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Study of the Level of Gene Expression of the GLUT2 Glucose Vector in the Small Intestine of the Meat Broiler and its Relationship to Productive Traits

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Abstract

The study was conducted in the field of animal production of the Agricultural Research Station / College of Agriculture and the Marshes / Dhi Qar University for the period from 10/11/2021 to 25/ 2/ 2022. Laboratory tests of the samples were carried out in the Molecular Genetics Laboratory of the College of Dentistry / Dhi Qar University, for the purpose of knowing the gene expression of the SLC2A2 gene encoding the glucose transporter GLUT2 and the abundance of its mRNA transcripts in different regions of the small intestine of broilers when fed at different levels of energy and to study the relationship of that abundance to some productive traits.

In this study, 135 non-naturalized of meat broiler (breed 308 Ross) were used. The broiler were randomly distributed to three experimental treatments, with three replicates for each group (15 chicks/repeat), used the adlibitum free feeding system for the duration of the experiment, which lasted (35) days. The study included three levels of energy, where the first treatment was a control diet, which represented 3065 and 3133 kilocalories/kg for the primer and final diet, respectively, as for the second treatment, it contained a high level of energy 3200 kilocalories/kg and 3297 kilocalories/kg for the primer and final diets, respectively, the third treatment contained a low level of energy 2769 kilocalories/kg and 2802 kilocalories/kg for primer and growth diets, respectively. The results of the study showed the following:-

- The possibility of amplifying the studied gene and knowing its abundance and according to the primers used.
- The highest level of mRNA transcription of the GLUT2 gene in the small intestine of males was in the jejunum region (9.41) at a representative energy level of 3297 kilocalories/kg, as for the duodenum, the expression of GLUT2 gene was lower than in the jejunum (0.535), the GLUT2 gene expression in males was slightly more than in females in the ileum region (0.578) at a representative energy level of 3297 kcal/kg.
- Gene expression was positively and significantly associated with live weight, weight gain and feed consumption rates, but it was inversely related to the efficiency of food conversion in the jejunum and duodenal regions.
- The abundance of the glucose transporter GLUT2 gene increased with the increase in the energy level in the diet.

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- Males gave a higher percentage of mRNA copies in all target organs compared to females.
- The activity of the gene responsible for the transfer of glucose can be modified through feeding and increasing energy levels and thus its reflection on the performance of birds.
- As for the productive traits, the differences were significant in the average live body weight and weight gain, as well as feed consumption compared with the control treatment, while the feed conversion efficiency did not show any significant differences.

I. INTRODUCTION

Advances in molecular genetics in the past decades have led to the identification of multiple genes that influence traits of importance in livestock, here, it provided an opportunity to enhance the response to selection, including traits that are difficult to improve by conventional selection, such as low heritability, in which phenotypic measurement is difficult or costly, or may only be possible later in life (Dekkers, 2004). The high growth rate and feed efficiency are the main factors in poultry production, but there are many safe factors also taken into consideration to achieve the optimal productive performance of poultry, which is represented by environmental conditions, diet, genetic potential of birds and diseases, regardless of the previous factors, recently the health of the intestines is the subject of an intensive study for poultry production (Rinttilä & Apajalahti, 2013).

Glucose is a major fuel for animal metabolism and glucose uptake by the plasma membrane stimulated by a family of facilitative transport proteins known as glucose transporter proteins (GLUT) encoded by the SLC2A2 gene represents the first step in determining the rate of glucose metabolism, GLUT family is characterized by the presence of 12 hypothetical transporters extending into the membrane, compared to mammals, the concentration of glucose is high in the blood of chickens even in the fasting state and is resistant to the injection of significantly high concentrations of insulin (Wood & Trayhurn, 2003).

GLUT2 was cloned 20 years ago and is a glucose-facilitating transporter in the pancreas, liver, kidney and intestine, it forms large, bidirectional flows of glucose into and out of the cell, because of its high capacity and low affinity, it also transports other dietary sugars such as galactose and fructose within the range of physiological concentrations of hormones and sugars to regulate their gene expression, in the past decade, three main features of the protein GLUT2 were revealed, the existing information for the genotypes of chicken breeds raised in Iraq (AL-soudl, 1971).

The traditional selection methods did not affect the further improvement of the quality and quality of meat if it was not evaluated at the genetic level (due to the difficulty of obtaining the parents), as well as the high cost of breeding and the length of this period when using the method of progeny testing, therefore, the aim of our study is to reveal the genes responsible for the transport of nutrients (glucose) and to know the role that these genes play through their gene expression in controlling the highest performance of the systems of transporting these nutrients in the membranes of absorption cells in the small intestine, which affects the availability of nutrients and energy for growth and development and to improve the economic characteristics of broilers, which is a good indicator for the election.

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II. MATERIALS AND METHODS

The study was conducted in the field of the College of Agriculture and the Marshes / University of Dhi Qar for the period from (10/11/2021) to (25/2/2022). The study period included two phases:

Field work stage:

Commercial broilers (Ross308), which included 135 unsexed birds from one day old to 35 days old, were bred in the ground system with three treatments, and each treatment was three replications of 15 birds for each replicate and spread with sawdust at a height of (5 cm) in the poultry field.

Nutrition

The fodder was prepared from Ghadeer Babil factory and the broilers were fed free feed from one day old to five weeks old, where the chicks were fed on two rations, the primer ration for a period of 1-21 days of age and the growth ration for a period of 22-35 days of age, the chemical analysis of the diets was done according to the general needs of the birds, as shown in Table No. (1) and Table No. (2), respectively.

Chemical Analysis

The quantity of the feed was calculated according to the nutritional needs based on the tables of chemical analysis of the feed materials according to the tables of (NRC 1994) and according to Table (1 and 2) Table (1) shows the proportions of the feed materials included in the composition of the primer diet for broilers.

Feed material	T1 control	T2 High Energy	T3 Low Energy
yellow corn	58	63	50
fodder wheat	5	4.75	0
wheat bran	0	0	18
Soybean meal 44% protein*	35	23	23.75
protein concentrate *	0	6.25	0

* The protein concentrate of Jordanian origin: it contains 48% protein (kilo calories / kg) represented energy, 6% fat, 8% calcium, 3% phosphorous, 0.75% methionine, 0.66% cysteine, 3% lysine.

* The protein concentrate of Jordanian origin: it contains 48% protein (kilo calories / kg) represented energy, 6% fat, 8% calcium, 3% phosphorous, 0.75% methionine, 0.66% cysteine, 3% lysine.

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Vegetable oil	1	2	7
Calcium carbonate	0.5	0.5	0.5
salt	0.25	0.25	0.25
Mixture of vitamins and minerals	0.25	0.25	0.5
Total	100	100	100

Calculated Chemical Analysis**

Represented energy (kilocalories/kg)	3065	3200	2769
Crude protein %	21.27	21.18	21.11
Energy ratio: protein	144.09	151.08	131.17
Raw fiber %	3.89	3.18	4.88
Calcium %	1.29	2.25	1. 23
Available phosphorous %	0.55	0.66	0.85
Lysine %	1.11	1.23	1.02
methionine and cysteine %	0.68	0.63	0.59

Table (2) shows the proportions of the feed materials included in the composition of the final diet for broilers

Feed material	**T1 Control**	**T2 High Energy**	**T3 Low Energy**
yellow corn	60	64	52
fodder wheat	5.5	7	0
wheat bran	0	0	16.75

** Chemical analysis of feed materials according to the (NRC.1994) tables.

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Soybean meal 44% protein*	20	20	23
protein concentrate *	12	6	7
Vegetable oil	1.5	2	0
Calcium carbonate	0.5	0.5	0.5
salt	0.25	0.25	0.25
Mixture of vitamins and minerals	0.25	0.25	0.5
Total	**100**	**100**	**100**

Calculated Chemical Analysis**

Represented energy (kilocalories/kg)	3133	3297	2802
Crude protein %	20.67	20.21	20.75
Energy ratio: protein	151.57	163.38	135.03
Raw fiber %	2.9435	3.06	4.7365
Calcium %	1.21	1.36	1.62
Available phosphorous %	0.44	0.63	0.80
Lysine %	0.71	0.88	1.00
methionine and cysteine %	0.50	0.59	0.67

The weight of the consumed feed was calculated at the end of each week, and through the difference between the weight of the provided feed and the weight of the remaining feed at the end of each week, we can calculate the amount of feed consumption according to the equation:

* The protein concentrate of Jordanian origin: it contains 48% protein (kilo calories / kg) represented energy, 6% fat, 8% calcium, 3% phosphorous, 0.75% methionine, 0.66% cysteine, 3% lysine.
* The protein concentrate of Jordanian origin: it contains 48% protein (kilo calories / kg) represented energy, 6% fat, 8% calcium, 3% phosphorous, 0.75% methionine, 0.66% cysteine, 3% lysine.
** Chemical analysis of feed materials according to the (NRC.1994) tables.

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The amount of feed consumed per week (gm/bird) = the amount of feed provided at the beginning of the week - the amount of feed remaining at the end of the week The food conversion factor was calculated weekly according to the following equation: (Al-Zubaidi, 1986)

Slaughter Process

The manual slaughter of the birds was carried out at the end of breeding at the age of five weeks, and after the blood was drained, the feathers were removed after the process of scalding at a temperature of (54°) and the head and legs were removed, the process of dissecting the bird from the esophagus to the end of the outlet and lifting the internal entrails according to the method (Al-Fayadh et al., 2011). Isolation of the edible internal organs (gizzard, liver and heart) from the non-edible viscera and weighing each of them using a sensitive balance and it was calculated as a percentage of the living body weight, the length of the intestine was measured using a measuring tape (100 cm) (Al-Fayadh et al., 2011). Samples were taken from the intestine area (duodenum, jejunum and ileum) and placed in liquid nitrogen (LN2) −196 °, the carcass was cut immediately after slaughter into the main segments, which are the thighs (Thighs), the chest (Breast) and the ankle-femoral joint (Drumstick), then weighed with a sensitive electronic scale, and the secondary segments, which are the back, neck and wings.

Studied traits:

Live body weight (gm/bird): Broilers were weighed using an electronic scale for each bird separately in the first week of life, and then weighed at the end of each week, average weekly weights were taken for each bird during the 35-day experiment period, according to the equation:

Weight gain rate (gm/bird) = live body weight at the end of the week - live body weight at the beginning of the week (gm/bird)

Extracting the values of weekly and total weight gain: The weight gain gained each week of the experiment was found for each bird through the following equation:

Weekly weight gain (gm / bird) = live body weight at the end of each week (gm / bird) - live body weight at the beginning of the week (gm / bird). (Al-Fayadh et al.,2011)

Finding the feed conversion efficiency, which is the result of dividing the amount of feed consumed by the live weight of the bird according to the equation:

$$\text{Food conversion efficiency} = \frac{\text{The amount of feed consumed during a specified period of time}}{\text{Average weight gain during the same period}}$$

(Al-Zubaidi, 1986)

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Laboratory work stage:

Laboratory tests of the samples were carried out in the Molecular Genetics Laboratory of the College of Dentistry / University of Dhi Qar, for the purpose of knowing the gene expression of the SLC2A2 gene, determining the abundance of mRNA in the duodenum, jejunum and ileum, and studying the relationship of mRNA abundance to some productive and qualitative traits.

Tissue collection process:

Samples were collected when the birds were slaughtered, cleaned and prepared, four samples were collected from each bird for the regions (duodenum, jejunum and ileum) and each sample was placed in an Eppendorf tube and the samples were kept in liquid nitrogen at a temperature of (-196) until the time of RNA extraction.

RNA Extraction Process

The workplace in the laboratory was well sterilized using ethanol alcohol at a concentration (70%) and the extraction steps were carried out according to the instructions recommended in Kit (Geneaid) as follows:

1. A sample of (40) gm of tissue was taken and transferred to a new Eppendorf, taking care of the sequence of numbers according to the type of tissue.
2. Adding (700) micro liters of genezol reaction (reagent), then mashing the samples, then shaking them using a vibrator.
3. Using a centrifuge, the duration is 1 minute by 15,000 revolutions / minute, and we take the filter located at the top in a new Eppendorf.
4. Add ethanol at a concentration of (70%) in equal amounts, then shake it with a vibrator.
5. Using the filters (colom) and placing them in a new collection tube, using half of the sample as the first stage, using the centrifuge for two minutes at 15,000 revolutions/min, then adding the remaining half to secure the sample after emptying the previous collection tube from the filtrate and using the centrifuge for two minutes at 15,000 revolutions/min.
6. Using the W1 substance, taking into account the replacement of the collection tube, adding it to an amount of (400) microliters, and spinning it for a minute in a centrifuge at 15,000 revolutions/min.
7. Add a wash with an amount of (600) micro liters after emptying the previous collection tube from the filtrate, then spinning for one minute at 15,000 rpm, then emptying the filter from the filtrate, then re-spinning for three minutes in a centrifuge at 15,000 rpm for drying.
8. Using a free-DNase substance with changing the collection tube, which works to lower the suspended DNA in the filter, using the substance in an amount of (25) microliters, and leaving it for three minutes to saturate the substance with the filter, then spinning for two minutes at 15,000 cycles / minute, after which the RNA comes down.

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RNA reverse transcription (conversion of RNA to cDNA):

Before starting the phase of the replication chain reaction, the genetic expressions were measured, the RNA was converted to cDNA, as the reverse transcription steps were carried out according to the instructions recommended in TRANS and as follows:

1- Dissolve the chip to room temperature.
2- Preparing the PCR tube with a volume of (0.5) micro liters.
3- Add (15) microliters of the extracted RNA.
4- Adding (1) microliter of Olica primer and adding the rest of the mixture with the use of a centrifugal device (15) seconds.
5- The samples are placed in the PCR machine at a temperature of (42°) for a period of (15) minutes, then at (25°) for a period of (15) minutes, then the temperature is raised to (85°) for five seconds in order to undo the work of the enzymes.

RNA Electrophoresis Method for preparing the gel:

Acarose gel is prepared at a concentration (1X) by adding (0.5g) of agarose powder to (50ml) of SB solution, then heat the mixture using a (Micro Wave) microwave device for about a minute and a half with continuous stirring from one moment to another until the mixture turns from a gelatinous consistency to a clear solution and then left to cool to a temperature (50-60°C), then we add the Red save stain to the prepared agarose solution and then pour the gel into the tray attached to the electric relay device continuously and quietly while fixing the comp for the formation of the holes, taking care to avoid the occurrence of bubbles, then leave a period of (5-10) minutes for the gel to solidify, then withdraw the comb quietly and place the frozen gel in the basin of the electrophoresis device, which contains the solution (SB 1X) as this solution covers the surface of the gel.

(3 µ L of Dye Loading Baffer) is mixed with (5 µl) of each sample of extracted RNA to be migrated using an appropriate sized micropipette over a piece of parafilm paper and fixed with tape on the table from both ends and withdraw from this mixture (5 µl) and put it in the pits of the gel, taking into account that the samples do not come out to the surface of the pits, then we determine the voltage of the electrophoresis device into two phases as shown in Table No. (3)

Table (3) shows voltage stages, electric relay current and time

First stage		Second stage	
Voltage	45V	Voltage	90V
Current	60 V	Current	120A
Time	15 min	Time	45min

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After completing the electrophoresis process, we display the gel in a UV Transilluminator equipped with an imaging system to determine the quality of the packages and to confirm whether they contain RNA in order to determine the possibility of continuing to work or not.

Define the Primers

As a result of molecular detection and knowledge of the gene expression of the SLC2A2 gene, the primers were selected as in the table:

Table (4) the sequence of primers that were used in the study

GLUT2 R	CACACTATGGGCGCATGCT
GLUT2 F	ATTGTGCCTGGAGGTGTTGGT
β-actin R	GTCCACCGCAAATGCTTCTAA
β-actin F	TGCGCATTTATGGGTTTTGTT

Real time PCR amplification steps

- **Materials required for the reaction:**

1- cDNA output safe extraction
2- Primer
3- Master mix syber green contains nitrogenous bases (T.A.C.G) + Polymerase enzyme.
4- Syber green + Mg + K . dye
5- D.D.W . water
6- RNA Extraction Kit / Promega / USA.

- **Mitigation primer**

1- Add (300) micro liters of D.D.W to become (100 Picomol) and prepare a stoke for work.
2- Add (90) of D.D.W to the new tube, then add (10) micro liters of stoke to become the quantity (10 Pimol).

- **PCR reaction**

Table (5) Volume of materials used in the RT-PCR reaction

Reaction volume	Ingredients
3 µl	cDNA template
1.5 µl	Forward primer

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1.5 µl	Reverse primer
12.5 µl	PCR SYBER
1.5 µl	Nuclease free water
20 µl	Final Volume

PCR program RT-

Table (6) The program used to run the RT-PCR technique

S.	steps	Temperature	Number Courses	Time
1	Initial mutant	95 °C	1	3 min
2	Mutant	95 °C	40	20 sec
3	Docking	60 °C		30 sec
4	Elongation	72 °C		15 sec

Statistical analysis

The experiment was designed according to a fully randomized design (CRD) to analyze the studied data in a one-way analysis using the statistical analysis program (Genstat) at a probability level of (0.01) to test the significant differences between the studied averages and based on the equation of the mathematical model:

$$Yij = \mu + Ti + eij$$

Whereas:

Yij = the value of the viewer j of the transaction i.

µ = the general average of the trait studied.

Ti = the impact of the transaction.

eij = the impact of the experimental random error of the transaction.

Duncan's polynomial test was used to determine the significance of differences between the averages of the transactions (Al-Rawi and Khalaf Allah, 1980).

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III. RESULTS AND DISCUSSION

Gel electrophoresis results: After total RNA was extracted from tissues of the intestine (jejunum, duodenum and ileum) and liver, the quality of the RNA collected was tested by electrophoresis on a 2% agarose gel, or the results were identified in a column with two strands corresponding to 28s and 18s on the gel, the extracted RNA was loaded onto the agarose gel as in the picture:

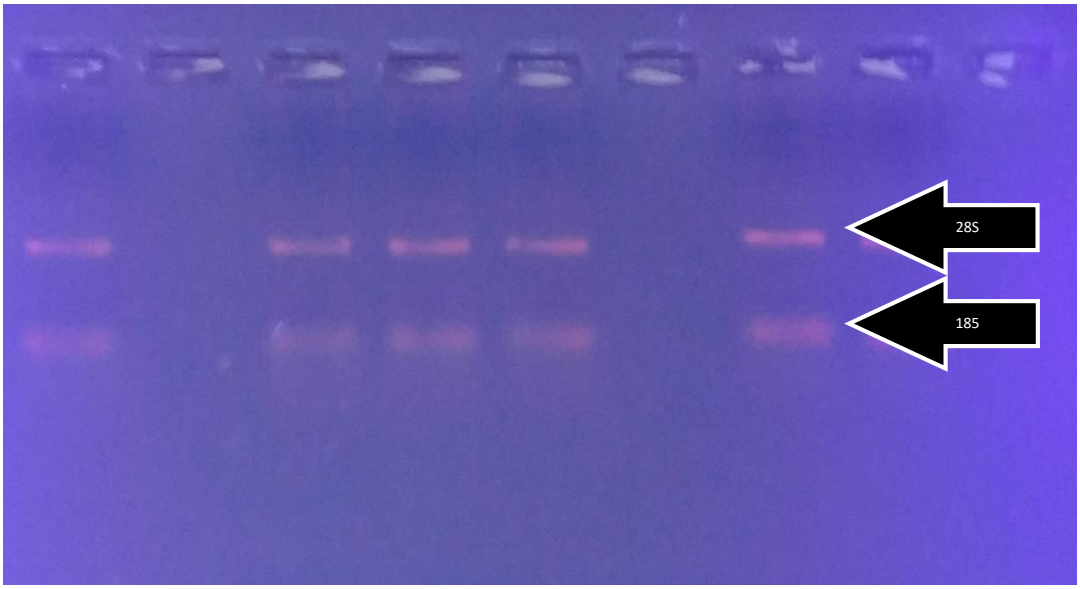

Diagram showing the electrical transfer of genetic material

Image 28s and 18s rRNA on a karose gel

The amount of gene expression

GLUT2 gene expression in females and males in the jejunum

As for the impact of gender on the performance and activity of the gene action, the study showed that males were the highest level in terms of gene expression and for all regions studied, the study showed that the amount of GLUT2 gene expression in females is less than in males, where the results were significant at the level (0.05), as shown in Table (7) and (8), this is in agreement with the study conducted by (Benyi et al., 2015) where commercial broiler chickens were selected for their rapid growth rate, as males grow faster than females.

The highest level of expression of the SLC2A2 gene, as shown in Table (7) at 35 days of age, was in the jejunum region in relation to the small intestine of birds fed on the feed in which the representative energy ratio is high, as the birds recorded the

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highest values of weight and weight gain and this increase it may be due to the high levels of energy represented, which made the regulation of gene performance well and with high activity

Table No. (7) results of gene expression in the jejunum with average live body weight and rate of increase in weight

Weight gain (gm)	Weight (gm)	Jejunum	Feed type
433	1747	0.244	Control
460	2080	0.941	High energy
138	932.3	0.2	Low energy
343.7	1587	0.462	average

Table No. (8) Results of gene expression in the jejunum with the average amount of feed consumption and the rate of feed conversion efficiency

Feed Conversion Efficiency Rate	Feed consumption rate (gm)	Jejunum	Feed type
1.6	1145	0.244	Control
1.49	1156	0.941	High energy
1.81	635.3	0.2	Low energy
1.63	978.6	0.462	average

GLUT2 Gene Expression in the Duodenum:

The study showed, as shown in Tables (9) and (10), that the amount of gene expression of GLUT2 gene in females in the duodenum is less than it is in males, meaning that the amount of gene expression is two times higher in males than females, where the results were not significant at the level of (0.05). Nutrient utilization can be the basis for the increase in body weight and rate of weight gain in males compared to females. This is in agreement with (Abdulkareem et al. (2016))who proved that broiler chickens are usually raised with high-calorie foods for the purpose of meat production because it has been proven to improve body weight, growth rate and feeding efficiency.

Table No. (9) Results of gene expression in the duodenum with average live body weight and rate of weight gain

Weight gain (gm)	Weight (gm)	Duodenum	Feed type
433	1747	0.207	Control

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460	2080	0.535	High energy
138	932.3	0.142	Low energy
343.7	1587	0.295	average

Table No. (10) results of gene expression in the duodenum with the average amount of feed consumption and the rate of feed conversion efficiency

Feed Conversion Efficiency Rate	Feed consumption rate (gm)	Duodenum	Feed type
1.6	1145	0.207	Control
1.49	1156	0.535	High energy
1.81	635.3	0.142	Low energy
1.63	978.6	0.295	average

The amount of gene expression in the ileum of GLUT2 gene: The study showed that the amount of gene expression of GLUT2 gene in males is slightly more than in females, where the results were significant at the level (0.05), as shown in Table (11) and (12), the results of the current study agree with the study conducted by Kaminski and Wong (2018) on broiler chickens, which showed that there are no differences in gene expression at advanced ages between males and females.

Table No. (11) Results of gene expression in the ileum with average live body weight and rate of weight gain

Weight gain (gm)	Weight (gm)	Ileum	Feed type
433	1747	0.119	Control
460	2080	0.578	High energy
138	932.3	0.049	Low energy
343.7	1587	0.249	Average

Table No. (12) results of gene expression in the ileum with the average amount of feed consumption and the rate of feed conversion efficiency

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Feed Conversion Efficiency Rate	Feed consumption rate (gm)	Ileum	Feed type
1.6	1145	0.119	Control
1.49	1156	0.578	High energy
1.81	635.3	0.049	Low energy
1.63	978.6	0.249	average

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