intake (RFI) with growth performance, blood metabolic parameters, and body composition factors in growing lambs. Individual body weight (BW) and dry matter intake (DMI) were determined in 137 male Hu lambs that were given a pelleted feed four times a day for 50 d. RFI did not show a correlation with metabolic BW (MBW) or average daily gain (ADG), but it showed a positive correlation with DMI and feed conversion ratio (FCR). Organ weight and intestine length had a large influence on RFI in lambs. The low-RFI lambs have smaller rumen and longer duodenum indicating the less feed intake and more sufficient absorption rate of low-RFI lambs. The smaller organs like liver, lung and kidney in low-RFI lambs may be related to lower energy consumption and slower metabolic rate. The observed bigger testis was in low-RFI lambs was another cause of the improved feed efficiency. Finally, the plasma concentrations of thyroxine (T4) and adrenocorticotropic hormone (ACTH) were lower in the ELow-RFI group than in the EHigh-RFI group. This study provides new insight into the biological processes underlying variations in feed efficiency in growing lambs.

Feed accounts for 65–70% of the cost in the sheep industry, and thus, improving feed efficiency (FE) is important for the economy and the environment. FE is a major indicator of the efficiency of feed utilization. When FE is low, there are negative consequences on the environment and the production cost is higher¹². FE is represented by the feed conversion ratio (FCR) or residual feed intake (RFI). FCR is defined as the ratio of feed intake to weight gain over a specific period of time, and is traditionally used in meat and egg production; however, it has certain statistical and biological limitations³–⁵. Besides, measuring the FCR is not cost-effective. It was Koch et al. who proposed the use of RFI⁶, which is regarded as a sensitive and accurate method to estimate FE⁷–⁹. RFI is defined as the difference between the actual feed intake and the predicted intake based on the body size and performance of each animal. A low RFI indicates less feed consumption and less waste generation with no effect on the weight, production and body size of the animals. Thus, RFI may be a reliable indicator of the differences in FE that account for the diverse genetic background of animals. Further, studying the regulation mechanism of RFI can not only reduce the cost of feed but also protect the environment by reducing the emission of carbon and methane¹⁰.

Many factors affect RFI, including body composition, the digestion and metabolism of nutrients, energy output, body activity and body temperature regulation¹¹,¹². Most studies focus on pigs, cattle and poultry, and studies on sheep are few. The aim of this study was to determine the associations between RFI, slaughtering performance, blood metabolic parameters and body composition in growing sheep.

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was positively associated with the eye muscle area and backfat thickness ($P < 0.05$). There was no significant difference ($P > 0.05$) between carcass traits, DMI, ADG, MBW, FCR, and RFI ($P > 0.05$) between the RFI groups with regard to the other parts of the gastrointestinal tract (Table 5).

### Table 1. Characterization of intake and growth performance in lambs with high, medium, and low residual feed intake (RFI).

| Trait                        | RFI group1 |     |     | SE2 | $P$-value |
|------------------------------|------------|-----|-----|-----|-----------|
| No. of animals               | 42         | 54  | 41  |     |           |
| RFI, kg/d                    | $-0.10^b$  | $0.06^a$ | $0.11^a$ | 0.05 | $<0.0001$ |
| Feed conversion ratio, kg of DM/kg of BW gain | 4.51$^c$ | 4.84$^b$ | 5.39$^a$ | 0.01 | $<0.0001$ |
| DMI, kg/d                    | 1.09$^c$   | 1.25$^b$ | 1.33$^a$ | 0.02 | $<0.0001$ |
| Metabolic BW, kg25           | 12.97      | 13.18 | 13.16 | 0.11 | 0.724     |
| ADG, kg/d                    | 0.25       | 0.26  | 0.26  | 0.00 | 0.108     |
| Initial BW, kg               | 24.44      | 24.76 | 24.94 | 0.34 | 0.933     |
| Final BW, kg                 | 36.66      | 37.66 | 37.34 | 0.39 | 0.935     |
| Relative growth1, %          | 40.33      | 41.9  | 40.25 | 0.51 | 0.303     |

Table 1. Characterization of intake and growth performance in lambs with high, medium, and low residual feed intake (RFI). *–1Least square means within a row with different superscripts differ significantly ($P < 0.05$). 1High = RFI was > 0.5 SD above the mean; Medium = RFI was 0.5 SD above and below the mean; Low = RFI was < 0.5 SD below the mean. 2SE = pooled SE. 3Relative growth = 2(W2 − W1)/(W1 + W2) *100%, W1 = Initial BW, W2 = Final BW.

### Results

#### Growth performance and feed efficiency.

In this study, the mean DMI of the animals was 1.22 kg/d (SD = 0.18); ADG, 0.25 kg/d (SD = 0.03); and FCR, 4.90 kg of DM/kg of BW gain (SD = 0.59). The mean RFI was 0.00 kg/d (SD = 0.09) and ranged from $-0.31$ to 0.22 kg/d, which represents a difference of 0.53 kg of feed per day between the animals with the highest and lowest RFI. The intake, growth performance, and FE data are presented in Table 1.

Low-RFI lambs consumed 12.8% and 18.0% less feed than their medium- and high-RFI counterparts, respectively ($P < 0.001$). The least-square means for RFI and FCR in the high-RFI lambs were higher than those in the medium-RFI lambs ($P < 0.001$), while the least-square means for the medium-RFI lambs were greater than those for the low-RFI lambs ($P < 0.001$). There was no significant difference in ADG, initial BW, MBW, and final BW ($P > 0.05$) between the high-, medium-, and low-RFI groups. There was no significant difference in the relative growth rate either.

RFI was not significantly correlated with MBW, ADG, initial BW or final BW, but it was highly significantly correlated ($P < 0.001$) with DMI ($r = 0.51$) and FCR ($r = 0.62$). Further, DMI showed a highly significant correlation ($P < 0.001$) with ADG ($r = 0.65$), initial BW ($r = 0.68$), final BW ($r = 0.82$) and MBW ($r = 0.77$), and moderate correlation ($P < 0.001$) with FCR ($r = 0.54$). FCR showed a negative correlation with ADG ($P < 0.01$) (Fig. 1).

#### Carcass traits.

Differences in carcass traits between the three RFI groups are presented in Table 2. There was no significant difference ($P > 0.05$) between the RFI groups with regard to carcass traits, but a positive association was found between carcass traits, DMI, ADG, MBW, FCR, and RFI ($P < 0.05$) (Fig. 2). The association of DMI with each of the carcass traits was positive ($P < 0.05$). Further, the association of ADG with body weight, carcass weight, GR value, and tail fat weight was positive ($P < 0.05$), but ADG was not significantly correlated with RFI ($P > 0.05$). The correlation between FCR, body weight, crass weight, and GR value was positive. Moreover, RFI was positively associated with the eye muscle area and back fat thickness ($P < 0.05$).

#### Tissue and visceral organs.

The weight and percentage of tissue and visceral organs are presented in Table 3. The weight of the liver and lung were lower in low-RFI lambs than in medium- and high-RFI lambs ($P < 0.01$), but the percentage weight of these two organs was not significantly different between the RFI groups. The weight and percentage weight of the testes were higher in the low-RFI and medium-RFI lambs than in the high-RFI lambs ($P < 0.01$). The total weight of the stomach and the total intestinal weight in the low-RFI lambs were 5.28% ($P < 0.01$) and 5.27% ($P < 0.01$) lesser than those in the high-RFI lambs. The results of correlation analysis indicated that RFI showed a positive correlation with the weight of liver and a negative correlation with the weight of testis ($P < 0.01$) (Fig. 2).

#### Gastrointestinal tract.

The gastrointestinal tract data for the high-, medium-, and low-RFI groups are presented in Table 4. Rumen weight was greater in medium- and high-RFI lambs than in low-RFI lambs ($P < 0.01$). Further, the reticulum weight in low-RFI animals was lesser than that in medium-RFI animals ($P < 0.05$), but it was not significantly different from that of the high-RFI animals ($P > 0.05$). The weight of the jejunum and colon in low-RFI animals was less than that in the medium- and high-RFI animals ($P < 0.05$).

With regard to the intestinal length, the absolute and relative length of the duodenum and ileum in the low-RFI animals was greater than that in the medium- and high-RFI animals, respectively ($P < 0.05$). However, the length of the cecum was the shortest in the low-RFI lambs ($P < 0.05$). No significant differences were observed with regard to the other parts of the gastrointestinal tract (Table 5).

The results of correlation analysis showed that the weight of rumen was positive correlated with RFI, FCR, ADG, DMI, initial BW, MBW, and final BW ($P < 0.05$). The length and weight of duodenum were negative correlation with RFI ($P < 0.01$) and FCR ($P < 0.05$) (Fig. 2).
Blood hormones and metabolites. Metabolic hormone and metabolite data for the EHigh-RFI and ELow-RFI animals are presented in Table 6. Correlation coefficients for the association of intake, performance, and FE traits with the metabolic variables are presented in Table 7. The plasma concentrations of T4 and ACTH were lower in the ELow-RFI group than in the EHigh-RFI group ($P < 0.01$). However, the concentrations of insulin, leptin, IGF-1, GC, and TRH were not significantly different between the groups ($P > 0.05$). RFI showed a positive correlation with T4 ($R^2 = 0.435$, $P < 0.05$) and ACTH ($R^2 = 0.534$, $P < 0.01$). FCR was positively correlated with T4 ($R^2 = 0.413$, $P < 0.05$), but no other significant associations were observed with regard to the remaining traits ($P > 0.05$).

**Discussion**

In the present study, the base RFI regression model (DMI explained by MBW and ADG) accounted for 80% of the variation in DMI; in the current study, this is similar to the data of other studies on cattle\cite{13,14}. DMI, FCR and RFI in low-RFI lambs were significantly lower than those in medium- and high-RFI lambs, but there were no differences in the initial BW, final BW, ADG, MBW and relative growth rate between the three groups, which was in agreement with the findings of Faure et al.\cite{15}. The results indicate that selection of RFI in sheep could...
increase FE by reducing feed consumption without affecting the growth performance of sheep. In agreement with our findings, Cai et al. and Barea et al. found that RFI was generally not correlated with ADG, but was correlated with DMI\textsuperscript{16,17}. Lancaster et al. considered RFI to be moderately correlated with FCR\textsuperscript{18}. However, Nkrumah observed that RFI was strongly correlated with FCR, and that high-RFI steers that were fed a concentrate-based diet consumed 15% more feed than low-RFI steers, which was generally consistent with our results\textsuperscript{19}. Previous studies have revealed that FCR is negatively correlated with ADG in lambs\textsuperscript{20}, which concurs with our findings that applying selection pressure over a long period for FCR results in an increase in growth rate and mature size and leads to greater maintenance energy costs and thus an increase in feed requirement\textsuperscript{21}. Nevertheless, previous results and our conclusion both imply that RFI has no association with ADG, initial BW, and final BW. Because the inheritance of RFI is independent of weight and ADG, genetic improvements using RFI as an index for FE can eliminate the effect of growth on RFI. Therefore, RFI is an accurate and sensitive index for measuring FE.

Controversies over the relationship between RFI and carcass traits have existed over the last few years. Most research results show that RFI has a weakly positive phenotypic and genetic correlation with body fat content\textsuperscript{14,22,23}. In contrast to these findings, Herd and Bishop reported that RFI and carcass lean content had a negative phenotypic (r = −0.22) and genetic (r = −0.43) association\textsuperscript{24}. In our study, the correlation coefficient between RFI and back fat (BF) was 0.227, and the BF in low-RFI animals was lower than that in high-RFI animals. This finding indicates although the potential benefit of selection of low-RFI animals is the reduction of BF deposition, it may mean an increase in the lean content of the carcass\textsuperscript{22,25}.

At present, there is no known association of RFI with the depth of the longissimus dorsi or growth in steers, bulls, and heifers\textsuperscript{5,14,19}. This is in agreement with our results, which show that there are no differences in GR, relative growth and slaughter rate between the three RFI groups. Our conclusion thus supplements the findings of previous studies. However, RFI has been reported to be weakly positively correlated with the eye muscle area\textsuperscript{18,26}. Our correlation data also revealed that RFI showed a positive correlation with the eye muscle area (r = 0.188) and BF (r = 0.227). Further, the association of RFI and DMI with the eye muscle area and BF indicates that sheep with a higher RFI have greater feed intake and better muscle development, as well as more fat deposition.

The weight of the internal organs and its contribution to the total body weight reflect the health condition in animals\textsuperscript{27}. The size of visceral organs is related to the level of feed intake\textsuperscript{28}, as the energy expenditure of these organs increases after feeding and is dependent on feed intake\textsuperscript{29}. In agreement with the findings of Basarab et al.\textsuperscript{7}, who observed that low-RFI cattle had an 8% and 10% lighter liver and lung, respectively, than high-RFI cattle, the weight of the liver and lung in our lamb population were different among the RFI groups: the basal metabolic rate
tract. with FE (measured using RFI) in an unselected line of rams. This report is in agreement with the findings reported that an animal’s serum cortisol response to exogenously administered ACTH is strongly correlated with RFI in an unselected line of rams. With increasing FE, serum concentrations of ACTH were lower in low-RFI lambs than in high-RFI lambs; thus, thyroid hormones are active in growing lambs.

Walker et al. reported that T4 was not affected by BW but by RFI; this difference may be due to the role of thyroid hormones, as the developing heifers required thyroid hormones to remain active. The results of previous studies are consistent with our findings that the plasma concentration of T4 was lower in ELow-RFI lambs than in EHigh-RFI lambs; thus, thyroid hormones are active in growing lambs.

The systemic concentrations of various metabolic and nutrient uptake variables and inhibitors of tissue catabolism have been found to be potential physiological biomarkers of FE in cattle. According to Stick et al. and Wood et al. (2004), the blood concentrations of IGF-1 are potential physiological markers of FE and are phenotypically positively correlated with RFI in beef cattle. However, Kelly et al. reported significant negative correlations between RFI and IGF-1 receptors in heifers divergent for RFI. Furthermore, Kelly et al. reported that the correlations between serum IGF-1 concentrations and RFI varied between different sampling times on the same day. In the present study, the serum concentrations of IGF-1 did not differ between different RFI groups; this is in agreement with the study of Richardson et al. Thus, the environment has a bigger impact on the concentration of metabolites than genetic mechanisms.

| Items            | RFI group1 | SEM2 | P-value |
|------------------|------------|------|---------|
|                  | Low (n=42) | Medium (n=54) | High (n=41) |
| **Heart** | | | |
| Weight, g       | 158.75     | 180.25     | 161.55     | 2.17 | 0.883 |
| Percentage, %   | 0.40       | 0.39       | 0.40       | 0.00 | 0.186 |
| **Liver** | | | |
| Weight, g       | 633.31b    | 694.12a    | 696.39a    | 8.87 | 0.005 |
| Percentage, %   | 1.62b      | 1.68b      | 1.73a      | 0.02 | 0.056 |
| **Spleen** | | | |
| Weight, g       | 51.40      | 52.71      | 52.62      | 0.66 | 0.681 |
| Percentage, %   | 0.13       | 0.15       | 0.13       | 0.00 | 0.547 |
| **Lung** | | | |
| Weight, g       | 375.87b    | 405.41b    | 408.04b    | 5.27 | 0.022 |
| Percentage, %   | 0.98       | 1.02       | 0.98       | 0.01 | 0.192 |
| **Kidney** | | | |
| Weight, g       | 110.12a    | 116.39a    | 114.74a    | 0.82 | 0.056 |
| Percentage, %   | 0.28       | 0.28       | 0.28       | 0.00 | 0.067 |
| **Perirenal fat** | | | |
| Weight, g       | 191.67     | 210.43     | 218.77     | 6.61 | 0.263 |
| Percentage, %   | 0.50       | 0.52       | 0.55       | 0.02 | 0.392 |
| **Head & feet** | | | |
| Weight, g       | 2.95       | 3.01       | 3.05       | 0.03 | 0.315 |
| Percentage, %   | 7.57       | 7.34       | 7.63       | 0.06 | 0.120 |
| **Skin & wool** | | | |
| Weight, g       | 4.70       | 4.84       | 4.76       | 0.06 | 0.655 |
| Percentage, %   | 11.99      | 11.69      | 11.83      | 0.11 | 0.517 |
| **Testis** | | | |
| Weight, g       | 234.10a    | 210.92a    | 158.43b    | 8.15 | 0.001 |
| Percentage, %   | 0.60a      | 0.53b      | 0.40b      | 0.02 | 0.001 |
| **TWS3** | | | |
| Weight, g       | 900.25b    | 956.52a    | 956.51a    | 7.85 | 0.004 |
| Percentage, %   | 0.28       | 0.24       | 0.22       | 0.01 | 0.246 |
| **TWI4** | | | |
| Weight, g       | 1188.71b   | 1254.63a   | 1254.87a   | 9.90 | 0.007 |
| Percentage, %   | 3.06       | 3.16       | 3.16       | 0.02 | 0.331 |

Table 3. Characterization of tissues and organs in lambs with high, medium, and low residual feed intake (RFI). *Least square means within a row with different superscripts differ significantly (P < 0.05). 1High = RFI was > 0.5 SD above the mean; Medium = RFI was 0.5 SD above and below the mean; Low = RFI was < 0.5 SD below the mean. SE = pooled SE. 3TWS = total weight of the stomach. 4TWI = total weight of the intestinal tract.
that the genetic relationship between plasma IGF-I concentration and RFI becomes less positive as cattle mature physiologically. Thus, the genes associated with systemic IGF-I concentration differ between the post-weaning and finishing stages of development. As a consequence, the concentration of serum IGF-1 may not be an appropriate indicator of RFI in sheep.

Leptin is known to regulate BW, feed intake, energy expenditure, reproduction, and immunocompetence. The plasma concentration of leptin is correlated with body lipid depots. Richardson et al. observed that the serum leptin concentration had a significant phenotypic correlation (r = 0.31) with RFI. In contrast, Brown et al. reported that systemic leptin concentration was not associated with intake, performance, or FE traits; this is similar to our findings that the serum concentrations of leptin were not significantly different between ELow-RFI animals and EHigh-RFI animals. The contradictory results may be explained by the differences in the environment, animal breed and physiological status.

Previous studies have reported that the systemic insulin concentration in high-RFI steers is greater than that in low-RFI steers; this is believed to result from the decrease in leanness caused by an increase in fat deposition, as insulin can reduce lipolysis and stimulate lipogenesis in adipose tissue. In contrast, Nascimento et al. found higher blood insulin concentrations in low-RFI animals. In the present study, plasma insulin concentrations had no relationship with intake, performance, or FE in sheep; this was similar to the results of Kelly et al. that were reported in growing beef heifers.

Conclusions
The findings of the current study indicate that there are significant differences in performance and FE in growing lambs. We observed a 18% decrease in DMI between low and high RFI lambs, but no difference in growth performance was detected between RFI groups. Organ weight and intestine length had a large influence on RFI in lambs. The low-RFI lambs have smaller rumen and longer duodenum indicating the less feed intake and more

| Items                   | RFI group¹ |          |          | SEM² | P-value |
|-------------------------|------------|----------|----------|------|---------|
| No. of animals          | Low        | Medium   | High     |      |         |
|                         | 42         | 54       | 41       |      |         |
| Rumen                   | Absolute   | 546.39a  | 587.49a  | 587.02a | 5.09    | 0.001   |
| Relative weight, %      | 62.26      | 61.51    | 61.20    | 0.31 | 0.455   |
| Reticulum               | Absolute   | 93.75b   | 102.81b  | 98.86b | 1.29    | 0.041   |
| Relative weight, %      | 10.47      | 10.70    | 10.58    | 0.15 | 0.798   |
| Omasum                  | Absolute   | 108.06   | 117.33   | 112.40 | 1.81    | 0.137   |
| Relative weight, %      | 11.93      | 12.24    | 11.92    | 0.17 | 0.665   |
| Abomasum                | Absolute   | 144.94   | 149.52   | 152.59 | 2.16    | 0.427   |
| Relative weight, %      | 16.00      | 15.55    | 16.31    | 0.24 | 0.321   |
| Duodenum                | Absolute   | 37.39    | 37.54    | 36.19  | 0.75    | 0.736   |
| Relative weight, %      | 3.18       | 2.93     | 2.88     | 0.06 | 0.110   |
| Jejunum                 | Absolute   | 764.01b  | 811.72a  | 785.63b | 7.63    | 0.031   |
| Relative weight, %      | 63.8       | 62.72    | 62.30    | 0.35 | 0.199   |
| Ileum                   | Absolute   | 25.24    | 24.59    | 24.29  | 0.50    | 0.748   |
| Relative weight, %      | 2.11       | 1.91     | 1.94     | 0.04 | 0.108   |
| Cecum                   | Absolute   | 47.32    | 52.37    | 49.21  | 0.88    | 0.052   |
| Relative weight, %      | 3.97       | 4.02     | 3.90     | 0.05 | 0.666   |
| Colon                   | Absolute   | 320.39b  | 372.38a  | 369.41a | 7.23    | 0.005   |
| Relative weight, %      | 26.94b     | 28.41a   | 28.97b   | 0.35 | 0.060   |

Table 4. The weight of the gastrointestinal tract in lambs with high, medium, and low residual feed intake (RFI). a–cLeast square means within a row with different superscripts differ significantly (P < 0.05). ¹High = RFI was > 0.5 SD above the mean; medium = RFI was 0.5 SD above and below the mean; low = RFI was < 0.5 SD below the mean. ²SE = pooled SE.
sufficient absorption rate of low-RFI lambs. The smaller organs like liver, lung and kidney in low-RFI lambs may be related to lower energy consumption and slower metabolic rate. The observed bigger testis was in low-RFI lambs was another cause of the improved feed efficiency, but the underlying mechanism remains to be investigated. Some level of association was observed between physiological markers and FE, for example, between T4 and ACTH and RFI during the growing period in lambs. Our present data show that high RFI lambs have physiology differences from low RFI lambs that control intake and conversion, but since this study was limited to a single breed and specific environmental conditions only, more research on larger populations of different breeds in different environments would be useful.

### Table 5. Intestinal length in lambs with high, medium, and low residual feed intake (RFI).

| Items   | RFI group1 | SEM2 | P-value |
|---------|------------|------|---------|
|         | Low        | Medium | High   |
| No. of animals | 42 | 54 | 41 |
| Duodenum | Absolute length, cm | 75.04a | 61.47b | 66.31b |
|          | Relative length, % | 2.25a | 1.82b | 1.92b |
| Jejunum  | Absolute length, m | 25.39a | 26.01a | 26.53a |
|          | Relative length, % | 75.87b | 76.71a | 77.15a |
| Ileum    | Absolute length, cm | 36.68a | 32.98ab | 31.92b |
|          | Relative length, % | 1.10a | 0.97b | 0.93b |
| Cecum    | Absolute length, cm | 32.53b | 35.91a | 33.23ab |
|          | Relative length, % | 0.98 | 1.06 | 0.97 |
| Colon    | Absolute length, m | 6.64 | 6.61 | 6.56 |
|          | Relative length, % | 19.83 | 19.45 | 19.03 |
| Total intestine | Total length³, m | 33.46 | 33.92 | 34.40 |

Table 5. Intestinal length in lambs with high, medium, and low residual feed intake (RFI). a–cLeast square means within a row with different superscripts differ significantly (P < 0.05). ¹High = RFI was > 0.5 SD above the mean; medium = RFI was 0.5 SD above and below the mean; low = RFI was < 0.5 SD below the mean. ²SE = pooled SE. ³Total length represents the total length of the intestinal tract.

### Table 6. Characterization of metabolic hormones and metabolites in lambs with EHigh and ELow residual feed intake (RFI).

| Items   | RFI group1 | SEM2 | P-value |
|---------|------------|------|---------|
|         | ELow     | EHigh |
| No.     | 15 | 15 |
| T4³, ng/mL | 57.28b | 79.52ª | 4.32 | 0.007 |
| Insulin, μIU/mL | 9.69 | 11.44 | 0.69 | 0.211 |
| ACTH⁴, pg/mL | 24.39b | 34.77ª | 1.78 | 0.002 |
| Leptin, ng/mL | 7.98 | 7.97 | 0.13 | 0.977 |
| IGF-1⁵, ng/mL | 15.40 | 18.20 | 2.10 | 0.519 |
| GC⁶, ng/mL | 21.73 | 21.71 | 1.17 | 0.991 |
| TRH⁷, ng/L | 49.87 | 41.49 | 2.27 | 0.066 |

Table 6. Characterization of metabolic hormones and metabolites in lambs with EHigh and ELow residual feed intake (RFI). a,bLeast square means within a row with different superscripts differ significantly (P < 0.05). ¹The EHigh group comprised 15 lambs with the highest RFI; the ELow group comprised 15 lambs with the lowest RFI. ²SE = pooled SE. ³T4 = thyroxine. ⁴ACTH = adrenocorticotropic hormone. ⁵IGF-1 = insulin-like growth factor-1. ⁶GC = glucocorticoid. ⁷TRH = thyrotropin-releasing hormone.
At the start of the performance test, the mean BW was 24.72 kg (SD 5.3). The plasma concentrations of leptin, insulin, T4, and ACTH were determined with commercial RIA kits (Beijing North Institute of Biological Technology, China), and the concentrations of GC and TRH were determined by ELISA. The ingredients and chemical composition of the pellet feed in the experiment are shown in Table 8. The diet was processed into granules; the granulating temperature was 70 °C and the grain diameter was 6 mm. The health condition of lambs was observed by the veterinarian routinely, the lamb did not suffer from acidosis and they were all in good condition during the study.

**Feed intake and growth data.** The lambs were fed four times a day, at 0630, 1130, 1530 and 1900 h, and the amount of feed consumed was recorded. An independent crib was assigned to each lamb. To avoid the accumulation of feed in the crib, feed remaining in the crib in excess of 20% of the feed offered daily was replaced by fresh feed. The amount of residual feed was weighed every morning before feeding; at the time of analysis, they were centrifuged at 1,500 × g at 4 °C for 15 min. The plasma was then split and stored in a new EP tube at −20 °C until analysis. The concentration of plasma IGF-I was determined using a validated RIA, according to a previously reported method by Spicer et al. The plasma concentrations of leptin, insulin, T4, and ACTH were determined with commercial RIA kits (Beijing North Institute of Biological Technology, China), and the concentrations of GC and TRH were determined by ELISA.

### Materials and Methods

#### Ethics Statement.
All experiments in this study were carried out in accordance with the approved guidelines from the Regulation of the Standing Committee of Gansu People’s Congress. All experimental protocols and the collection of samples were approved by the Ethics Committee of Gansu Agriculture University.

#### Animals and management.
In total, 137 male Hu lambs were purchased from Jinchang Zhongtian Sheep Industry Co. Ltd., Gansu, China, and transferred to Minqin Zhongtian Sheep Farm, at 90 days of age. Healthy lambs with good growth and intact genealogical records were randomly selected and treated with a standardized immunization program before they were weaned. They were reared indoors and housed in individual pens (0.8 × 1 m) until 165 days of age. The feeding and housing conditions and the management environment were standardized.

Briefly, the animals were exposed to an acclimatization period of 15 days, during which the proportion of pellet feed in the diet was gradually increased by 6.7% per day while the forage proportion was concurrently reduced until they were only fed the pellet feed. During this adaptation period, the animals were allowed to accustom themselves to the pellet feed and ad libitum feeding. After that, the lambs were on ad libitum intake for 10 days (pre-test period) before the experimental period, which lasted for 50 days. The feed intake for each animal was recorded in the pretest period in order to customize the feed intake in the experimental period. The animals were also given ad libitum access to fresh drinking water. Each sheepfold was thoroughly disinfected twice a month. At the start of the performance test, the mean BW was 24.72 kg (SD = 3.95). Experimental rations were formulated according to the recommendations of the feeding standard for sheep in China (NY/T1816-2004). The feeds were stored at −20 °C in triplicate before they were analyzed for dry matter (DM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF). The other feed parameters were calculated according to the feed composition and nutritive values reported in China (2015). In order to determine the DM, the feed was oven dried at 104 °C for a minimum period of 16 h. The CP (Total N × 6.25) was determined using a previously reported method (Sweeney, 1989) with a VELP UDK 192 nitrogen analyzer. The NDF and ADF concentrations of the feed were determined using developed by Van Soest et al. The ingredients and chemical composition of the pellet feed in the experiment are shown in Table 8. The diet was processed into granules; the granulating temperature was 70 °C and the grain diameter was 6 mm. The health condition of lambs was observed by the veterinarian routinely, the lamb did not suffer from acidosis and they were all in good condition during the study.

#### Blood collection and analysis.
At the end of the experimental period, blood samples for 30 lambs (15 lambs with the highest RFI and 15 lambs with the lowest RFI) were obtained by jugular venipuncture in the morning. Samples were collected in EP tubes to determine the plasma concentrations of thyroxine (T4), insulin, ACTH, leptin, IGF-1, glucocorticoid (GC) and thyrotropin-releasing hormone (TRH). The samples were immediately stored in ice water; at the time of analysis, they were centrifuged at 1,500 × g at 4 °C for 15 min. The plasma was then split and stored in a new EP tube at −20 °C until analysis. The concentration of plasma IGF-I was determined using a validated RIA, according to a previously reported method by Spicer et al. The plasma concentrations of leptin, insulin, T4, and ACTH were determined with commercial RIA kits (Beijing North Institute of Biological Technology, China), and the concentrations of GC and TRH were determined by ELISA.

| Ingredient | Percentage (%) | Chemical composition1 | Content |
|------------|----------------|-----------------------|---------|
| Barley straw | 27.00 | DM (%) | 87.55 |
| Corn | 44.00 | CP (%) | 16.28 |
| Soybean meal | 2.20 | DE (MJ/kg) | 12.38 |
| Rapeseed meal | 2.60 | NDF (%) | 36.54 |
| Cottonseed meal | 4.20 | ADF (%) | 14.12 |
| Concentrate feed2 | 20.00 | Ca (%) | 0.60 |
| Total | 100.00 | P (%) | 0.30 |
| Starch (%) | 28.48 |

**Table 8.** Ingredients and chemical composition of the experimental diet (air-dry basis). 1Concentrate feed consists of dried malt root, Urea, NaHCO₃, Premix (2.5%), and NaCl (2.5%). 2Premix provides the following mineral elements (mg/kg) and vitamins (IU/kg): S, 200; Fe, 25; Zn, 40; Cu, 8; Mn, 40; I, 0.3; Se, 0.2; Co, 0.1; VA, 940; VD, 111; VE, 20. DM, CP, NDF, and ADF are the measured values, while the others are calculated values.
Slaughtering measurements. Ten days after the experimental period finished, lambs were transported to a commercial slaughterhouse. Lambs were weighed after 24 h of fasting and slaughtered in a standardized procedure. All procedures were in accordance with the guidelines of the Biological Studies Animal Care and Use Committee, Gansu Province, P. R. of China. All lambs were bled to death with a clean small cut to the jugular vein. Internal organs were removed from the body, residual blood was allowed to drip out, and the organs were then weighed. Each carcass was weighed within 30 min after slaughtering, and the dressing percentage was calculated after determining the BW from the carcass weight. The longissimus dorsi (LD) excised from the left carcass side at the 12th rib was used to determine the eye muscle area (EMA). EMA was measured by planimeter on traced outlines of a cross section of the eye of LD at the 12th rib. The fat thickness represents the fat content of the carcass, which is based on the soft tissue depth at the GR site. The GR site is present over the 12th rib, at 110 mm away from the midline. The gastrointestinal tract was separated and ligatured with a cotton thread. The weight of the gastrointestinal tract was measured after cleaning and eliminating the contents. The length of the intestinal tract was measured using a tape.

Determination of RFI. The feed intake of each animal was recorded over 50 days, from 115 days of age to 165 days of age, and the data were used to calculate the RFI for each lamb. RFI is defined as the difference between the actual daily feed intake and the expected daily feed intake of each individual. RFI was calculated using a linear regression model, into which the DMI, ADG and mid-test metabolic body weight (MBW) data of all the lambs were entered. Total daily DMI was considered as the sum of all the meals consumed in a day, after correction for DM content. In the test period, ADG was calculated as the coefficient of the linear regression of BW (kg) on time with the REG procedure (SAS Inst. Inc., Cary, NC). MBW was calculated using the methods of Basarab et al. The base model used was $Y_i = \beta_0 + \beta_1 \text{MBW}_i + \beta_2 \text{ADG}_i + \epsilon_i$, where $Y_i$ represents the DMI of the $i$th animal; $\beta_0$, the regression intercept; $\beta_1$, the regression coefficient on MBW; $\beta_2$, the regression coefficient on ADG; and $\epsilon_i$, the uncontrolled error of the $i$th animal.

For analysis of growth performance, FE, carcass traits, tissue and visceral organs, and gastrointestinal tract, the animals were divided into three groups based on the RFI values: high RFI (RFI $> 0.5$ SD above the mean), medium RFI (RFI $0.5$ SD above and below the mean), and low RFI (RFI $< 0.5$ SD below the mean).

For blood hormone and metabolite analysis, the 15 lambs with the highest RFI (called the Extreme-High-RFI group, or the EHHigh-RFI group for short) and the 15 lambs with the lowest RFI (called the Extreme-Low-RFI group, or the ELow-RFI group for short) were selected from the 137 lambs.

Statistical analysis. In the data analysis, lambs were used as the experimental units. Statistical analysis was performed using SPSS 16.0 for Windows (SPSS, Chicago, IL, USA). Differences in growth performance, FE, carcass traits, the weight of tissue and visceral organs, and the weight and length of the gastrointestinal tract between the high-, medium-, and low-RFI groups were analyzed using ANOVA and LSD post-hoc test differences. In blood parameters between the EHHigh-RFI and ELow-RFI groups were determined using a $t$-test. A $P$ value of $< 0.05$ was considered to indicate statistical significance. Data are presented as the mean ± SE values. Pearson correlation coefficient was calculated using the PROC CORR procedure.

References

1. Nkromah, J. D. et al. Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. *Journal of animal science* 84, 145–153 (2006).
2. Crews, D. H. Jr. Genetics of efficient feed utilization and national cattle evaluation: a review. *Genetics and molecular research: GMR* 4, 152–165 (2005).
3. Aggrey, S. E., Karnuah, A. B., Sebastian, B. & Anthony, N. B. Genetic properties of feed efficiency parameters in meat-type chickens. *Genetics, selection, evolution: GSE* 42, 25, https://doi.org/10.1186/1297-9686-42-25 (2010).
4. Aggrey, S. E. & Rekaya, R. Dissection of Koch's residual feed intake: implications for selection. *Poultry science* 92, 2600–2605, https://doi.org/10.3382/ps.2013-03302 (2013).
5. Do, D. N. et al. Genome-wide association and systems genetic analyses of residual feed intake, daily feed consumption, backfat and weight gain in pigs. *BMC genetics* 15, 27, https://doi.org/10.1186/1471-2156-15-27 (2014).
6. Koch, R. M., Swiger, L. A., Chambers, D. & Gregory, K. E. Efficiency of feed use in beef cattle. *Journal of animal science* 22, 486–494 (1963).
7. de Verdal, H. et al. Improving the efficiency of feed utilization in poultry by selection. 2. Genetic parameters of excretion traits and correlations with anatomy of the gastro-intestinal tract and digestive efficiency. *BMC genetics* 12, 71, https://doi.org/10.1186/1471-2156-12-71 (2011).
8. Eya, J. C., Ashame, M. F., Pomeroy, C. F., Manning, B. B. & Peterson, B. C. Genetic variation in feed consumption, growth, nutrient utilization efficiency and mitochondrial function within a farmed population of channel catfish (Ictalurus punctatus). *Comparative biochemistry and physiology. Part B, Biochemistry & molecular biology* 163, 211–220, https://doi.org/10.1016/j.cbpb.2012.05.019 (2012).
9. Saintilan, R. et al. Genetics of residual feed intake in growing pigs: Relationships with production traits, and nitrogen and phosphorus excretion traits. *Journal of animal science* 91, 2542–2554, https://doi.org/10.2527/jas.2012-5687 (2013).
10. de Oliveira, P. S. et al. Identification of genomic regions associated with feed efficiency in Nelore cattle. *BMC genetics* 15, 100, https://doi.org/10.1186/s12863-014-0100-0 (2014).
11. Luiting, P. & Uff, E. M. Residual feed consumption in laying hens. 1. Quantification of phenotypic variation and repeatabilities. *Poultry science* 70, 1655–1662 (1991).
12. Herd, R. M. & Arthur, P. F. Physiological basis for residual feed intake. *Journal of animal science* 87, E64–71, https://doi.org/10.2527/jas.2008-1345 (2009).
13. Arthur, P. F. et al. Genetic and phenotypic variance and covariance components for feed intake, feed efficiency, and other postweaning traits in Angus cattle. *Journal of animal science* 79, 2805–2811 (2001).
14. Basarab, J. A. et al. Residual feed intake and body composition in young growing cattle. *Canadian Journal of Animal Science* 83, 189–204 (2003).
15. Faure, J. et al. Consequences of divergent selection for residual feed intake in pigs on muscle energy metabolism and meat quality. *Meat science* 93, 37–45, https://doi.org/10.1016/j.meatsci.2012.07.006 (2013).
16. Cai, W., Casey, D. S. & Dekkers, J. C. Selection response and genetic parameters for residual feed intake in Yorkshire swine. *Journal of animal science* **86**, 287–298, https://doi.org/10.2527/jas.2007–0396 (2008).

17. Barea, R. et al. Energy utilization in pigs selected for high and low residual feed intake. *Journal of animal science* **88**, 2062–2072, https://doi.org/10.2527/jas.2009–2395 (2010).

18. Lancaster, P. A., Carstens, G. E., Ribeiro, F. R., Tedeschi, L. O. & Crews, D. H. Jr. Characterization of feed efficiency traits and relationships with feeding behavior and ultrasonic carcass traits in growing bulls. *Journal of animal science* **87**, 1528–1539, https://doi.org/10.2527/jas.2008–1352 (2009).

19. Nkrumah, J. D. et al. Different measures of energetic efficiency and their phenotypic relationships with growth, feed intake, and ultrasonic and carcass merit in hybrid cattle. *Journal of animal science* **82**, 2451–2459 (2004).

20. Snowder, G. D. & Van Vleck, L. D. Estimates of genetic parameters and selection strategies to improve the economic efficiency of postweaning growth in lambs. *Journal of animal science* **81**, 2794–2713 (2003).

21. Arthur, P. F., Archer, J. A. & Herd, R. M. Feed intake and efficiency in beef cattle: overview of recent Australian research and challenges for the future. *Animal Production Science* **44**, 361–369 (2004).

22. Richardson, E. C. et al. Body composition and implications for heat production and Angus steer progeny of parents selected for and against residual feed intake. *Animal Production Science* **41**, 1065–1072 (2001).

23. Schenkel, E. S., Miller, S. P. & Willon, J. W. Genetic parameters and breed differences for feed efficiency, growth, and body composition traits of young beef bulls. *Canadian Journal of Animal Science* **84**, 177–185 (2004).

24. Herd, R. M. & Bishop, S. C. Genetic variation in residual feed intake and its association with other production traits in British Hereford cattle. *Livestock Production Science* **63**, 111–119 (2000).

25. Drennan, M. J., McGee, M. & Keane, M. G. The value of muscular and skeletal scores in the live animal and carcass classification scores as indicators of carcass composition in cattle. *An international journal of animal bioscience* 2, 752–760, https://doi.org/10.1017/S1753731108001754 (2008).

26. Crews, D. H. Jr. et al. Genetic parameters for net feed efficiency of beef cattle measured during postweaning growing versus finishing periods. *Proceedings of the Western Section, American Society of Animal Science* **54**, 1–4 (2003).

27. Smith, R. M. et al. Effects of selection for decreased residual feed intake on composition and quality of fresh pork. *Journal of animal science* **89**, 192–200, https://doi.org/10.2527/jas.2010-2861 (2011).

28. Johnson, D. E., Johnson, K. A. & Baldwin, R. L. Changes in liver and gastrointestinal tract energy demands in response to physiological workload in ruminants. *The journal of nutrition* **120**, 649–655 (1990).

29. Seal, C. J. & Reynolds, C. K. Nutritional implications of gastrointestinal and liver metabolism in ruminants. *Nutrition research reviews* **6**, 185–208, https://doi.org/10.1079/NRR19930012 (1993).

30. Heaton, K., ZoBell, D. R. & Cornforth, D. Effects of delayed castration of British crossbred cattle on weight gain, carcass traits, and consumer acceptability. *Proceedings, WSASAS 55*, 130–133 (2005).

31. Arthur, P. F., Archer, J. A. & Herd, R. M. Feed intake and efficiency in beef cattle: overview of recent Australian research and challenges for the future. *Animal Production Science* **44**, 361–369 (2004).

32. Wood, B. J., Archer, J. A. & van der Werf, J. H. H. Response to selection in beef cattle using IGF-1 as a selection criterion for residual feed intake under different Australian breeding objectives 91, 1–2 (2004).

33. Nkrumah, J. D. et al. Genetic and phenotypic relationships of serum leptin concentration with performance, efficiency of gain, and carcass merit of feedlot cattle. *Journal of animal science* **85**, 2147–2155, https://doi.org/10.2527/jas.2006–764 (2007).

34. Walker, R. S., Martin, R. M., Gentry, G. T. & Gentry, L. R. Impact of cow size on dry matter intake, residual feed intake, metabolic response, and cow performance. *Journal of animal science* **93**, 672–684, https://doi.org/10.2527/jas.2014–7702 (2015).

35. Walker, R. S., Martin, R. M. & Buttry, B. Effects of residual feed intake and dam body weight on replacement heifer intake, efficiency, performance, and metabolic response. *Journal of animal science* **93**, 3602–3612, https://doi.org/10.2527/jas.2015–9040 (2015).

36. Brockman, R. P. & Laarveld, B. Hormonal regulation of metabolism in ruminants: a review. *Livestock Production Science* **14**, 313–334 (1986).

37. Knott, S. A., Cummins, L. J., Dunshea, F. R. & Leury, B. J. Rats with poor feed efficiency are highly responsive to an exogenous adrenocorticotropic hormone (ACTH) challenge. *Domestic animal endocrinology* **34**, 261–268, https://doi.org/10.1016/j.dame.2007.07.002 (2008).

38. Luiting, P., Decuypere, E., G. P. N. d., Buyse, J. & Room, G. In 45th Annual meeting EAAP 104 (Edinburgh, 1994).

39. Stick, D. A., Davis, M. E., Loerch, S. C. & Simmen, R. C. Relationship between blood serum insulin-like growth factor I concentration and postweaning feed efficiency of crossbred cattle at three levels of dietary intake. *Journal of animal science* **76**, 498–505 (1998).

40. Kelly, A. K. et al. Expression of key genes of the somatotropic axis in longissimus dorsi muscle of beef heifers phenotypically divergent for residual feed intake. *Journal of animal science* **91**, 159–167 (2013).

41. Kelly, A. K. et al. Effect of divergence in residual feed intake on feeding behavior, blood metabolic variables, and body composition traits in growing beef heifers. *Journal of animal science* **88**, 109–123, https://doi.org/10.2527/jas.2009–2196 (2010).

42. Richardson, E. C., Herd, R. M., Colditz, L. G., Archer, J. A. & Arthur, P. F. Blood cell profiles of steer progeny from parents selected for and against residual feed intake. *Australian Journal of Experimental Agriculture* **42**, 901–908 (2002).

43. Welch, C. M. et al. An examination of the association of serum IGF-I concentration, potential candidate genes, and fiber type composition with variation in residual feed intake in progeny of Red Angus sires divergent for maintenance energy EDP. *Journal of animal science* **91**, 5626–5636, https://doi.org/10.2527/jas.2013–6609 (2013).

44. Houseknecht, K. L., Baile, C. A., Matteri, R. L. & Spurlock, M. E. The biology of leptin: a review. *Journal of animal science* **76**, 1405–1420 (1998).

45. Garcia, M. R. et al. Serum leptin and its adipose gene expression during peripubertal development, the estrous cycle, and different seasons in cattle. *Journal of animal science* **80**, 2158–2167 (2002).

46. Lord, G. M. et al. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* **394**, 897–901, https://doi.org/10.1038/29795 (1998).

47. Chilliard, Y., Anne, F., Carole, D. & Boququier, F. Plasma leptin in underfed or overfed adult Holstein and Charolais cows, and its relationship with adipose tissue cellularity. *International Journal of Obesity* **22**, S717 (1998).

48. Nkrumah, J. D. et al. Different measures of energetic efficiency and their phenotypic relationships with growth, feed intake, and ultrasonic and carcass merit in hybrid cattle. *Journal of animal science* **82**, 2451–2459 (2004).

49. Snowder, G. D. & Van Vleck, L. D. Estimates of genetic parameters and selection strategies to improve the economic efficiency of postweaning growth in lambs. *Journal of animal science* **81**, 2794–2713 (2003).

50. Arthur, P. F., Archer, J. A. & Herd, R. M. Feed intake and efficiency in beef cattle: overview of recent Australian research and challenges for the future. *Animal Production Science* **44**, 361–369 (2004).

51. Xiong, B. H., Luo, X. R., Zhao, F. & Pang, Z. H. The effect of lamb eye muscle depth and width on loin eye area, shape and meat yield. *Asian Australasian Journal of Animal Sciences* **13**, 225–226 (2000).
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Author Contributions
F.D.L., X.X.Z. and W.M.W. designed the study. X.X.Z., W.M.W., F.T.M., Y.F.L. and C.L. collected the tissue and blood samples. X.X.Z., W.M.W., F.T.M. and Y.F.L. contributed to growth performance and feed efficiency. X.X.Z., W.M.W. and C.L. contributed to carcass traits, organ weight and intestine length. F.T.M. and Y.F.L. contributed to blood hormones and metabolites. F.D.L., X.X.Z., W.M.W. and F.T.M. analyzed the data. X.X.Z and W.M.W. wrote the manuscript. All authors have read and approved the final manuscript.

Additional Information
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