Case Study 13: Design of an experiment to compare performance and meat quality characteristics of three Ethiopian goat genotypes under varying nutritional conditions

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Summary

An experiment is planned to compare and select an indigenous Ethiopian goat genotype that best meets the body weight and meat quality import requirements of Middle East countries. Three genotypes are to be used in an experiment in which three different feeding regimes are also to be compared.

The case study illustrates how a study protocol is prepared to describe the genotypes and feeding regimes to be used, the details of the experimental design, the measurements to be made and the statistical analysis proposed for a completely randomised factorial design., , The case study then goes on to discuss the appropriateness of the study design used in the protocol and uses a number of study questions to allow the student to investigate the issues further.

The important need for good biometrician researcher collaboration is emphasised.

Glossary

A few terms may be unfamiliar to the reader.

Chevon quality:	a complex parameter used to assess the quality of goat meat based on an overall assessment of the chemical composition of the meat, its colour, cooking loss, taste etc.
Chilled carcass weight:	the weight of the carcass after storage for 24 hours at about 4^{o}C. It is also called cold carcass weight.
Concentrate roughage ratio:	the types of feed contained in a diet. The concentrate portion in this experiment is made up of feed ingredients such as wheat bran, oil cake (agro-industrial by products) and salt as a supplement; the roughage is native pasture hay. The proportion of the two components can be varied depending on the animal performance required.
Dressing percentage:	calculated as hot carcass weight as a percentage either of slaughter weight or of empty body weight (see next page). It can also be calculated using cold carcass weight as the numerator.
Dry matter intake:	estimates the daily feed requirement of the animal (mainly providing energy, protein, minerals) on a dry matter basis. It is calculated as the difference of the daily feed offered and refused.
Empty body weight:	the weight obtained when the weight of the contents of the digestive tract (contents in the alimentary canal) is subtracted from the slaughter weight.

Fasting weight loss:	the weight loss recorded after an animal is fasted (from feed but not water) usually for 16 hours before it is slaughtered.
Feed conversion efficiency:	the ratio of the kg of feed fed per kg gain of the animal.
Hot carcass weight:	the weight usually taken within 45 minutes after slaughter and excluding body components such as head, feet, skin, blood, visceral organs (kidneys, liver, heart, lungs), testicles, fat depots (scrotal fat, kidney and pelvic fat and omental fat) and intestines.
Shrinkage weight loss:	the difference between hot and chilled carcass weight following storage of carcass for 24 hours at about 4^{o}C.

Background

About one tenth of the goat population of Africa is found in Ethiopia. Goats contribute to the rural economy and provide livelihoods to the poor, supplementing their food with nutritious meat and milk. Meat from small ruminants represents approximately one fifth of the meat eaten in sub-Saharan Africa. Goats also provide fiber and other by-products such as hide and skin and offal and offer sources of cash income to smallholder farmers. Goats also have the potential to become an increasingly valuable foreign exchange earner. Expansion of the human population, coupled with increasing urbanisation, is resulting in higher demands for meat, yet increases in livestock production are lagging behind the corresponding increases in human populations. There are thus considerable opportunities to tap further the potential of the small ruminant for the expanding international markets.

Ethiopia is currently benefiting from foreign currency achieved through export of goat meat, especially to Middle East countries. But international markets for meat are becoming more competitive and livestock producers need to meet quality standards laid down by importing countries both for live animals and for meat. The Livestock Marketing Authority (LMA) of Ethiopia recently assessed the needs of importing countries and reported that research is needed on ways to attain higher animal weights at a younger age, to achieve acceptable fat content of the carcass and acceptable meat quality, and to solve a darkening problem of the carcass observed in highland goats.

Goats purchased for export are mainly from Afar, Somali and Borena areas of Ethiopia. Purchases from some highland parts of the country have been discontinued due to an observed darkening of the meat during storage. Exporters at abattoirs suspect that this phenomenon may be due to poor fat coverage of the carcass, which in turn may reflect inadequate provision of dietary nutrients. Indigenous goats are not currently attaining the required marketing weight per age and acceptable fat content. The major factors that affect their productivity are feed supply, animal genotype and management. Feed resources vary in the different parts of Ethiopia but generally involve grazing of native pasture, crop residues of cereals and grain legumes. Oil cakes, mill products and other unconventional feeds are also sometimes provided as supplements. However, grazing land is increasingly being brought

under cultivation and so further exploitation of goat production may be necessary under stall-fed conditions.

Indigenous goats appear to have the potential to meet the export market provided they can be fed optimum nutritional inputs, and appropriate genotypes are selected that meet the necessary chevon quantity and quality required for the export market. Therefore, there is research needed to compare the performance of different genotypes using improved feeding systems made of available feed ingredients in Ethiopia in order to identify those best able to achieve the live weight, carcass yield and meat quality required at a younger age for the export market. An experiment was planned to achieve this aim at the International Livestock Research Institute's Debre Zeit Research Station in Ethiopia.

This case study focuses on the study protocol written for the experiment and also discusses certain elements of the study design. The results have since been published and can be found in Sebsibe (2006).

Questions to be addressed

The main question to be addressed is: how does one go about designing an experiment and preparing a protocol? We discuss what needs to be included in an experimental protocol and describe its contents. The protocol, as presented for this case study is not perfect, deliberately so.

The objective of the experiment is to compare different indigenous goat genotypes under different feeding regimes for performance and meat quality characteristics that meet the export market requirements to meet import standards of Middle East countries.

Study protocol

The protocol (simplified for the purpose of this case study) was the one used for the experiment. Another example of an experimental protocol is included in Case Study 5. Protocols need to cover various aspects of a study. The items included in this case study are listed below. Particular aspects about each item are noted in green italics.

Study protocol/Background

This has already been given to introduce the case study. Normally it would appear here.

There should also be a literature review.

Study protocol/Objectives

These are very important and should be clearly and unambiguously written.

- Compare different indigenous goat genotypes and select the one that best meets the import standards of Middle East countries.
- Identify an optimum feeding regime that meets the required target weight at a younger age for the selected genotype.
- Assess the effect of the feeding regimes and genotypes on feed conversion efficiency, growth performance, carcass characteristics and meat quality.

These objectives can be turned into the null hypothesis that:

Indigenous goat genotypes under the same nutritional regimes have similar growth performance and produce similar carcass characteristics and meat quality.

Study protocol/Location

Next we describe where the experiment is to be done.

The study is to be carried out at Debre Zeit (ILRI) research station, 45 km East of Addis Ababa at an altitude of 1900m above sea level. The average annual rainfall of the area is 851mm and the average minimum and maximum temperatures are 8.9°C and 24.3°C, respectively.

Source: Jean Hanson

Study protocol/Genotypes

Next we describe the goat genotypes that have been chosen.

The following three indigenous genotypes will be evaluated. Each belongs to a different goat family population.*(Photographs will not normally be included in a protocol.)*

1. Afar. This goat is well adapted to semi-arid and arid environments. It has a concave facial profile, narrow face, pricked ears and long, thin, upward-pointing horns. It is leggy and has a patchy coat colour, dominated by white, followed by light brown and black. Other genotypes, namely Worre and Abergelle, are related to the Afar. The meat can be consumed fresh, air-dried or cooked and is currently being exported.

2. Long-eared Somali. This is a large white goat with predominantly straight facial profile. Horns are mainly curved and pointing backwards. Polledness has been recorded in males. Most males have beards. The genotype is related to the short-eared Somali goat type. Goat meat is preferred to mutton in areas where the long-eared Somali goat is kept. The meat may be consumed fresh or preserved through various traditional methods, and is currently being exported.

3. Central Highland. This goat is a medium-sized, broad-faced animal with thick horns and is mainly reddish brown in colour. It has a predominantly straight facial profile. The horns are mainly straight and pointed backwards. Most males have a beard and a ruff. This genotype is related to Western highland and Keffa goat types. The meat is currently not being exported for the fear of rejection due to the reported meat-darkening problem. However, this genotype is included because of its high proportion in the goat population of Ethiopia and because it represents a different goat family.

Study protocol/Feeding regimes

Now the diets.

The experiment also presents an opportunity to observe the effect of feeding regime on meat colour during storage.

Three feeding regimes will be used:

- Low feeding regime: a diet to support a growth of 50 g/d (concentrate: roughage ratio, 50:50)
- High feeding regime: a diet to support a growth of 75 g/d (concentrate: roughage ratio, 80:20)
- Medium level: a diet to support a growth of 62 g/d (concentrate: roughage ratio, 65:35).

The ration will be formulated following nutrient recommendations for developing countries. The feed ingredients will be native pasture hay (as a basal roughage diet) supplemented with wheat bran, oil cake (agro-industrial by-products) as concentrates, and salt.

Goats will be housed in individual pens and fed individually daily. Individual feed intakes will be recorded daily by weighing both the feed offered and refused. Goats will have access to clean

water and a mineral block.

Study protocol/Experimental design

Details of the animals, their management and assignment to diet.

Twenty four male goats, and a few replacements, will be purchased for each genotype from farmers in their respective regions. The goats will be of similar age (milk teeth) and weighing 13-15 kg. Goats from each genotype will be randomised to the three diets and then all 72 goats assigned at random to individual pens.

The animals will be dewormed, dipped and vaccinated against recommended parasites and diseases before they are moved to the pens. There will then be an adaptation period of 14 days before the experiment starts. Thereafter, the experimental diet will be offered to each goat as planned. Goats will be weighed weekly in the morning before watering and feeding. However, the initial and the final weight will be recorded for two consecutive days and the averages taken as the initial and final body weights.

Each goat will be monitored until it reaches a body weight of about 20kg. This selection is based on the range of weights demanded by the major importing Arabian countries. The number of days taken to reach this target weight will be recorded.

Study protocol/Measurements

Then details of what is measured and how.

The following measurements will be made.

1. Days to reach the target slaughter weight

2. Feed measurements

Dry matter (DM) intake

Feed conversion efficiency
Nutritional value of the feeds

3. Body measurements

Body weight will be recorded weekly.

Body measurements will be taken before slaughter on the following body parts with the help of a measuring tape. The goat will be measured while standing on four legs on an even surface.

- Body length (point of shoulder to the pin bone-see photograph)
- Body height (base of the hoof to the highest point of the wither)
- Heart girth (circumference of the body immediately behind the forelegs)
- Paunch girth (circumference around the umbilicus)
- Neck girth (circumference at the base of the neck)
- Thigh circumference (around the middle of the thigh)

4. Carcass measurements

After reaching the pre-determined final weight an animal will be fasted for 16 hours with free access to water and then weighed before slaughter. Slaughtering will be done following the 'Halal' method. The carcasses will be eviscerated and all body components such as head, feet, skin, blood, visceral organs (kidneys, liver, heart, lungs), testicles, fat depots (scrotal fat, kidney and pelvic fat and omental fat), intestines, when full and empty, will be weighed.

The following carcass characteristics will be recorded:

- Final weight
- Fasting weight loss
- Slaughter weight
- Empty body weight
- Hot carcass weight
- Chilled carcass weight
- Shrinkage weight loss
- Dressing percentage
- Proportion of whole sale cuts
- Proportion of lean, bone and fat
- Muscle/bone/fat ratios
- Fat depth (of the 12th rib, 110 mm from the spinal cord).
- Rib eye area
- Fat thickness (at first and last rib and last lumbar vertebrae)
- Non-carcass components
- Carcass length
- Leg length
- Circumference of the buttock

Empty body weight will be computed as the difference between slaughter weight and the sum of the digestive tract contents. The chilled carcass weight will be measured after 24 hours of chilling at 4^0C and used to determine chilled dressing percentage and shrinkage weight loss.

After removing the tail the carcass will be split along the dorsal middle line with a band saw and carcass length(from the caudal edge of the last sacral vertebra to the dorso-cranial edge of the atlas (first cervical) vertebra) measured. Eye muscle area will be measured after tracing the muscle at the 12/13th rib position.

Commercial cuts will be made from the left half of the carcass and disjointed to leg, loin, racks, shoulder and neck, and breast and shank. Then the rib section (8-9-10th rib) of the carcass will be dissected by knife and tissues will be separated to determine the proportions of muscle, bone and fat.

5. Chevon quality

Description of scientific method can become quite technical; nevertheless, it needs to be described precisely so that others can check what was done.

Meat quality measurements will include:

- Chemical composition (CP, EE, ash and DM)
- Tenderness
- pH
- Colour
- Cooking loss
- Fatty acid composition

The dissected lean and fat from the half carcass will be minced and sub-sampled for analysis of CP, EE, moisture and ash using standard AOAC methods.

Tenderness will be measured in a standard sized, cooked sample using shear force tests. The loins (m. longissimus dorsi, L1- L6) will be vacuum packed and frozen at -20^{0}C for shear force determinations. The frozen loins will then be thawed for 24h at 2^{0}C and cooked in an electric oven until the meat internal temperature reaches 65^{0}C. Muscle cores with a cross section of 1x1 cm and at least 3 cm long will be cut parallel to the muscle fibres and shear force values taken using Warner-Bratzler shear force / Instron apparatus mounted in a texture analyser testing machine. An average of at least six replicates will be taken of the maximum force needed to shear a sample perpendicular to the axis of the fibre direction.

Muscle pH will be determined using a pH meter with a combined electrode by insertion into the Longissimus muscle at the 12/13th rib site on the chilled carcass. It will be done 24 hours post mortem and the ultimate pH will be determined. Muscles will be weighed and cooked in a plastic bag immersed in a water bath at 85^{0}C for 45 minutes until an internal temperature of 70^{0}C is achieved. Samples will be cooled and weighed and the percentage loss in weight recorded to measure cooking loss.

The lean and subcutaneous fat from the half carcass will be minced and sub-sampled. The samples will then be subjected to a gas liquid chromatography technique to determine fatty acid composition. Meat colour will be estimated in the Rectus abdominis muscle 24 hours after slaughter and after 30 minutes of exposure to air using the L*a*b* system by chronometer (Minolta CR 300).

Study protocol/Statistical analysis

It is important to describe in detail how the data will be analysed.

The three genotypes and three feeding regimes will be factorially arranged in a completely randomised, individually fed experiment. Each variable will be subjected to a least squares analysis of variance by GenStat. The model will be designed to determine the effect of genotype, feeding regime and their interaction on body weight, daily gain, nutrient utilisation, carcass traits and meat quality. The daily live weight gain for each animal will be calculated by linear regression of live weight on time.

The structure of the analysis of variance will be as follows:

Source of variation	d.f.	S.S.	M.S.
Genotype	2		
Feeding regime	2		
Genotype x Feed	4		
Residual	63		
Total	71		

When an F-test is significant the least squares means will be compared using Duncan's multiple range test. Variables measured as percentages will be transformed using the arc sine function. *(Biometricians may question the use of Duncan's test here, also possibly the need for a transformation. Two study questions address these issues.)*

An experiment will be carried out using a taste panel to assess meat eating quality. The assessment will be carried out on Longissimus muscle samples cooked in the same way as for shear force measurements. The attributes (flavour, tenderness, aroma and juiciness) will be assessed by eight panelists using an eight-point descriptive scale (1-8) with the larger scores indicating a more favourable rating. For example, flavour will be described as 1=extremely undesirable, 2=very undesirable, 3=moderately undesirable, 4=slightly undesirable, 5=slightly desirable, 6=moderately desirable, 7=very desirable and 8= extremely desirable. Similarly, juiciness will be assessed as 1=extremely dry to 8=extremely juicy, aroma as 1=not intense to 8= extremely intense and tenderness as 1=extremely tough to 8=extremely tender. The panelists will be trained in standard attribute assessments.

There will be two sessions per day. During each session each panelist will be presented with four samples. Samples will be coded with a unique randomly selected three digit code and the serving sequence will also be randomised. Tap water and fresh carrot rings will be used as a mouth cleanser before tasting and in between different samples. The panellists will be expected to take a bite of the carrot followed by a sip of water and wait for 10 minutes between samples in order to restore the normal fluid environment in their mouths. The samples will be offered warm.

Findings, implications and lessons learned

1. The above study protocol is typical of protocols that need to be prepared before any study is undertaken. A number of details are included deliberately in this protocol to stimulate further discussion on possible improvements that might be made. The first point to note about the protocol is that many of the aspects of experimental design are concerned with scientific rather than statistical method. This is important. There is no point designing a good statistical design if the scientific conduct of the experiment is poor. A biometrician should be able to follow what is proposed without necessarily understanding the details.

2. A protocol will start with an introduction that describes the background to the study, previous work that has been done and reasons for approaching the study in the way proposed. Here we have separated the introduction from the protocol and included it as the Background at the beginning of this case study. The introduction/background to the study has also been intentionally abbreviated to simplify it for the purpose of this case study. Usually the introduction will contain a literature review referring to work already done and into which the proposed study can be put into context.

3. The objectives should then be clearly stated. Researchers sometimes find these difficult, but they are essential for ensuring that an appropriate study is designed to meet the specified objectives and that the results can be suitably analysed to fulfil the study expectations.

4. The protocol then contains details of where the study is to be conducted, the experimental materials to be used, the type of experiment and how it is to be designed. A list of measurements to be made is then given with details of methods to be used. Finally, a summary of proposed methods for statistical analysis are outlined. Each of these is important.
5. Details of a sensory analysis experiment to assess taste qualities are also given. These have intentionally been described somewhat loosely in order to provide study questions for the student.
6. So what might be missing? There is a long list of measurements to be made during the experiment. This will certainly require some care in data recording. It may not be obvious to the reader which measurements are observed and which measurements are derived from other measurements: this could be made clearer. A section on data recording would focus the researcher's mind on how the data will best be assembled and which variables can be derived later by computer. Draft data recording sheets could usefully be attached to the protocol to demonstrate how data recording is to be done. A statement should also be included on how data are to be entered into the computer and the software to be used. Data management is likely to be an intricate part of the study and plans are best set out at the start. This is an important component of research methods.

Study design

1. Goats were assigned at random to pens. But complete randomisation can increase the difficulties of day-to-day management of the experiment. In contrast, grouping goats receiving the same feeding regime together in the same block of pens would simplify feeding and reduce possible errors. This might be suitable for an experiment in a well controlled environment provided that there are insignificant variations associated with the position of a pen within the house. Experimental design can thus become a compromise between what is theoretically desirable and what is practically manageable and may well depend on the availability and quality of experimental attendants.

2. It might be possible to design the experiment as a randomised block with genotypes and feeding regimes randomised within blocks in order to accommodate variations caused by the positions of the pens within the building. Pens close to the edge of the house may be more influenced by outside air flows than those inside. This would not help with ease of experiment management but may reduce the size of the residual variance.

3. Appropriate sample size is an important issue to address, especially as large animal to animal variation can occur. Knowledge of likely error variation from previous experiments can help to determine a suitable sample size for each group. In this experiment it was deduced, rightly or wrongly, that eight goats would be sufficient per group. Should there be no significant interactions then the experiment would result in more than adequate sample sizes of 24 goats for comparisons of average genotype differences on the one hand, and of average feeding regime differences on the other. The summing of one factor over another illustrates a powerful feature of factorial experiments. In this case, however, the researcher is interested in determining optimal feeding regimes for each genotype and it is likely that significant interactions could occur. It is important, therefore, to ensure that adequate numbers of goats are included for comparisons between individual groups.
4. When a researcher is discussing an experimental design with a colleague or biometrician he/she should be expected to be asked to justify reasons for his/her decisions not only on sample size but also on questions on numbers of treatments, doses etc. Thus, it would not be unreasonable to discuss the numbers of different genotypes that are proposed for comparison or different levels or types of feeding regimes. For example, the researcher might have wished to compare four genotypes but have realised that experimental constraints prevented this. The option of using two feeding regimes and four genotypes could, however, be explored. In this particular case the 3x3 experiment proved to be the

most desirable arrangement, but in other situations adjustments to the initial proposal for the design can be fruitful.

Study questions

1. Suppose that the researcher really wished to assess four genotypes but was constrained to having no more than 80 goats on experiment. Meet with another student to discuss the problem and prepare a discussion in class with one student playing the role of researcher and the other as biometrician.
2. A few extra goats were purchased as replacements for each genotype. Discuss why it might be useful to have these extra goats and when such goats might be used to substitute other goats during the course of the study. How would the researcher decide which 24 goats from the total number available for each genotype to allocate to the experiment?
3. The layout of a typical animal experiment is illustrated in one of the photographs. It can be seen that pens are situated in parallel rows. As explained in the case study it is sometimes a good idea to form blocks of goats so that each genotype x feeding regime is included within each block in order to account for temperature or air movement variation across the building. Include a sentence or two within the protocol to describe how this should be done for the experiment described and modify the structure of the analysis of variance accordingly.
4. Often in animal experiments researchers elect to order animals according to their body weights and 'block them' into groups (in this case groups of nine) and then assign them at random to treatment from within each group. What might be the advantage of this and why do you think that the researchers decided not do so in this study.
5. The methods for statistical analysis in the experimental protocol proposed that Duncan's multiple range test be used for comparing least squares means. Discuss when Duncan's test should be applied and whether it is appropriate in this case. If not, suggest what approach you would adopt.
6. It is proposed that the arc sine function be used for variables that are calculated as percentages. Discuss when and why an arc sine transformation is used? Is it appropriate to apply it to the percentage variables in this case study? Discuss any disadvantages that may occur in reporting results when transformations are used.
7. Details of the sensory analysis experiment are incomplete. Using the suggestions provided prepare a suitable experimental design and describe how the statistical analysis will be done. Write appropriate paragraphs that might be added to the protocol.
8. Go through the various measurements that are proposed. Decide which measurements are to be calculated from others, and rewrite the lists separating those measurements that are to be taken directly from the animals and those that are to be derived. Note the cases where the details on how measurements are to be obtained are unclear.
9. Design recording sheets to take the recorded data.

Related reading

Sebsibe, Ameha 2006. *Meat quality of selected Ethiopian goat genotypes under varying nutritional conditions.* PhD thesis, Department of Animal and Wildlife Sciences, Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa. Abstract

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Unless otherwise mentioned photographs were taken by the senior author.