



Reproductive and metabolic response and body changes in ewes fed with two energy levels

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ABSTRACT

Objective. To evaluate the reproductive response, metabolic state and body changes in Dorper and Katahdin ewes supplemented with 2 energy levels prior to insemination. Materials and methods. The animals used in this experiment were 14 Katahdin and 13 Dorper ewes distributed in two treatments, in which estrus had been synchronized. Fourteen days prior to synchronization of estrus, the ewes were distributed in two treatments according to the energy level of the diet, T1:2.0 and T2:2.5 Mcal kg⁻¹ of ME. Feeding consisted of 14 days of adaptation and 14 of feeding with integral diet. At the start and finish of each study, measurements were made of backfat thickness, area of the ribeye, body weight, and glucose and insulin concentrations. The principal reproductive parameters were measured, such as response to estrus, percentage of gestation and prolificity. Analyses of variance were made along with ji-squared tests to determine the effect of the energy level and genotype. **Results.** The genotype and energy level did not affect any of the variables measured, although it was found that with both energy levels there was an increase in glucose concentrations, as well as an increase in weight, body fat and area of the rib-eye. **Conclusions.** Because there was no difference in the reproductive parameters and body changes with feeding in the 2 energy levels, it is preferable to use the feed with 2.0 Mcal kg⁻¹ of ME, given that it reduces costs.

Keywords: Body condition; estrus; fertility; flushing; fat (Source: AIMS, MeSH).

RESUMEN

Objetivo. Evaluar la respuesta reproductiva, estado metabólico y cambios corporales en ovejas Dorper y Katahdin suplementadas con dos niveles de energía previo al empadre. Materiales y métodos. Se utilizaron 14 hembras Katahdin y 13 Dorper distribuidas en 2 tratamientos a las cuales se les sincronizó el celo. Catorce días previo a la sincronización de estros, las ovejas se distribuyeron en 2 tratamientos de acuerdo con el nivel de energía en la dieta, T1:2.0 y T2:2.5 Mcal kg⁻¹ de EM. La alimentación abarcó 14 días de adaptación y 14 de alimentación con dieta integral. Al inicio y

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al final del estudio se midió el espesor de grasa dorsal, área del ojo de la costilla, peso corporal y concentraciones de glucosa e insulina. Se midieron los principales parámetros reproductivos como respuesta a estro, porcentaje de gestación y prolificidad. Se realizaron análisis de varianza y pruebas de ji cuadrada para determinar el efecto del nivel de energía y el genotipo. **Resultados.** El genotipo y el nivel de energía no afectaron ninguna de las variables medidas, aunque se encontró que con ambos niveles de energía existió un incremento en las concentraciones de glucosa, así como en ganancia de peso, grasa corporal y área del ojo de la costilla. **Conclusiones.** Debido a que no existió diferencia en los parámetros reproductivos y cambios corporales en ovejas al alimentarlas con los dos niveles de energía, es mejor la alimentación con 2.0 Mcal kg⁻¹ de EM, ya que reduce costos de alimentación.

Palabras clave: Condición corporal; estro; fertilidad; flushing; grasa (Fuente: AIMS, MeSH).

INTRODUCTION

An adequate diet is determinant for improving the reproductive response of ewes. During lactation, there is weight loss (1), especially in ewes which are fed with low levels of metabolizable energy (2). A correct feed supplementation (3) improves body condition of the ewes and consequently the reproductive response. However, in most flocks this practice is not carried out due to the lack of knowledge of its benefits or to the lack of economic resources of the producers; therefore, the ewes reach weaning with low body condition. It is desirable for ewes to be inseminated shortly after weaning the lambs, however, this is sometimes difficult due to the low body condition of the ewe. It has been reported that the ewes with medium condition have a higher rate of conception than those of low condition (4). One alternative is supplementation prior to insemination, given that this has been shown to improve reproductive response (5, 6); to this respect it has been demonstrated that supplying high energy diets for a short or long period increases the ovulatory rate of ewes (7). The improvement in the reproductive parameters is accompanied by changes in glucose and insulin concentration in blood plasma (8).

To carry out flushing for short periods with producers who do not supplement with concentrates in their production system is difficult, due to the fact that these diets are based on the use of grains to increase the energetic contribution, requiring periods of adaptation to the diet to avoid digestive problems; therefore, flushing with an adaptation period is viable in these cases. It is important to note that in addition to incrementing the reproductive response, the ewes recover part of their body condition, therefore, supplementation for a period prior to insemination could help (6). It has been demonstrated that ewes with a body condition lower than 2 on a scale of 1 to 5 have a lower ovulatory rate than those with a condition higher than 3 (9). Although there are recommendations of the requirements of ewes prior to insemination, above all based on weight (10), there could be differences due to the genotype, climate of the location and body fat at the moment of initiating supplementation, thus the body condition and backfat thickness at the start of synchronization of estrus may modify the reproductive response. It is also necessary to consider that on occasions or in some locations there is lack of availability of grains or some other energy source. This generates the need to test diets with different energy levels without affecting the results of insemination.

In some states of Mexico, the introduction of Dorper and Katahdin studs has been popularized, thus achieving ewes of these races. Therefore, the objective of the present study was to evaluate the reproductive response, metabolic state and body changes in Dorper and Katahdin ewes supplemented with two energy levels prior to insemination.

MATERIALS AND METHODS

Animal handling. The animals were managed according to the official Mexican norm NOM-062-ZOO-1999, of technical specifications for the production, care and use of laboratory animals.

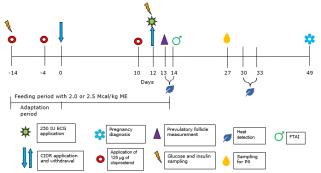
Study site. The study was carried out in the locality of San Pedro Añañe, pertaining to the municipality of San Bartolo Soyaltepec, Oaxaca, Mexico, located at 17° 31′ 15.5″ latitude north, 97° 22′ 6″ longitude west at an altitude of 2189 m. The climate is sub-humid with rains in summer.

Population of the study and experimental design. The population consisted of 13 Dorper and 14 Katahdin ewes of 1 to 3 births, with mean and standard deviation of weight of 36.44±5.52 kg, which had been weaned between 2 and 3 weeks prior to the start of the study. After weaning, the ewes were fed with bales of corn straw and Rhodes grass (Chloris gayana). The body condition was 3.08±0.09 on a scale of 1 to 5. The ewes were housed individually and were distributed in two treatments according to genotype and body condition, so that both treatments had an equal distribution of ewes with different body condition. T1: ewes fed with an integral diet with 2.0 Mcal kg⁻¹ of ME (6 Dorper and 6 Katahdin ewes) and T2: ewes supplemented with an integral diet with 2.5 Mcal kg⁻¹ of ME (7 Dorper and 8 Katahdin ewes). The percentage of crude protein was 13% for both diets (Table 1). The bromatological composition of the ingredients was made prior to the formulation of the diets. This period of adaptation to the diet began with the pre-synchronization of estrus. This period consisted of providing 200 g of feed in the first week and 400 g in the second week, the ground maize straw (3.06% PC, 0.97 Mcal of ME kg⁻¹ and 32.95% of Total Digestible Nutrients) at free access. From the start synchronization of estrus until artificial insemination, 1.5 kg of integral diet per ewe was provided, administering 700 g at 8:00 h and 800 q at 16:00 h, thus the supplementation period consisted of 28 days (14 days of adaptation and 14 days of feeding with integral diet) (Figure 1).

Table 1. Ingredients and chemical composition of
diets with 2 energy levels provided to the
ewes prior to insemination.

Ingredient	T1	Т2	
Maize grain (%)	27.4	50.5	
Soybean paste (%)	12.8	10	
Bean straw (%)	25	14	
Maize straw (%)	24	14.6	
Soybean oil (%)	0	3	
Urea (%)	0.8	0.9	
Mineral salt (%)	2	2	
Common salt (NaCl) (%)	1	1	
Molasses (%)	7	4	
Chemical composition			
Crude protein (%)	13.0	13.0	
Mcal kg ⁻¹ of ME	2.0	2.5	
Crude fiber (%)	16.98	10.68	
Cost per kg of feed (\$, mexican pesos)	4.11	4.52	

T1: ewes supplemented with 2.0 Mcal kg⁻¹ of ME and T2: ewes supplemented with 2.5 Mcal kg⁻¹ of ME.



*FTAI: fixed time artificial insemination

Figure 1. Protocol of synchronization of estrus and taking blood samples for measurement of glucose, insulin and progesterone in ewes fed with 2.0 and 2.5 Mcal kg⁻¹ of ME prior to insemination.

Measurement of body state. Body weight, backfat thickness and area of the rib-eye were measured at the start of the period of adaptation to the diet and at the end of feeding (Figure 1). For this purpose, a Pie Medica[®] model Aquila ultrasound was used, with transductor of 3.5 MHz and acoustic coupler.

Pre-synchronization and reproductive management. Prior to synchronization of estrus, a double dose of 125 µg of cloprostenol was applied with 8 days interval, so that all of the ewes would be at the same phase of the estrous cycle at the start of the experiment. Six days after the second application, an intervaginal insert impregnated with 0.3 g of progesterone was placed, and left for 12 days; 2 days prior to its withdrawal, a 125 µg intramuscular injection of cloprostenol was applied. When the CIDR was withdrawn, 250 UI of eCG was applied, initiating the detection of estrus 24 hours later, with an interval of 6 hours until the day of artificial insemination.

At 48 hours after the withdrawal of the insert with progesterone, measurements were made of the number and size of the preovulatory follicles located in both ovaries. For this purpose, an EMP 2000vet[™] ultrasound was used with a linear transductor of 5.0 MHZ. Artificial insemination was performed by laparoscope at a fixed time, 52 hours after the withdrawal of the insert with progesterone.

The return to estrus occurred between days 16 and 19 post insemination, and the diagnostic of gestation was made at 35 days (Figure 1). The number of ewes that gave birth and lambs born were registered.

Determination of glucose, insulin and **progesterone.** To measure the concentration of glucose and insulin, a blood sample was taken from the jugular vein in a vacutainer tube BD[®], at the start of pre-synchronization, which was also the start of the period of adaptation to the diet. Another sample was taken when the insert with progesterone was withdrawn. The samples were then centrifuged in a VELAB model VE-4000[®] centrifuge at 1600 *g* for 10 minutes. The serum obtained was separated to be deep frozen at -70°C in a Thermo Scientific model 803CA until its analysis. Thirteen days after artificial insemination, another blood sample was taken to determine progesterone (P4). This sample was treated similarly to that obtained to measure glucose and insulin.

For the determination of glucose, the reagent "Glucose-SL. assay" (DCL) was used, employing an automated biochemical analyzer ES-218 (Kontrol LAB). The quantification was made using the final point and kinetic methods, making the reading at a wave length of 340 nm. The insulin concentration was measured by means of Elisa with the kit "insulin-ELISA" by Calbiotech. The analyses of P4 were made by means of an immune-enzymatic test (Immunometrics, UK Ltd., 280 Muster Road, London SW6 6BQ). The analytic sensitivity was 0.13 ng mL⁻¹ with coefficient of variation intra and inter assay of 9.59 and 13.7%, respectively.

Statistical analysis. For the analysis of the concentration of glucose, insulin, P4, weight gain, area of ribeye and backfat thickness, an analysis of variance was made using as fixed effect the treatment, the genotype and their interaction. The variables response to estrus, return to estrus, percentage of gestation at 35 days and prolificity were analyzed with a ji-squared test, and the variable hours at the start of estrus was analyzed with an analysis of survival, using the statistical package SAS version 9 (11).

RESULTS

There was no effect of the genotype Dorper or Katahdin and their interaction with the energy level in the variables evaluated, thus the results in the tables are presented only by the effect of the energy level. The energy level did not affect (p>0.05) weight gain, fat thickness and area of the ribeye during supplementation prior to insemination. Table 2 shows that during the time of feeding prior to insemination, the ewes fed with 2 and 2.5 Mcal kg⁻¹ gained 3.68 and 4.21 kg of weight, 0.271 and 0.286 cm of fat thickness and 2.024 and 1.92 cm² of ribeye area, respectively.

Table 2.	Mean \pm standard error of weight gain,
	backfat thickness and area of the ribeye
in Dorper and Katahdin ewes fed with two	
energy levels prior to insemination.	

Variable	T1	Т2
Initial weight (kg)	36.44 ± 1.82	36.75 ± 1.63
Final weight * (kg)	41.79 ± 2.29	42.1 ± 2.05
Weight gain (kg)	3.68 ± 0.64	4.21 ± 0.57
Initial fat thickness (mm)	1.79 ± 0.10	1.88 ± 0.10
Final fat thickness (mm)	2.71 ± 0.18	2.86 ± 0.17
Fat thickness gain (mm)	0.97 ± 0.17	1.02 ± 0.16
Initial AOC (cm ²)	7.606 ± 0.365	7.592 ± 0.310
Final AOC (cm ²)	10.089 ± 0.456	9.518 ± 0.435
Gain of AOC (cm ²)	2.024 ± 0.312	1.92 ± 0.298

*Weight at the end of the study or at insemination; AOC: area of the ribeye; T1: ewes supplemented with 2.0 Mcal kg⁻¹ of ME prior to insemination and T2: ewes supplemented with 2.5 Mcal kg⁻¹ of ME.

There was no effect (p>0.05) of the feeding prior to insemination with the two energy levels (2 or 2.5 Mcal kg⁻¹ of ME) in the reproductive variables such as hour of entrance in estrus after the withdrawal of the progesterone, percentage of gestation, prolificity, number and size of the preovulatory follicles (Table 3).

 Table 3. Reproductive variables in Dorper and Katahdin ewes fed prior to insemination with two energy levels

two energy levels.		
Variable	T1	Т2
Entrance to estrus (h)		
24	33.3	13.3
30	16.7	20.0
36	25.0	46.7
42	25.0	20.0
Return to estrus (%)	8.4	13.4
Percentage of gestation	91.6	86.6
Prolificity	1.27	1.38
Number of preovulatory follicles	3 ± 0.3	3 ± 0.3

Size of preovulatory follicles (mm) $5.17 \pm 0.22 + 0.22 \pm 0.22$

T1: ewes supplemented with 2.0 Mcal kg⁻¹ of ME prior to insemination and T2: ewes supplemented with 2.5 Mcal kg⁻¹ of ME.

When the concentrations of progesterone were compared on day 13 post-insemination and of glucose and insulin at the end of feeding with both energy levels, it was found that there was no effect (p>0.05) of the supplementation with 2.0 or 2.5 Mcal kg⁻¹ of ME, genotype and interaction (Table 4).

Table 4. Mean ± standard error of the concentrations		
of progesterone, glucose and insulin in Dorper		
and Katahdin ewes supplemented with two		
levels of energy prior to insemination.		

Variable	T1	T2
Progesterone (ng/mL)	8.67±1.67	9.67±1.02
Initial glucose (mmol/L)	3.56±0.07	3.67±0.07
Final glucose (mmol/L)	4.75±0.35	4.51±0.35
Initial insulin (pmol/L)	13.98±1.62	17.62±2.95
Final insulin (pmol/L)	13.06±0.84	12.84±0.80

T1: ewes supplemented with 2.0 Mcal $kg^{\rm -1}$ of ME prior to insemination and T2: ewes supplemented with 2.5 Mcal $kg^{\rm -1}$ of ME.

DISCUSSION

Feeding with 2.0 or 2.5 Mcal kg⁻¹ of EM during 14 days of adaptation and 14 days of feeding with integral diet prior to insemination in Dorper and Katahdin ewes did not modify the reproductive parameters, concentrations of glucose, insulin and progesterone, or the changes in weight and dorsal fat thickness.

Similar conception rates have been found in Katahdin and Dorper ewes (4); on the other hand, different results have been observed in the reproductive response when supplementing with energetic diets prior to insemination. It has been reported that supplementation at 1.5% of live weight for 35 days with a concentrate with 3.54 Mcal kg⁻¹ of digestible energy to grazing ewes during the reproductive season and with availability of fodder, does not affect the number of corpus luteum and the estrus response (12). In a later study under similar conditions, but conducted during the summer, considered the non-reproductive season and with scant fodder, it was found that supplementation increased the number of ovulated ewes and the number of corpus luteum (6); therefore, the availability and amount of grass should be considered when formulating the rations to be used for supplementation. In the present study, the feeding with integral diet with 2 or 2.5 Mkal

kg⁻¹ of ME prior to artificial insemination did not affect the reproductive response. In contrast, it has been reported that feeding with 3.0 Mcal kg⁻¹ of DE for a short period (5 days before to 1 day after the withdrawal of progestogen) or a long period (15 days before to 1 day after withdrawal of the progestogen) increase the percentage of double ovulations (7). Although energetic supplementation, whether for a long period of 16 days or a short period of 6 days increment double ovulations, it is necessary not to exceed the percentage of protein in the diet (13). The use of forage plants of high nutritive quality is also an alternative in places where there is availability, given that it improves the ovulation rate (5). Therefore, good diet prior to insemination has been demonstrated to improve the reproductive parameters. In the present study there was no difference between the diets with 2.0 and 2.5 Mcal kg⁻¹ of ME in Dorper and Katahdin ewes, although the percentages of fertility found are good. However, if higher prolificity is desired, diets with more than 2.5 Mcal kg⁻¹ of ME could be tested.

Diets high in energy (130% of the energy requirement for maintenance) for a short period have no effect on the number of follicles employed; however, the duration of estrus is increased, along with the size of the ovulatory follicle and ovulation rate (14). In our study, the diet with 2.0 or 2.5 Mcal kg⁻¹ of ME in Dorper and Katahdin ewes did not affect the number and size of the preovulatory follicles and prolificity; in contrast, in Ossimi ewes an increase has been found in the number of medium (3 to 5 mm) and large (> 5 mm) follicles, as well as in ovulation by providing a diet with 2.6 Mcal kg⁻¹ of ME with respect to those fed with 2.0 Mcal kg^{-1} of ME (15). These differences may be due to multiple factors, such as the different nutritional requirements and different reproductive response of the genotypes, or that the diet with 2.6 Mcal kg⁻¹ of ME was provided intermittently on different days of the estrous cycle, whereas in the present study the supplementation was carried out for a long period prior to artificial insemination. It is probable that contrary to what was reported by other authors where the ovulatory rate and size of the follicles have been increased, in the present study, providing a diet with 2.0 Mcal kg⁻¹ of ME for a period of 14 days of adaptation and 14 with integral diet was sufficient to increase the body reserves and the concentrations of glucose and insulin, which are involved in the development of the follicles (16).

Body condition is related to the amount of fat in the body of the ewes (17). It has been reported that body condition has an influence on the reproductive response of ewes, where a higher rate of conception and number of lambs born has been registered in ewes with a body condition at insemination of between 3 and 3.5 in contrast with those with a lower condition (18). Similarly, it has been determined that in Malpura ewes, the optimal body condition for insemination is between 3.0 and 3.5, given that the conception rate and litter size decrease with a lower or higher condition (19), in addition, incrementing body condition above 3.5 may be an inefficient utilization of energy (17). In the present study, the ewes were distributed in such a way that both groups were balanced according to their body condition; at the start of the study there was an average condition of 3.0 and a backfat thickness of 1.8 mm for those that consumed 2.0 Mcal kg⁻¹ of ME and 1.88 mm for those that consumed 2.5 Mcal kg⁻¹ of ME. Providing both diets generated a recovery of muscle and fat, therefore, it is probable that in the case of feeding the ewes over a longer period with 2.5 Mcal kg⁻¹ of ME, they would have gained greater fat thickness and area of the ribeye. However, incrementing the body state beyond what is accepted could generate adverse effects in reproduction.

In the present study backfat thickness and area of the ribeye were used as more exact measurements for measuring the body state of the ewes, where it was observed that during the supplementation for 14 days of adaptation and 14 of feeding with integral diet with 2.0 or 2.5 Mcal kg⁻¹ of ME, the body condition and fat reserves of the ewes increased.

In supplementation plans for long periods prior to insemination, the use of diets with 2.0 Mcal kg of ME is more recommendable, given that it was shown that similar reproductive results are obtained and with a lower cost of feeding. When backfat thickness is increased, it is probable that the ewes also store fat in different parts of the body, as it has been shown that there is a high positive correlation between the fat in the carcass and internal, omental, mesenteric and pelvic fat (20). Fat thickness at the moment of insemination does not affect the birthing rate and litter size; however, the weight of the litter increases at birth and at weaning (21). Therefore, in addition to considering the importance of recuperation of the ewe at insemination in the reproductive parameters, it is necessary to evaluate the benefits in the future development of the lambs. Better productive and reproductive parameters have been found in ewes with body fat of 1.5 to 2 mm compared with those with a fat thickness of less than 1.5 mm (22). In the present study it would not have been possible to make a classification of such a low level of fat thickness at the moment of insemination, given that only 8.6% of the ewes had a thickness of under 2 mm, with 69.5% concentrated between 2 and 3 mm of fat.

Glucose supplies energy to the ovarian cells, while insulin regulates its absorption (23). In response to nutritional deficiency, changes can be generated in the concentrations of metabolic hormones, permitting the alteration of follicular growth, guality of the oocytes and the subsequent survival of the embryo. It has been reported that Ile-de-France ewes with a body condition lower than 2.0 presented a lower concentration of glucose and insulin than those with higher than 3.0, while it was increased with supplementation. (9) It has also been reported that by incrementing body condition due to good nutrition, insulin concentration increases when the body condition increases from 1.25 to 3.0, to later become stabilized or show a slight decrease when body condition increases from 3.0 to 4.0 (24). In the present study, although the backfat thickness and rib-eye area increased during the stage of feeding with 2 and 2.5 Mcal kg⁻¹ of ME, the insulin concentration did not increase, and to the contrary, there was a decrease, which was more pronounced in the treatment with 2.5 Mcal. Therefore, it is possible that with the ewes in the present study, by having an average body condition of 3.08 at the start of the study, their insulin concentration remained stable when provided with the diets.

The increment in glucose concentration occurs even with the supplementation for a short period with high energy diets (14). Both the amount of glucose and of insulin are rapidly modifiable, given that one day after the start of energetic supplementation, their concentration increases (13). Similarly, it has been reported that besides glucose, insulin and leptin also increase in grazing and supplemented hair ewes, but this is not reflected in fertility and prolificity (25). It has been found that when there is diet restriction, folliculogenesis is inhibited, perhaps due to the decrease in the circulation of glucose, insulin and glucagon, whereas with supplementation this effect is reverted and the number of follicles larger than 3.5 mm increases (8). At the end of the present study the concentrations of glucose and insulin did not vary between the ewes fed with 2.0 and 2.5 Mcal kg⁻¹ of ME, probably because during the long period during adaptation and feeding with the integral diet, the ewes increased their concentrations, weight gain, fat thickness and ribeye area in both treatments. Therefore, the ewes were able to recover when they were supplemented over a long period of time even with the supplementation with 2.0 Mcal kg⁻¹ of ME.

Progesterone plays an important role in preventing luteolysis, thus aiding the maternal recognition of pregnancy (26), whereas the deterioration of steroidogenesis, or reduced capacity of progesterone production leads to the death of the luteal cells (27). In this sense, progesterone secreted by the corpus luteum is critical for the establishment and maintenance of early gestation (28). The extra supply of energy in the diet of ewes through the inclusion of 4% palm oil in ewes increments the lipidic metabolism, including cholesterol, which in turn increases the concentration of progesterone. However, by increasing the inclusion of palm oil to 6 %, the lipidic metabolism and concentration of progesterone are not further modified (29). Similarly, in cows with low body condition in grazing and supplemented with a concentrate with 4% of corn oil, an increase in the concentration of progesterone and cholesterol has been reported. In contrast, in dairy cows it has been found that the difference of the 12% in the consumption of dry matter of fodder does not alter the metabolism of progesterone (30). In the present study 3% of soy oil was added to the diet with 2.5 Mcal kg⁻¹ of ME and the concentration of progesterone was measured on day 13 post-insemination, given that this is when the natural drop in progesterone of the estrous cycle occurs. In gestating ewes, the corpus luteum should produce enough progesterone to aid in the maternal recognition of gestation. To this respect, supplementation prior to insemination with 2 or 2.5 Mcal did not influence the later production of progesterone by the corpus luteum.

Under the conditions of the present study and due to the similar results in the variables evaluated and even greater cost of the diet with 2.5 Mcal, the use of 2.0 Mcal kg^{-1} of ME is recommended in ewes prior to insemination.

It is concluded that feeding with a diet containing 2.0 or 2.5 Mcal kg⁻¹ of ME for a period of 14 days of adaptation and 14 days integral diet prior to insemination in Dorper and Katahdin ewes did not affect the reproductive parameters, metabolic state and body changes; however, with both diets there is an increase in live weight and the body reserves. Therefore, in order to reduce costs of feed, the use of 2.0 Mcal kg⁻¹ of ME is suggested.

Conflict of interest

The authors declare that they have no conflict of interest related to the publication of the present article.

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